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ANTIMICROBIAL ACTIVITY AND CHEMICAL CONSTITUENTS OF DIFFERENT EXTRACTS OF RHIZOMES OF TURMERIC (*CURCUMA LONGA* L.) FROM WEST ANATOLIA

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ABSTRACT

To speciticate the chemical context and antimicrobial characteristic of rhizomes of turmeric (*Curcuma longa* L.) rhizomes extratcs obtained from Muğla, Turkey. The extract of ethyl acetate (EA), methanol (M) and water (W) of turmeric rhizomes were studied for antimicrobial characteristic against 11 bacteria and one yeast by disc diffusion method. Among the extracts assayed, the ethyl acetate extracts of turmeric rhizomes exhibited good activity against *B. subtilis* ATCC 6633, *B. cereus* CCM 99 at 100mg for example 20 mm was recorded as diameter zone of inhibition. The least inhibition zone of rhizomes is 9 mm against *K. pneumoniae* CCM 2318 at 50 mg. *C. albicans* ATCC 10239 showed no inhibition zone both 50 mg and 100mg doses. The M extracts of turmeric rhizomes displayed good activity against *S. aureus* ATCC 6538/P 100 mg for example 14 mm was recorded as diameter zone of inhibition. When we compared MIC value of the EA, M and W, the EA extract (MIC 1-16 mg/ml) was found to be very effective followed by the W extract (MIC 2-64 mg/ml). The least effective was the M extract (MIC 16-128< mg/ml). The W extracts presented the best activity (MIC 2 mg/ml) against *S. aureus* ATCC 6538/P compared with standart drugs.

KEYWORDS:
*Curcuma longa* L., turmeric, rhizomes, extract, antimicrobial activity, GC-MS

INTRODUCTION

Nowadays, usage of natural antibacterial compounds is take attention, herbs and spices for the preservation of foods, as these posses a characteristic flavour and sometimes show antioxidant activity and also antimicrobial characteristic [1]. To achieve this purpose, the food industry has used synthetic additives (added or already present naturally in the foods) which reduction microbial growth or inhibit microorganisms and prevent or delay, in a significant way. However, even we discovered many antibiotic drug, we still facing multidrug resistance to bacteria [2,3] and the side effect of antibiotic treatment for patients like allergia. Some of the plant family have a antimicrobial activity like Zingibereaceae, Oleaceae, Liliaceae and Lamiaceae. Several plant parts are used for medicinal purposes ie., bulb, gel, leaves, roots, barks, peels etc. *Curcuma longa* L. (Family Zingiberaceae) is a a perennial herb with, pulpy, orange, tuberous roots that grows to about 2 feet in length and is cultivated extensively in India, China, Bangladesh and other Asian countries with a tropical climate [4]. Curcumin has been reported to show anti-inflammatory, antioxidant and chemopreventive property [5]. And also, antimicrobial activity of *C. longa* have against various microorganisms [6-13].

Our paper is the first report on the antimicrobial activity of West Anatolian turmeric against pathogenic bacteria. Therefore, we have investigated for the antimicrobial activities of three different solvent extracts from West Anatolian turmeric. The aim of this study is to carry out a comparative analysis of the antimicrobial activity of extracts obtained from West Anatolian turmeric. For this reason, we have practiced antimicrobial effect against some microorganisms including opportunistic pathogens: Gram-negative bacteria; *Escherichia coli* O157:H7, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 583, *Aeromonas hydrophila* ATCC 19570, *Klebsiella pneumoniae* CCM 2318. Gram-positive bacteria; *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* CCM 99, *Staphylococcus aureus* ATCC 6538/P, *Streptococcus faecalis* ATCC 8043. Fungus; *Candida albicans* ATCC 10239.

Disk diffusion method and minimal inhibitory concentration (MIC) were used for antimicrobial activity of tested extracts.
MATERIALS AND METHODS

Sample collection and storage: turmeric preparations were obtained from various retail outlets in West Anatolia, including supermarkets, shops and market stalls in Cine, Turkey in 2011. Turmeric sample was stored at ambient temperature until initial sample preparation, after which they were stored at 4°C until required for analysis.

Preparation of Curcuma longa L. rhizomes extracts. Two 100 g portions of powder of dried rhizomes of \textit{C. longa} were soaked separately in 1000 ml of ethyl acetate (EA) and methanol (M) for 72 h at room temperature. For water (W) extraction, 100 g of powdered sample were boiled in 1000 ml of hot water for 60 min [9].

Test microorganisms. The bacterial and fungal strains used for the screening were Gram-negative bacteria; \textit{E. coli} O157:H7; \textit{E. coli} ATCC 35218; \textit{P. aeruginosa} ATCC 27853; \textit{S. typhimurium} CCM 583; \textit{A. hydrophila} ATCC 19570; \textit{K. pneumoniae} CCM 2318. Gram-positive bacteria, \textit{S. epidermidis} ATCC 12228, \textit{B. subtilis} ATCC 6633, \textit{B. cereus} CCM 99, \textit{S. aureus} ATCC 6538/P, \textit{S. faecalis} ATCC 8043. Fungus, \textit{C. albicans} ATCC 10239.

Nutrient agar (NA) (Merck) and Potato-Dextrose Agar (PDA) (Merck) were used for testing the antibacterial and anticandidal activity.

Study of antimicrobial activity by disc diffusion assay. For the first screening, the paper disc diffusion method was used to determine antibacterial activity, which is based on the method described previously [14]. Tobramycin (10 µg/disc) (Oxoid), ampicillin (10 µg/disc) (Oxoid) and nystatin (30 µg/disc) (Oxoid) were used as positive controls and paper discs treated with ethyl acetate, methanol and DMSO were used as a negative control. The plates were then incubated at 35 °C for 24 h in an incubator. Inhibition zone diameters around each of the discs were measured and recorded at the end of the incubation time.

Determination of minimum inhibitory concentrations (MICs). MICs were determined by the agar dilution method, which is based on the method described previously [9]. The MICs of ampicillin (Oxoid) and oxacillin (Oxoid) were also determined. A final inoculum of 1×10^4 CFU/ml was spotted onto agar plates. The plates were then incubated at 35°C for 24 h in the incubator. The MIC was defined as the lowest concentration of extracts at which no visible growth was observed.

RESULTS AND DISCUSSION

In this work, the extracts of ethyl acetate, methanol and water of turmeric rhizomes were tested for antimicrobial activity against 11 bacteria and one yeast by disc diffusion method. The results are presented in Table 1. According to these data, the EA were the most active against tested bacteria both 50 mg and 100 mg doses. As compared with standart antibiotics. Among the extracts assayed, the EA of turmeric rhizomes exhibited good activity against \textit{B. subtilis} ATCC 6633, \textit{B. cereus} CCM 99 at 100mg for example 20 mm was recorded as diameter zone of inhibition. This followed by \textit{S. aureus} ATCC 6538/P and \textit{ATCC 19570} with 16 mm inhibition zone. The least inhibition zone of rhizomes is 9 mm against \textit{CCM 2318} at 50 mg. \textit{ATCC 10239} showed no inhibition zone both 50 mg and 100mg doses.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate extract (mg)</td>
<td>Methanol extract (mg)</td>
</tr>
<tr>
<td>\textit{E. coli} O157:H7</td>
<td>C 50 100</td>
</tr>
<tr>
<td>\textit{E. coli} ATCC 35218</td>
<td>- 13 15</td>
</tr>
<tr>
<td>\textit{P. aeruginosa} ATCC 27853</td>
<td>- 11 14</td>
</tr>
<tr>
<td>\textit{S. epidermidis} ATCC 12228</td>
<td>- 12 14</td>
</tr>
<tr>
<td>\textit{B. subtilis} ATCC 6633</td>
<td>- 15 20</td>
</tr>
<tr>
<td>\textit{B. cereus} CCM 99</td>
<td>- 15 20</td>
</tr>
<tr>
<td>\textit{S. typhimurium} CCM 583</td>
<td>- 11 13</td>
</tr>
<tr>
<td>\textit{S. faecalis} ATCC 8043</td>
<td>- 10 12</td>
</tr>
<tr>
<td>\textit{A. hydrophila} ATCC 19570</td>
<td>- 13 16</td>
</tr>
<tr>
<td>\textit{K. pneumoniae} CCM 2318</td>
<td>- 9 14</td>
</tr>
<tr>
<td>\textit{S. aureus} ATCC 6538/P</td>
<td>- 12 16</td>
</tr>
<tr>
<td>\textit{C. albicans} ATCC 10239</td>
<td>- 9 14</td>
</tr>
</tbody>
</table>

C, Control (only ethyl acetate, methanol or water); -, no inhibition; NT, not tested
The M extracts of turmeric rhizomes displayed good activity against *S. aureus* ATCC 6538/P 100 mg for example 14 mm was recorded as diameter zone of inhibition. This followed by 13 mm *B. cereus* CCM 99 at 100 mg.

While the W extract showed antibacterial activity against listed bacteria at 100 mg, none of the bacteria displayed antibacterial activity at 50 mg. The reason for these, curcumin is the product obtained by solvent extraction of turmeric i.e., the ground rhizomes of *Curcuma longa* and also curcumin is an oil-soluble pigment, practically insoluble in water [16]. All the extracts showed no anticanical activity.

When we compared MIC value of the EA, M and W extract, the EA extract (MIC 1-16 mg/ml) was found to be very effective followed by the W extract (MIC 2-64 mg/ml). The least effective was the M extract (MIC 16-128< mg/ml). In contrast to, Aly and Gumgumjee (2011) [12], tested antimicrobial activity of methanolic and butanolic extracts of *C. longa*. The diameter of inhibition zone ranged from 25 to 27 mm with mean antimicrobial index of 25 and from 14 to 24 mm with mean index of 19 mm for methanol and butanol *C. longa* extracts, respectively.

The EA extract displayed the best activity (MIC 1 mg/ml) against *B. subtilis* ATCC 6633, *A. hydrophila* ATCC 19570 and *S. aureus* ATCC 6538/P. The W extracts presented the best activity (MIC 2 mg/ml) against *S. aureus* ATCC 6538/P compared with standart drugs. In a similarly, Niamsa and Sitiwit [17] reported that aqueous extract of *C. longa* exhibited antimicrobial activity against *K. pneumoniae* ATCC 10031, *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25924, and *S. epidermidis* ATCC 12228 (MIC 4-16 gL\(^{-1}\)) and minimum bactericidal concentration (MBC) 16-32 gL\(^{-1}\). And also, Chandrana and his friend [18] reported that antimicrobial activity of turmeric was effective against *E. coli*, *B. subtilis* and *S. aureus* and suggested that the activity is due to the presence of curcuminoid, a phenolic compound. In another similar work, Naz and his friends [19] reported that both curcuminoid and oil showed antibacterial activity against all tested microorganisms. Because of intrinsic tolerance of microorganisms change to varying degrees of sensitivity of the bacterial test organisms.

Pundir and Jain (2010) [13] reported that the ethanolic extract being strongly active against *E. coli* isolates while aqueous extracts strongly active against *S. aureus* isolates (25mm-30mm). The ethanolic extract of turmeric was effective in extraction of antimicrobially active substances as compared to water and hexane reported by Gur and his friend [16]. The ethanol extraction of herbs and spices was better because ethanol is an organic solvent and dissolves more organic compounds. Therefore, the greater amounts of active antimicrobial components have been acquired [20]. Irhsad and his friends [21] reported that the ethanol extract of turmeric displayed the highest zone of inhibition (11 mm) against *B. subtilis* followed by *E. coli* and *S. aureus* that were 10 mm and 9 mm, respectively. The methanol extract of turmeric displayed maximum activity against *B. subtilis* (9 mm) followed by 8 mm for *E. coli* and *S. aureus*. And also, Mirbod and his friends [22] reported that feeding incremental levels of CRP(*C. longa* rhizome Powder) decreased *E. coli* enumeration in the ileal content of laying hens. No and his friends [23] reported that the curcumin nanoparticles (CNPs) formulated with positively charged surfactant cetrimonium bromide (CTAB) exhibited the highest antimicrobial activity against *L. monocytogenes*, indicating that there is a strong relationship between surface charge and antimicrobial activity of curcumin. The enhanced

### TABLE 2

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (mg/ml)</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Water extract</th>
<th>Ampicillin</th>
<th>Oxacillin</th>
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<td>64</td>
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<tr>
<td><em>P. aeruginosa ATCC 27853</em></td>
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<td>128&lt;</td>
<td>16</td>
<td>16</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis ATCC 12228</em></td>
<td>16</td>
<td>128&lt;</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td><em>B. subtilis ATCC 6633</em></td>
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<td>16</td>
<td>4</td>
<td>4</td>
<td>32</td>
<td></td>
</tr>
<tr>
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<td>4</td>
<td>4</td>
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<tr>
<td><em>S. typhimurium CCM 583</em></td>
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<td>8</td>
<td>16</td>
<td>16</td>
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<tr>
<td><em>S. faecalis ATCC 8043</em></td>
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<td>128</td>
<td>64</td>
<td>64</td>
<td>4</td>
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<tr>
<td><em>A. hydrophila ATCC 19570</em></td>
<td>1</td>
<td>32</td>
<td>8</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae CCM 2318</em></td>
<td>2</td>
<td>32</td>
<td>4</td>
<td>16</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus ATCC 6538/P</em></td>
<td>1</td>
<td>16</td>
<td>2</td>
<td>8</td>
<td>4</td>
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</tr>
</tbody>
</table>

128<, no activity

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antimicrobial action of CNPs-CTAB was concluded to be due to the increased cell-antimicrobial interaction, which resulted from the opposing electrical charges between CNPs-CTAB and L. monocytogenes cells, as well as increased antimicrobial penetration endowed by the small size. One study about phytochemical composition of turmeric, Zhang and his friends reported that [23] 5% and 10%, are β-sesquiphellandrene, aromadendreneoxide and germacrone for C. longa main components.

Conclusion, all the extracts of rhizomes of turmeric showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these extracts were more effective than conventional antibiotics to combat the pathogenic bacteria and fungus tested. Among the tested extracts the EA extracts were the most active against the microorganisms tested compared to the antibiotic standard. All the extracts showed no anticanidal activity. So, pharmacological test is essential to isolate and characterize their effective compounds. Moreover, these plants extract should be researched in vivo to better understand their safety, activity and properties.

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We require that our authors reveal any possible conflict of interest in their submitted manuscripts.

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The availability of safe water is as important as the water crisis, due to various types of pollutants in water. Therefore, the aim of this research is to determine the concentrations and to characterize the spatial distribution of Cr, Cu, Fe, Mn, Ni, Pb, and Zn heavy metals in surface waters of the Inegol Plain in both winter and summer periods. We use ordinary kriging interpolation technique to estimate a variable at an unmeasured location using observed values at nearby locations, and indicator kriging to map the probability of heavy metals to exceed the threshold fixed by the Turkey Water Pollution Control Regulation (SKKY). The concentrations of heavy metals in surface water show substantial seasonal differences in summer and winter in the Inegol Plain. Cr, Fe, Pb and Zn concentrations were recorded within the permissible limits throughout the plain. However, according to the background value of Cu, Mn and Ni, results show that the water in the area is under high pollution risk from these pollutants, particularly in areas distributed around the industrial areas. It is evident from the study that heavy metal concentrations were higher in water samples in the vicinity of hot water industries and of the Inegol Organized Industrial Zone.

KEYWORDS: Heavy Metals, GIS, Inegol Plain, Surface Water Pollution, Spatial Correlation, Spatial Variation.

INTRODUCTION

Environmental problems are considered to be the most important hazard nowadays because they threaten the welfare of all living organisms and the natural function of ecosystems. Air, water and soil of the ecological system, which are crucial for the continuity of life, are faced with mounting ecological problems as a result of the increasing industrialism and rapid urbanization. As water is necessary for all forms of living beings, and constitutes one of the basic components of the living environment, its pollution is generally afforded more importance than that of soil and air [1]. Water bodies are also a habitat for the living organisms it contains. For this reason, the presence of water in a living environment, the habitats that it provides, and its quality are extremely important [2].

Water pollution is identified as exceeding of the normal values of material concentrations in water resources as a result of anthropogenic and natural activities, and the degeneration of the natural composition of water resources or the identification of some materials in the water environment that have not before existed in the water resource [3]. Physical, chemical, and biological properties of water resources that are polluted as a result of various activities can change too.

Surface water is the most vulnerable water body as it is affected by the surface flow of agricultural lands and domestic and industrial waste in wide drainage basins [4]. Heavy metals such as Cr, Cu, Ni and Zn, which are discharged into the water by industrial establishments, fertilizers and chemicals used in the agriculture, wastewater from the paper industry, and derivations of Pb, which are added to benzene, comprise the contamination sources of surface waters [5]. Along with anthropogenic effects (urban, industrial, and agricultural activities, and increasing consumption of the water resources), natural processes (changes in precipitation supply, erosion, and disintegration of crust material) also decrease the quality of surface waters [6-8].

Heavy metals are among the primary contaminants of surface waters [9, 10]. It is known that some heavy metals (Cu, Fe, Mn and Zn) are necessary for life; however, when the levels of these metals exceed certain limits in organisms they lead to toxic effects [9, 10]. Hence, heavy metals are of huge importance in the research of the quality of surface waters. The studies available in the literature show that heavy metal contamination in water increases and that waters are affected negatively by industrialization and settlement [4, 7, 11]. Furthermore, if the settlement
and industrial areas are close to the water resources then the additional problem occurs of the use of water resources being used for waste storage. Indeed, many industries use metals for various operations, and frequently make use of nearby water bodies to store their waste, which deteriorates the local water quality [12]. Moreover, areas surrounding highways and cities also constitute areas where heavy metal contamination may be high [13, 14].

Heavy metal pollution is important due to their toxicity for living organisms, human beings, and environment [15]. Furthermore, heavy metals affect the health of living beings in proportion to their concentration levels. Therefore, it is important to study heavy metals in aquatic environment. For example, the toxic effects of Pb are spread into the environment [16] from batteries, metallurgy plants, cables, iron and steel manufacturing and automobile exhausts [17]. This metal (Pb) is carcinogenic, teratogenic, and will negatively affect the mental development of children [18].

Inegol, in western Turkey, is under the influence of rapid urbanization and increasing industrialization. The determination of heavy metal pollution caused by rapid urbanization and increasing industrialization, and the effects that can occur later, is important in terms of environmental management and monitoring. Heavy metals of urban origin that lead to water pollution were examined in several studies [5, 19-23].

The heavy metal pollution due to the clear images of the rivers is not noticeable. However, mapping improves visibility. Therefore, in this study, we intend to demonstrate the spatial variation of dissolved heavy metal (Cr, Cu, Fe, Mn, Ni, Pb and Zn) concentrations in the surface waters of the Inegol Plain, in order to detect the potential contaminant sources, as spatial variability of water heavy metals is important for environmental supervision and ecosystem evaluation.

**DATA AND ANALYSIS**

**Site description.** The study area is the Inegol Plain in the Bursa province in the southeast of the Marmara region, western Turkey (Fig. 1). This area is located at approximately 40° 09’ northern latitude and in 29° 49’ eastern longitude. The research area shows basin characteristics in terms of morphology, hydrology, and geology [24-26]. The Inegol Plain lies at the base of this basin. The plain is approximately 142 km² ellipse-shaped area, large axis of which extends in a NW–SE direction, and average height above sea level is 300 meters [24]. The study area is within the domain of the Marmara climate [25]. Generally, average temperatures for January are 4–5°C and for July are 22–24°C. The mean annual temperature is 13.8°C and the average annual rainfall is 427 mm. The plain consists of older Quaternary alluvium. The Kocadere and its tributaries were influential in the formation of the study area. The waters of this river reaches Sakarya River by the Inegol-Yenisir merge throat [24, 25, 27]. Therefore, it is included in the Black Sea river basin in the distinction of Turkey river basins.

The largest settlement in the study area is Inegol. Other important settlements are the towns of Alanyurt, Yenicekoy, Kursunlu and Cerrah (Fig. 1). The population of Inegol has increased constantly over the recent years, from 76,908 in 1965 to 215,375 in 2009, and 242,232 in 2014. The most important economical activities in the area are agriculture, woodworking, furniture, wood products, textiles, tourism, food, and machinery industries [28]. Although Inegol has very fertile soil for agriculture functions, industry is the most important economical activity in the plain.

**Data and evaluation of surface water samples.** A sampling grid method was used to determine the spatial variation of contaminants at a site. For
characterization of surface water contamination, samples were obtained using a systematic grid-system. For this, the study area was divided into a grid system of 3.0 × 3.0 km. Samples were collected using a “Systematic Random” method. Surface water samples were taken from 17 different locations in February and July 2014, from sites most likely to be affected by industrial and anthropogenic activities, during two seasons (i.e., summer (July) and winter (February)).

The bottle (point) sampling method used to measure the levels of chemical contaminants [29]. From various points of the Inegol Plain, surface water samples were taken from approximately 25 cm depth via the direct method. To avoid contamination and pollution arising from the sampling bottle and to minimize the contamination, sample bottles were washed using pure water and rinsed three times with water samples to be taken at each sampling point in the area. At each sampling point, water samples were collected in 100 ml polyethylene containers, and the samples were filtered through 125 mm filter paper in the field. In order to minimize the effects of evaporation, each water sample was stored in a portable freezer when being carried.

All water samples taken to the Laboratory of the Department of Chemistry at Balikesir University were passed through cellulose filters of 0.45 mm thickness using a vacuum filtration system. In order to prevent cation absorption or precipitation, three drops of nitric acid (HNO3) were added into each sample bottle. All water samples were stored in refrigerators at the Laboratory of the Department of Chemistry at Balikesir University until analysis was carried out. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine the total contents of dissolved heavy metals (Cr, Cu, Fe, Mn, Ni, Pb, and Zn), at the Bulent Ecevit University Science and Technology Application and Research Center. The pollutants concentration data was interpolated over the whole study area using geostatistical tools on the developed GIS.

Statistic and geo-statistics. The data obtained from the experiments were subjected to statistical analysis to determine descriptive statistics. The Pearson correlation coefficient was used to determine the interrelationships between the heavy metals.

Kriging method, which is in “ArcGIS Geostatistical Analyst Model”, was used to analyze the spatial distribution of heavy metals in the study area with the help of analysis results of surface water samples. Using the kriging method we can predict the variable values of un-sampled locations using the values from a sampled location [30]. Ordinary kriging and indicator kriging are widely used methods in geostatistical analysis of contamination [31]. Ordinary kriging was used to create regional distribution maps and indicator kriging was used to characterize the hazard posed by heavy metal concentrations at specific threshold values in the study area. Indicator kriging was carried out by considering the attributes of the Cr>20ppb, Cu>20ppb, Fe>300ppb, Mn>100ppb, Ni>20ppb, Pb>10ppb, and Zn>200ppb as point measurements with values 0 and 1. Limit values set for the 1st quality waters (high quality water) in the Turkey Water Quality Control Regulation were used in the risk analysis for Cr, Cu, Ni, Pb, and Zn in the study [32].

RESULTS AND DISCUSSION

Statistical distribution of heavy metals in surface water on the Inegol Plain. The descriptive statistics of the heavy metal concentration data are listed in Table 1, including the mean, standard deviation, coefficient of variation, skewness, kurtosis, minimum, and maximum. The maximum values of the water Cr, Cu, Mn, and Ni concentrations (Table 1) in winter are much larger than those of the background concentrations (20, 20, 100, and 20ppb, respectively), and Cu, Mn, and Ni in summer (20, 100, and 20ppb, respectively). All heavy metals in winter and summer did not show a normal distribution considering the criteria proposed by George and Mallery [33] of skewness and kurtosis values within ± 2. According to this, Cr, Cu, Mn, and Ni concentrations in winter and Cr, Mn and Zn in summer are positively skewed in the plain, and also peak indexes (kurtosis) are very high due to the greater disturbance from human activities. The high skewness and large variance also indicate that there are some extreme values in the data sets. The descriptive statistics show that the spatial distributions of these heavy metal concentrations were not homogeneous in the plain. Thus, delineation of hazardous areas was done cautiously, and an appropriate GIS method was applied in the study.

Pearson’s correlation coefficients of heavy metals studied in the river water in the plain are summarized in Tables 2 and 3. Correlation analysis showed very strong correlation between Fe and Mn (r = 0.786) and Fe and Ni (r = 0.667) at p < 0.01 level, forming one group of Fe-Mn-Ni in winter. Another group in winter, represented by Pb-Cu, also displayed a significant strong correlation (r = 0.630, p < 0.01). Pb also showed positive correlations with Zn (r = 0.612) indicating its relationship with the Cu-Pb-Zn group. On the other hand, correlation analysis showed very strong correlation between Cr and Mn (r = 0.730) and Cr and Fe (r = 0.675) at p < 0.01 level forming one group of Cr-Fe-Mn in summer. Cu showed strong correlation with Pb (r = 0.718) and Zn (r = 0.606) at p < 0.01 level forming one group of Cu-Pb-Zn in summer. Another group in summer was represented by Ni and Fe (r = 0.667, p < 0.01), and by Ni and Mn, which also displayed a significant strong correlation (r = 0.878, p < 0.01), indicating its relationship with the Ni-Fe-Mn group.
### TABLE 1

The statistics for the heavy metals (ppb) in winter and summer.

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Variance</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
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<tbody>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>1.22</td>
<td>24.06</td>
<td>4.72</td>
<td>5.79</td>
<td>33.57</td>
<td>2.72</td>
<td>8.02</td>
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<td>Cu</td>
<td>5.38</td>
<td>95.53</td>
<td>14.26</td>
<td>21.22</td>
<td>450.28</td>
<td>3.95</td>
<td>15.96</td>
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<td>Fe</td>
<td>24.51</td>
<td>138.65</td>
<td>56.79</td>
<td>27.99</td>
<td>783.50</td>
<td>1.59</td>
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<td>Mn-1</td>
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<td>Ni</td>
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<td>Pb</td>
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<td>20.16</td>
<td>18.80</td>
<td>353.59</td>
<td>2.09</td>
<td>4.42</td>
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</table>

### TABLE 2

Correlations between the heavy metals in winter.

<table>
<thead>
<tr>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
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<td>.064</td>
<td>-1.31</td>
<td>.082</td>
<td>.313</td>
<td>-0.66</td>
<td>-1.66</td>
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<tr>
<td>Sig. (2-tailed)</td>
<td>.807</td>
<td>.617</td>
<td>.755</td>
<td>.221</td>
<td>.800</td>
<td>.524</td>
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<td>Cu</td>
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<td>.234</td>
<td>.304</td>
<td>.630(**)</td>
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<tr>
<td>Sig. (2-tailed)</td>
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<td>.234</td>
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<td>.667(**)</td>
<td>.312</td>
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<td>Sig. (2-tailed)</td>
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<td>Sig. (2-tailed)</td>
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<td>.236</td>
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<td>.000</td>
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<td>.003</td>
<td>.000</td>
<td>.395</td>
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<tr>
<td>Sig. (2-tailed)</td>
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<td>.818</td>
<td>.820</td>
<td>.740</td>
<td>.800</td>
<td>.009</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).

### TABLE 3

Correlations between the heavy metals in Summer.

<table>
<thead>
<tr>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
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<td>Sig. (2-tailed)</td>
<td>.730(**)</td>
<td>.849(**)</td>
<td>.012</td>
<td>1</td>
<td>.730(**)</td>
<td>.606(**)</td>
</tr>
<tr>
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<td>1</td>
<td>.253</td>
<td>.011</td>
<td>-.329</td>
</tr>
<tr>
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<td>.849(**)</td>
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<tr>
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<td>.000</td>
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<td>.011</td>
<td>.849(**)</td>
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<td>-.125</td>
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<td>Sig. (2-tailed)</td>
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<td>.967</td>
<td>.000</td>
<td>.631</td>
<td>.927</td>
<td>.271</td>
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<tr>
<td>Ni</td>
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<tr>
<td>Sig. (2-tailed)</td>
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<td>.181</td>
<td>.927</td>
<td>.430</td>
<td>.003</td>
</tr>
<tr>
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<td>.606(**)</td>
<td>.696(**)</td>
<td>.283</td>
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<td>Sig. (2-tailed)</td>
<td>.501</td>
<td>.010</td>
<td>.902</td>
<td>.271</td>
<td>-.554</td>
<td>.003</td>
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</table>

** Correlation is significant at the 0.01 level (2-tailed).
Heavy metals showing very high correlation may indicate that they come from the same source [34]. According to Handa and Jadhav [35] positive correlation can be attributed to same origin, while the metals with negative correlations are an indication of distinct sources for the metals in the river. Cr, Cu, Fe, Mn, Ni, and Pb come mainly from industrial activities/effluents via untreated domestic sewage discharges, and traffic sources. The main source of Zn and Cu is from processing units and chemical weathering of the minerals.

**Spatio-temporal distribution of heavy metals in surface water on the Inegol Plain.** The spatial distribution patterns of seven heavy metals in the surface waters of the plain are shown in Figure 2 (a–
the riverbed, while during the summer the rise in temperature for zones with lower values. These maps illustrate distinct concentration gradients in the study area. The heavy metal concentrations display greater seasonal differences at the sampling sites and the variation range is relatively greater. In general, Fe, Mn, Ni, and Pb concentration levels in summer are higher than in winter in the study area (Fig. 2), while concentration levels of Cr, Cu, and Zn are higher in winter than summer. This finding is in agreement with those reported in a previous study concerning rivers contamination [36]. According to Lekka et al., during the low-flow season, water quality appeared poorer than during the high-flow season. We know that river systems in Turkey are characterized by an extended summer period of dryness, as the climate of the study area is Mediterranean in nature, with a pronounced summer drought and a mild winter. Due to the turbulence created by increased flow in winter, some heavy metals are carried away from the riverbed, while during the summer the rise in temperature and evaporation cause the rise in heavy metal concentrations in water in the plain.

Based on Figure 2, the major areas with higher concentrations of Cr, Fe, Mn, Ni, Pb, and Zn were in the northwestern side of the plain. However, Figure 2b presents the spatial distribution and regional differences of Cu concentrations, which were different to that of other pollutants. When the distribution of Cu concentration levels in the winter season was evaluated (Fig. 2), a significant increase was observed in the surface waters, which were the tributaries of Koca Cay around Kursunlu in the southeast of the plain. Similar to Cu, Zn and Mn also showed relatively high concentrations in the southeastern plain. On this side of the plain, Cu, Zn, and Mn could originate from activities taking place in the river’s upper catchment and the activity of the surrounding thermal springs. However, Cr, Ni, Pb, and Zn concentrations demonstrate a decline from northwest to southeast.

However, in summer, high concentrations of Fe and Mn (Fig. 2c,d) are determined in both the southeastern and northwestern part of the plain. The highest concentrations of Fe and Mn were detected in and around the Yenicekoy (Koy Stream), Akhisar (Kara Stream), the north of Alanyurt (Koca Cay), the east of Kulaca, and the areas surrounding Kursunlu (Koca Cay). Whereas, high Cr concentrations were observed in and around the Kursunlu area located in the eastern part of the plain, and Kara stream located in the north of Alanyurt and Akhisar. During the summer period, the highest Cu and Zn concentrations were detected in the surface waters (Kara Stream) located in the west of the study area, starting from Inegol. The most significant increase overall was observed in surface waters around Ortakoy, Deydinler, and Kulaca in the center of plain for Ni (Fig. 2c). The Ni concentration in surface waters in these areas was higher than 20 ppb. High concentrations of Pb in summer were identified on the northwestern side of the plain.

Generally, the highest heavy metal concentrations both in winter and summer were identified in the surface water located in the west of the Inegol Plain. Surface waters in this area, which experiences extensive human activities, such as intensive agriculture, industry, and urban emissions, showed relatively higher concentration levels than other areas. Mustapha and Nabegu [37] also reported that intensive agriculture, urbanization, and industrialization were responsible for surface water deterioration by heavy metals. Furthermore, the western part of the study area is densely populated, and as a result, increased waste and more intensive industrial activities are concentrated here, along with the Organized Industrial Zone, which is located in this region. Besides, the majority of the heavy metals resulting from human activity are common in urban and industrial areas [38–40]. On the other hand, the relatively high levels of accumulation discovered in the southeastern plains are thought to be associated with hot and cold water supplies, as this area is located within the domain of cold water sources and related factories, and the Oylat Hot Spring. No study or investigation was conducted on the cold water sources and related factories (Fresa and Kink Mineral Water). Additionally, the effect of the Oylat Hot Spring was not evaluated as it is located outside the study area.

Estimation of the areas where water quality limit value exceeds national levels: The use of indicator geostatistics allows for the mapping of the probability for attribute values to exceed a certain threshold value. These probability maps are very useful for decision makers because of their easy interpretation. The probability maps generated by indicator kriging are displayed in Figure 3. The heavy metal concentrations identified in the Inegol Plain surface waters in the summer and winter seasons were compared to the threshold values set for 1st quality waters by SKKY. The concentration of Cr, Fe, Pb, and Zn in both winter and summer seasons was under the threshold values. We found anomalies that indicate a high probability for Cu, Mn, and Ni water contamination, which imply that these waters should not be used for human consumption. As the entire study was located on the plains areas where there are no significant differences in soil type, parent material and land use, the strong spatial heterogeneity in the distribution of these three heavy metals was most likely directly related to human activities. Most of area has low pollution risk and its probability exceeds 0.7 (Fig. 3a–f). These waters are mainly located in the central areas and corresponding to the water around agricultural areas. The high-risk area (>0.7) is much smaller. This indicates that there proportionally less surface water exceeds the pollution threshold.
Figure 3a and b show indicator kriging and probability maps based on the thresholds of Cu. Figure 3a indicates that the areas with a probability greater than 0.5 for exceeding the threshold of the 20 ppb of Cu are strongly related to the location of hot water springs and industrial plants. In short, we can say that Cu mostly originates from the river’s upper catchment activities. Most areas southeast of the plain showed a probability greater than 0.7 for exceeding the thresholds during the winter seasons (Fig. 3a). Also, this shows that the high Cu could be related to industrial and mining activities in the sampling areas in the southeast of the plain. Thus, the industrial plants in the southeast, may be considered for further environmental monitoring for Cu. Cu is required in small amounts as an essential element but is toxic in certain critical doses. According to Acharya et al. [41], contamination of drinking water with high levels of Cu may lead to chronic anemia. Most common Cu toxicity includes acute haemolysis and abnormal kidney function.

Figures 3c and d show indicator kriging probability maps based on the thresholds of Mn. Figure 3c shows that the areas with a probability above 0.5 for exceeding the threshold of the 100 ppb of Mn are strongly related to the location of hot water springs and industrial plants in summer. Like Cu in winter, Mn in summer can originate from the river’s upper catchment activities. The probability map for the threshold in summer shows that the area with probability above 0.7 is located in the southeast, the same as Cu. In the winter, there is a probability above 0.5 for exceeding the threshold of Mn in the industrial area in the northeast. These results reveal that the northwest should be intensively monitored to remedy high water Mn concentration. Furthermore, industrial plants in the northwest and southeast of the study area should be prioritized for further Mn monitoring and remediation. Normally, Mn occurs naturally in many surface water and groundwater sources, and in soils that may erode into these waters. Human activities are also responsible for much of the Mn contamination in water. Although Mn is an essential element for many living organisms, including humans, it could have negative health effects in high values [42].

Figure 3e and f show indicator kriging probability maps based on the thresholds the 20 ppb of Ni. Most areas with high probability for exceeding 20 ppb of Ni are located in the northwest in winter, and are highly correlated with the location of industrial plants in the study area. However, high probability of Ni exceeding threshold values in the central plains during summer are highly correlated with the Ni-rich
fertilizers. Thus, the areas with horticultural farming activities in the center of the plain and industrial plants in the northeast may be considered as first and second priorities for further environmental monitoring of Ni. According to the Environmental Protection Agency [43], Ni is a potential carcinogen for lungs and may cause skin allergies and lung fibrosis. Moreover, a number of studies on the carcinogenicity of nickel compounds in experimental animals are available [44].

The concentration of Cu, Mn and Ni indicates the probability of increasing risk at certain locations. In this case, if heavy metal hazards to human health and the environment are of greater concern than the cost of remediation, the spatial distribution of the probability of heavy metals should be used to delineate hazardous areas based on a tolerable probability level of incorrect delineation.

CONCLUSION

Over the past three decades the Inegol Plain has experienced pronounced urban sprawl. In the plain the population density and the percentage of developed land use has decreased. In contrast, the rural areas experienced significant population growth and industrial development. Industrial development had important impact on water quality in the plain, which was established by analyzing the spatial and temporal disturbance of water quality indicators through GIS and statistical analyses. For that reason, spatial and temporal changes of heavy metals in the surface waters in the Inegol Plain were investigated here to identify industrial development has effected water quality.

It is evident from the study that heavy metal concentrations are higher in water samples in the vicinity of hot water industries and of the Inegol Organized Industrial Zone. The metal concentrations were found to decrease with increasing distance from the industrial areas. This clearly shows the influence of industrial activities on the Inegol Plain. Also, the study shows that the Inegol plain, in some parts, is polluted with heavy metals, which most likely originate from the rivers’ upper catchment activities, and surrounding horticultural farming activities.

The concentrations of heavy metals in surface water show substantial seasonal differences in summer and winter in the Inegol Plain. Recorded Cr, Fe, Pb and Zn concentrations were within the permissible limit throughout the plain. However, according to the background values of Cu, Mn, and Ni, the results show that the water in the area suffers high pollution risks from all of these pollutants, which are mainly distributed around the industrial areas.

The difference in metal accumulation is not correlated with tolerance to the heavy metal. Like we mentioned before, heavy metals pose a number of hazards to human health. For this reason, their concentration in the landscape and their effects on health must be regularly monitored. This study not only demonstrates the application of geostatistical methods in assessing the characteristics of the river water but also provides preliminary assessment of the river water quality that will serve as a database for future investigations and monitoring of river water quality in the study area. Also, the findings of the present study can be used as a baseline to assess the current status of habitats and the relative modification level due to anthropogenic impacts.

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THE GROWTH AND MORTALITY RATES OF SILVER CRUCIAN CARP (CARASSIUS GIBELIO BLOCH, 1982) IN SEYHAN DAM LAKE (SOUTHEASTERN MEDITERRANEAN REGION: ADANA, TURKEY)

Meltem Manasirli, Cansev Azgin*, Caner Enver Ozyurt, Munir Ziya Lugal Goksu
Cukurova University Fisheries Faculty 01330 Balcali, Adana, Turkey

ABSTRACT

In this study, growth parameters of the non-native Silver Crucian Carp (Carassius gibelio Bloch 1782) in the Seyhan Dam Lake were investigated between June 2013 and May 2014. The length ranged from 14.5 - 32.7 cm, weight varied between of 52 – 607.46 g while age composition changed from 0+ - V. the group. The length-weight relationship was $W=0.0107xL^{3.0095}$ ($R^2=0.9391$) for females. It was determined that feature positive allometric growth for growth females. von Bertalanffy growth constants in length and weight were as $L_x = 36.60$ cm, $W_x = 835.475$ g, $K = 0.320$ year$^{-1}$ and $t_0 = -0.2986$ year. Natural mortality rate $M = 0.74$ year$^{-1}$, total mortality rate $Z = 0.77$ year$^{-1}$, fishing mortality rate $F = 0.03$ year$^{-1}$ and exploitation rate $E = 0.04$ year$^{-1}$ was estimated. Silvery Crucian Carp in Seyhan Dam Lake have proliferated excessively and has already invaded our respective reservoir completely.

KEYWORDS: Seyhan Dam Lake, Silver Crucian Carp (Carassius gibelio), Growth parameters, Mortality Rates, Exploitation rate.

INTRODUCTION

The silver crucian carp (Carassius gibelio) is a native species of European continent, it is can be found in Western Asia, Siberia and all over Europe [1]. This specie was bred in Asia and was carried to Europe in the 17th century [2] it entered into Turkey via Greece or Bulgaria through the Meric River or it was brought in by the hand of man [3]. The genus of Carassius which belongs to Cyprinidae has a wide range tolerance, it adapts to a new environment in a short period of time and they dominated the environment by breeding extremely fast [4]. Today, all our internal water systems have been invaded by silver crucian carp [5]. The most significant reason for this specie becoming the dominant one in the environment is that the reproduction occurs via gynogenesis which is a special form of parthenogenesis reproduction [6], meaning it reaches sexual matura- tion faster when compared to other species with which it shares its ecosystem and it can have long spawning period depending on the water temperature [7]. Carp also adversely affects the food production in ecological environment due to its diet regimen (omnivore) [8, 9, 10, 11, 12, 13]. Seyhan Reservoir is a significant internal water reservoirs in South eastern Mediterranean Region; it was built on the Seyhan River and put into operation in 1956. With the aim of developing a professional fishery and to create a living for the community, first 500000 units of zander (Sander lucioperca) fry were brought from Directorate General for State Hydraulic Works Kovada Production Facilities in 1976. The fry were protected by the dam which was constructed with the aim of flood control, irrigation and energy generation. Also, the carp (Cyprinus carpio) and herbivorous grass carp (Ctenopharyngodon idella) were added by DSI in different times as of 1971. During the time the carp was being added to the various reservoirs of Turkey, it was observed that Carassius species may also enter into these aquatic ecosystems [14, 15]. These observations have shown that a large number of species have entered into the ecosystems of lakes over time beyond the fish that was added as planned. It has been observed that some of these species have reached a significant stock densities while some becomes lost within the ecosystem. However, one fish that has seen a huge increase in its population is Carassius gibelio which is an invasive and ecologically harmful fish species. It has even had a significant increase even though it has been placed on top of commercial fishing list. Beyond this there have been no studied conducted on the biology and population dynamic of C. gibelio in Seyhan Reservoir; only its distribution by age and the correlation that has to its length-weight was investigated by [16]; some reproduction properties in Karacaoren-I reservoir [17]; some biological characters in Egirdir Lake [18]; it distribution in Thrace [3]; its distribution in Turkish Freshwater System [5] and the growth characteristics of the population in Iznik Lake were investigated by [19].
MATERIALS AND METHODS

Study area. The Seyhan Reservoir is is 67 m above the sea level at the northern part of Adana and the width is approximately 4 km and length is 23 km. Its base is composed of siltstone, conglomerate, aluvion and ancient clays. The lakes deepest area reaches 45 m in the spring months and extends in an area of 9200 ha maximum.

Fish collections. The samples were collected from different regions and fishermen shown in Figure 1 between the dates of June 2013 and May 2014 on monthly basis by using the extension nets which had various net mesh-openings (20, 22, 24, 26, 28, 30, 32, 34, 36, 38mm) [20].

Methods. The fish that was caught was brought to Cukurova University Faculty of Aquaculture fresh by using ice; the sexual separation, total length, fork length, standard length (in mm), total weight (gr; sensitivity in 100) measurements and age determinations of each individual fish were made within the direction of principles stated by [21].

The Electronic Length-Frequency Analyses (ELEFAN) was implemented. The package program named FAO-ICLARM Stok Assessment Tools (FISAT II) including ELEFAN program was used in the implementation of Length-Frequency Analysis and other equations stated. Additionally the data obtained by the age determinations was also used in the mathematical evaluation of growth characteristics and determination of mortalities of specie investigated.

The equations developed by von Bertalanffy will be used for the determination of absolute growth by length and weight [22]:

\[ L_t = L_\infty \times (1 - e^{-K(t-t_0)}) \] and

\[ W_t = W_\infty \times (1 - e^{-K(t-t_0)}) \], respectively.

At this equation; \( L_t \) refers to total length at the time of (t) (cm), \( t \) refers to any time (day), \( L_\infty \) refers to asymptotic length, in other words, asymptote length (in cm), \( K \) refers to Brody Growth Coefficient (year\(^{-1}\)) and \( t_0 \) is a theoretical value and refers to the age before the hatching (year). \( W_\infty \) refers to asymptotic weight (g), \( W_t \) refers to the total weight (g) at the time of (t), \( t \) value being a theoretical value which cannot be calculated with FISAT package program, was calculated by using \( \log(-t_0) = (-0.3922) - 0.2752 \log L_\infty - 1.038 \log K \) equation suggested by [23]. The length-weight correlation was calculated with the equation suggested by [24].

The exponential coefficient of total deaths (Z) was calculated with the Length-converted Catch Curve suggested by [25] and the mortality due to natural reasons (M) was calculated with the [23] equation stated below; the growth constants were calculated by using von Bertalanffy estimate created by the Length-Frequency Analysis.

\[ \log M = (-0.0066) - 0.279 \log L_\infty + 0.6543 \log K + 0.4634 \log T \]

The exponential coefficient of deaths due to fishery was calculated by using (Z) and (M) relevant to the mortalities with the equation of \( Z=F+M \) [26]; the equation of \( E=F/Z \) given by [22] was used for calculating the ratio of benefitting from the stock (E).

FIGURE 1
Seyhan Dam Lake and stations, Turkey [45]
The temperature among the abiotic environmental factors was calculated by using YSI multiple measuring apparatus.

RESULTS

Temperature. As shown in Figure 2, the temperature values were measured monthly in Seyhan Reservoir between the date of June 2013-May 2014, the lowest temperature was 10.2 °C measured in February 2014 and the highest temperature was 28.5 °C measured in August 2013. Within the timeframe of the study, the average temperature was calculated to be 18.65±6.494 °C.

Growth. Length-Frequency and Weight-Frequency. The total length of 530 units of
*C. gibelio* female fish obtained from Seyhan Reservoir varied between 14.5 cm to 32.7 cm (Figure 3); total weight varied between 52 g to 607.46 g (Figure 4). It was determined that the length differences were within the average of 20-23 cm and that the 21 cm total length had the highest mode; average weight value and standard deviations were 22.43±2.82 cm (Figure 3). Total weight values average was 140±200 g; they were mostly at the group of 170-200 g and the average weight and standard deviations were determined to be 208.32±86.09 g (Figure 4).

**Age-Length Distribution.** In accordance with the age determination made, the ages of *C. gibelio* female individuals in Seyhan Reservoir ranged at the age of 0+–V. (Table 1). The percentage distribution of age groups were 0.56%, 3.2%, 42.3%, 41.30%, 7.2% and 5.0%, respectively. It is can be seen from the distribution by age that the total length groups mostly composed of 2nd and 3rd age groups at the range of 20-26 cm (Table 1).

**Growth Parameters.** The total length-frequency distribution and von Bertalanffy growth curve by length are given in Figure 5 and the growth constants are given in Table 2. The average total length values calculated as a result of solving them via the von Bertalanffy equation for age groups and the growth curves by length and weight are given in Figure 6 and Figure 7.

| TABLE 1 |
| --- | --- |
| **Age-Length Distribution of *C. gibelio* in the Seyhan Dam Lake** |
| Length Groups (cm) | Age Groups (year) |
|  | 0+ | I | II | III | IV | V |
| 14-15.9 | 3 | 14 | |
| 16-17.9 | 7 | 5 | |
| 18-19.9 | 130 | 93 | |
| 20-21.9 | 75 | 88 | 3 | |
| 22-23.9 | 7 | 29 | 13 | |
| 24-25.9 | 2 | 5 | 9 | 1 | |
| 26-27.9 | 28-29.9 | 12 | 17 | |
| 30-31.9 | 1 | 7 | |
| 32-33.9 | | | | | |
| Length Interval | 14.5-14.7 | 14.6-19.9 | 17.7-26.5 | 18.7-27.6 | 22.2-30.0 | 27.7-32.7 |
| Mean Length±St | 14.6±0.1 | 15.5±0.54 | 21.7±1.33 | 22.3±1.47 | 26.7±2.04 | 29.6±1.37 |
| N | 3 | 17 | 224 | 221 | 38 | 27 |

**FIGURE 5**

von Bertalanffy Growth Curve

| TABLE 2 |
| --- | --- |
| **von Bertalanffy Growth Constants** |
| Sex | TLm (cm) | K (year⁻¹) | TWm (g) | t0 (year) | Rn |
| Female | 36.60 | 0.320 | 835.475 | -0.2986 | 0.363 |
Length – Weight Relationships. The Length – Weight relationships of *C. gibelio* female individual sampled from Seyhan Reservoir was calculated as \( W = 0.017 \times L^{3.0095} \) (\( R^2 = 0.9391 \)) (Figure 8). The b value indicates the body form and the growth characteristic of the fish was determined to be 3.0095 and accordingly, it was understood that these individuals showed positive allometric growth characteristic and their body shapes had slightly a chunky structure.

Mortality and Exploitation Rates. The exponential coefficients of total mortality (Z), natural mortality (M) and mortality due to fishery and the level of exploitation from the stock (E) calculated for the silver crucian carp in Seyhan Reservoir are given in Table 3 and Figure 9.
TABLE 3
Mortality Rates (Total (Z), Natural (M), Fisheries (F)) and Exploitation Rate (E)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Z</th>
<th>M</th>
<th>F</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0.77(0.51-1.72)</td>
<td>0.74</td>
<td>0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

FIGURE 9
Length-Converted Catch Curve

As seen in Table 3, total mortality (Z) for the female individuals and natural mortality were determined to be very similar. The mortality due to fishery was determined to be very low; and almost non-existent. The level of benefitting from the stock was calculated to be E = 0.04 year⁻¹. The reason for this was that the fishery association had terminated its fishing activities in Seyhan Reservoir during the date of sampling and the relevant species had completely invaded the relevant area at that time.

DISCUSSION

From other research conducted in other lakes and reservoirs, it was determined that the distribution of C. gibelio by age varied between I-V in Seyhan Reservoir [16], 0-V+ [27] in Gelingüllü Dam, I-VII in Seyitler Reservoir [28], I-VII in Ulubat Lake [29], I-VI in Aksu River a hard water zone [30], I-VI in Buldan Reservoir [31], I-VI in Marmara Region [32], 0+IX [33], 0+V [34], 0+IV [35] in Eğirdir Lake, I-VI [17], 0-V [36] in Beyşehir Lake, I-VI in Kebea Reservoir [37], I-V in Karacaören I Reservoir [38], II-V in Yedigöller [39], I-V in Hamam Lake [40]. In this paper, 0+-V age group was determined; and this is similar to [41] [34] and [36].

In the previous studies, maximum length and weight values of C. gibelio population in Turkish lakes and reservoirs is given in Table 4.

It can be seen that this study’s value are lower than the values stated by [36] and [30] but very close to the value given by [27]. Along with that the exponential weight value (W₀ = 835.475 g) determined by growth constants is only higher than the value given by [31], this may be because the researchers in other evaluation, evaluated both the male and female fish. The reason why the exponential weight value was lower than the values given by other researchers was that the relevant
species had only been in the relevant area for the last 4 years as well as that the nutritional and climatic conditions in Seyhan Reservoir are different from other lakes and reservoirs.

In this paper, the “b” value in the Length-Weight Correlation was calculated to be 3.0095. As seen in Table 5 where the length-weight correlation of silver crucian carp calculated in different areas, had the b value of those fish vary between 2.597 – 3.186.

As the relevant species is an invasive species an action plan shall be prepared and put into practice immediately in order to improve Seyhan Reservoir ecologically in terms of fishery.

ACKNOWLEDGEMENTS

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A STUDY ON WATER QUALITY OF KARASU STREAM (VAN, TURKEY) AND ASSESSMENT OF USAGE IN DRINKING, IRRIGATION AND FISHERIES

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ABSTRACT

In this study, some water quality parameters were analyzed to investigate water quality changes in Karasu Stream which inflows to Lake Van. The in situ measurements and laboratory analyses were made on water samples taken from 4 sampling points on the Karasu Stream Bed monthly, between November 2009 and October 2010. In the study, water temperature, dissolved oxygen, dissolved oxygen saturation; conductivity, pH, calcium, magnesium, total hardness, total alkalinity, carbonate and bicarbonate, chloride, salinity, nitrate, nitrate, ammonium, ammonia, phosphorus and sulfate were analyzed.

They were determined that average water temperature was 13.4°C, dissolved oxygen between 10.03 mg/L, saturation 119.4%, pH 8.23, conductivity 601.4 µS/cm, salinity 0.08‰, chloride 47.66 mg/L, calcium 31.0 mg/L, magnesium 111.2 mg/L, total hardness 536 mg/L, bicarbonate 452.2 mg/L, total alkalinity 370.3 mg/L, nitrate 4.1 mg/L, nitrite 0.017 mg/L, ammonium 0.41 mg/L, ammonia 0.40 mg/L, total phosphorus 0.06 mg/L and sulfate 19.5 mg/L. At the all sampling points and all months, carbonate was not encountered. Karasu Stream is in first and second water quality classes all of water quality criteria.

KEYWORDS:
Karasu stream, Lake Van basin, water quality, drinking, irrigation, fisheries

INTRODUCTION

Water, in the simplest explanation, is described that consist of one oxygen and two hydrogen atoms, colorless, odorless, tasteless and transparent liquid. However, it is an indispensable substance to be utilized for the survival of all biological organisms. In addition to, water is an essential of human life and activities associated with drinking, industry, electricity production, agriculture, fisheries, recreation, transport etc. and it is considered one of the most dedicate parts of environment [1,2,3].

For centuries, people have chosen to settle in areas which have been rich in water and eked out their living nearby water resources where they can use from plenty of water, or in which aquatic resources can easily and abundantly be obtained. As a matter fact, we have seen this in Urartu water civilization with dams and water channels in Van and surrounding settlements [3, 4, 5]. On the other hand, water is also one of the basic elements in formation of the living, at that time it is a vital environment [1].

However, the perturbations in the hydrodynamics in the earth’s ecosystems and the population increases have imposed strains and stresses on water resources over time in these areas and necessitated water bodies to be constructed in order to have an adequate utilization and management of water resources. Furthermore, the amount of water in the world is limited, and it is a known fact that water can never be replaced with any other element in nature [3, 4].

Today, effective use of water resources is one of the most important issues. Because, the usage of water affects the water resources directly. The effective use requires better management of water resources, with a more explicit expression, together water quality and quantity [3]. Various factors are believed to contribute to a degradation and slump in water resources. For aquatic ecosystems, pollution is one of the most important problems nowadays [4]; therefore, aquatic resources must be protected very well.

Streams are source of life for people today that thirst is a major problem, and a cheap, clean and continuous resource for meeting our energy needs, the fir's condition and insurance of plant production potential. Also they have been the favorite of mankind the biological richness and valuable fish stocks besides an environment for transportation, tourisms, and recreation activities [3].

In the Lake Van basin, water quality studies were made by [6, 7, 8, 9, 10] and limnological characteristics and fish population properties by [11, 12, 13, 14, 15, 16, 17], and the effect of sand pits on Karasu stream to tarek (Alburnus tarichi) by [18].
This study was carried out the purpose of to determine the water quality criteria of the Karasu stream which is under serious threat due to very close to van city center, to estimate how effective the sand pits on the stream on the quality of water, and to determine the usability of the water in drinking, irrigation and fisheries.

MATERIALS AND METHODS

Karasu stream is located in Van province, Lake Van Basin, eastern Turkey and wit the border of Iran. The stream is formed by the merging of the small tributaries from the mountains terrain near the Iranian border. Flowing from east to west, the stream flows into the Lake Van from the region called Zeve Martyrdom east of the Lake (38° 34' 51.54" N and 43 ° 13' 21.42" E). Sarmhemet Dam Lake is built in 1991 on the stream for irrigation [11, 14], now also has been produced electricity.

On the stream, four sampling points, which were considered to be likely changed in the quality criteria and relatively reaching is easy, have been selected to make in situ measurements and to collect samples for laboratory analysis (Figure 1).

This study was carried out between November 2009 and October 2010. The in situ measurements and taking of samples were done 15-20th days of each month at 10-15 hours, from 15-20 cm depth and transported with thermos containers to laboratory and they stored at 2-4 ºC. measurements of water temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), dissolved oxygen saturation % (DOS), and salinity % were made in the field with multimeter device (ThermoElectron Orion 5 Star). In the laboratory, analysis was performed in replicates of two or three. Calcium, magnesium and total hardness (TH) were analyzed with EDTA, total chloride (TC) with Argentinometry (Mohr-Knudsen) and carbonate-bicarbonate, total alkalinity (TA) with HCl titration methods [19].

Ammonia with Nessler method (HACH method 8038), nitrate with cadmium reduction method (HACH method 8039), nitrite with diazotization method (HACH method 8507), phosphorus with PhosVer 3-ascorbic acid method (HACH method 8048), sulfate with SulfaVer 4 method (HACH method 8051) were analyzed with HACH -LANGE DR 5000 spectrophotometer [20]. Evaluation of the results was made with Turkish Standards (TS 266) [21], Water Intended for Human Consumption Regulation (WHCR) [22], Water Pollution Control Regulation (WPCR) [23], Surface Water Quality Obtained or Planned to Drinking Water Regulation (SWQPR) [24], Surface Water Quality Management Regulation (SWQMR) [25], World Health Organization (WHO), the European Union (EU) drinking (76/464/EEC) and fisheries (78/659/EEC) water directives [26], irrigation water quality characteristics [27] and other relevant legal and technical knowledge. Evaluation of the resulting data was made according to general statistical basis.

RESULTS AND DISCUSSIONS

When the stream bed is observed, soil erosion is seen intensely. In Dorutay, Kasmoglu and Zeve sampling points, coastal erosion is clearly seen, and in Ablanges bridge region no such erosion has been encountered due to river improvement studies conducted by DSI (General Directorate of State Hydraulic Works). However, due to these studies, it is observed that the original bottom and coastal structure of Karasu stream has been changed quite a bit. Between Kasmoglu and Ablanges regions, there are about 20 sand pits and from the stream bed, sand taking with working machines was done throughout the year except winter since years (Figure 2). After from this point, intensively turbidity is observed and as a result of this situation, there are various problems in maintaining vital functions for aquatic organisms. Especially during the reproduction period of tarek (Alburnus tarichi), the migration has been decreased in recent years observed in
1990’s, the living condition of Capoeta capoeta, Alburnus timarensis, and Cyprinus carpio in the stream are deteriorating. Because of the sand taking working, the sediments have been congested the gills of fish and occasionally intense fish deaths can be seen. It has also caused to death of fish eggs by covering eggs [18].

According to sampling months, the in situ measurements mean data are given in Table 1 and the laboratory analysis mean data in Table 2.

Water temperature is important in feeding, reproduction, growth and migration of fish and other aquatic organisms. Also, temperature affects parameters by increasing or decreasing such as solid solubility, EC, DO and pH [1].

Water temperature also directly affects the metabolism and physiology of aquatic organisms. Moreover, it has an impact on the speed of the chemical reactions, the solubility of the gases, taste, smell and other properties of water [26]. In the drinking waters, the temperature has a direct association with taste and the best taste is about at 10-12 ºC [28]. The temperature of irrigation water is the most important physical characteristic. It is directly influenced on the plant growth. The temperature of irrigation water should be around 15 ºC. A low temperature of 7-8 ºC can have negative effect on plant growth [27, 29]. In this study, we measured it between 0.8-24.5 ºC and mean 13.4 ºC (Table 1). The average temperature value of the samples may be included in the first class according to Turkish standards [21, 22, 23, 24, 25] and WHO and EU directives [26]. The pH value obtained from this study is suitable for drinking, aquaculture and irrigation. It was reported between 8.06-8.21 in Murat River [36], 8.08-8.21 in Murat River [36].

The average pH value was determined as 8.23 in Karasu stream (Table 1). The pH of waters is important for reproduction, growth and nutrition of aquatic organisms. The higher the pH increases, the toxic the effect of ammonia is [1]. Chemical reactions are controlled with pH. Most of biological activities are limited within a narrow range. pH value between 6.5 and 9.0 for aquatic organisms are optimum [26]. The pH of first class drinking waters are between 6.5 and 9.5 in Turkish standards [21, 22, 23, 24, 25], and 6.5-8.5 in EU directives [26]. The pH obtained from this study is suitable for drinking, aquaculture and irrigation. It was reported between 7.50-8.20 in Bendimahi stream [8], 7.59-9.39 in Arpit stream [9], 7.72-8.87 in Deliçay stream [10], 7.76-8.45 in Kelkit stream [35], and 8.08-8.21 in Murat River [36].

<table>
<thead>
<tr>
<th>Sampling Months</th>
<th>Temperature (ºC)</th>
<th>DO (mg/L)</th>
<th>DOS (%)</th>
<th>pH</th>
<th>EC (μS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2009</td>
<td>10.5</td>
<td>8.16</td>
<td>85.6</td>
<td>8.47</td>
<td>645.0</td>
</tr>
<tr>
<td>December 2009</td>
<td>6.7</td>
<td>12.69</td>
<td>119.7</td>
<td>8.41</td>
<td>630.6</td>
</tr>
<tr>
<td>January 2010</td>
<td>2.1</td>
<td>11.06</td>
<td>98.6</td>
<td>8.57</td>
<td>726.7</td>
</tr>
<tr>
<td>February 2010</td>
<td>6.2</td>
<td>13.49</td>
<td>134.8</td>
<td>8.11</td>
<td>637.0</td>
</tr>
<tr>
<td>March 2010</td>
<td>9.2</td>
<td>13.85</td>
<td>151.9</td>
<td>8.47</td>
<td>468.5</td>
</tr>
<tr>
<td>April 2010</td>
<td>13.1</td>
<td>-</td>
<td>-</td>
<td>8.19</td>
<td>531.2</td>
</tr>
<tr>
<td>May 2010</td>
<td>15.3</td>
<td>11.64</td>
<td>144.8</td>
<td>8.44</td>
<td>501.7</td>
</tr>
<tr>
<td>June 2010</td>
<td>15.9</td>
<td>6.76</td>
<td>85.5</td>
<td>7.52</td>
<td>615.5</td>
</tr>
<tr>
<td>July 2010</td>
<td>22.7</td>
<td>7.13</td>
<td>105.1</td>
<td>8.11</td>
<td>607.5</td>
</tr>
<tr>
<td>August 2010</td>
<td>21.5</td>
<td>9.43</td>
<td>139.9</td>
<td>7.95</td>
<td>616.5</td>
</tr>
<tr>
<td>September 2010</td>
<td>19.5</td>
<td>9.10</td>
<td>135.3</td>
<td>8.28</td>
<td>575.0</td>
</tr>
<tr>
<td>October 2010</td>
<td>16.1</td>
<td>7.64</td>
<td>112.3</td>
<td>8.40</td>
<td>649.0</td>
</tr>
<tr>
<td>Mean</td>
<td>13.4</td>
<td>10.03</td>
<td>119.4</td>
<td>8.23</td>
<td>601.4</td>
</tr>
</tbody>
</table>
The EC depends on the total amount of anions and cations dissolved in water and give an idea of the ionic richness of the water. Water with higher ion concentration has also higher EC values [37]. In a water resource, the EC is connected with chemical structure of stream bed and joins the speed and level of hydrocycle [1]. In drinking water, EC increase is an indicator of pollution or mixing of the sea water [28]. In this study, EC was measured as average 601.4 μS/cm (Table 1). The EC values were reported between 254.4-340.6 μS/cm [6], 480-590 μS/cm [35], 298.4-981.2 μS/cm [36] and as average 680.47 μS/cm [8], 350 μS/cm [9], 697.13 μS/cm [10], 5178 μS/cm [18], 779.6 μS/cm [30], 301.9 μS/cm [31] and 578.7 μS/cm [32]. According to Turkish standards [21, 22, 24] and EU drinking and fisheries directives and WHO [26], Karasu stream water is the first class, but other Turkish standards [23, 25] especially in irrigation it is second class. With regard to average EC value for irrigation is in C2 class. It can be used in soil with salt problem [27]. If the rainfall is irregular and the water used in agriculture is inadequate as in Van, C2 class water shall be used in irrigation [38].

Chlorine is not present in natural waters as gaseous, but it can be present in the form of chloride or salt. Chlorine will damage the gills of aquatic animals and lead to death. In a healthy drinking water it is one of the important indicators [1]. It may be an indicator of sewage mixture and taste threshold value is between 250-500 mg/L. 250 mg/L of high concentration of salts generates a bitter taste, and up to 1500 mg/L is harmless to health [26]. Excessive chlorine intake in humans, kidneys can be affected and in the case of chloride deficiency, malnutrition and muscle weakness can be occurred [34]. It has very high toxic effect and its content are above a certain value of irrigation water causes leaf blight in plants. The 100-200 mg/L sodium chloride can damage some vegetable [27]. The average chloride value was determined as 47.66 mg/L in this study (Table 2). It has been reported as 11.68 mg/L [8], 61.9 mg/L [9], 9.42 mg/L [10], 23.3 mg/L [18], 84 mg/L [30], 8.99 mg/L [31] and 53.53 mg/L [32]. Our average value is the first and second class with respect to Turkish WHO and EU standards [21, 22, 23, 24, 25, 26].

Ca and Mg are the most important two elements in total hardness. TH for fisheries and drinking is very important criteria. For skeleton structure of aquatic and terrestrial organisms, they are very important. In terms of human health, Ca and Mg have important functions besides forming kidney stones. There are in strengthening the immune system, teeth and bone structure, and preventing of infract and blood coagulation in human and animals [1, 34]. In irrigation waters, Ca restricts the acquisition of certain nutrients and leads to chlorosis in the soil and plants in extreme cases. Small amount of Mg is sufficient for plants [27]. The value of Ca, Mg and TH were determined as 31.0 mg/L, 111.2 mg/L and 536 mg/L, respectively in our samples (Table 2). In fisheries, TH is absolutely necessary criteria in water, up to 400 mg/L TH value are accepted as suitable [1, 33]. However, it is an undesirable feature in the industry that water uses as heating or as cooling water [1]. Mg value is high according to TS 266 [21]. Our value is similar to some studies [8, 9], but higher than others [10, 30, 31, 32].

TA is important because of impact on buffering capacity of water. Carbonate and bicarbonate are the main components in the formation of TA [1]. In this study, carbonate could not determine, bicarbonate was found as 452.2 mg/L and TA as 370.3 mg/L (Table 2). For rainbow trout farming, this value is near the upper limit. The stream may be included in the high alkaline water class [1, 30, 33]. Very small amount of carbonate is sufficient for plants. Its high concentration has toxic effect and also negatively affects the soil physical properties. It reduces soil permeability. If the soil dries, it hardens and binds the dust layer. Large cracks are formed in soil [27]. TA was reported as 490.55 mg/L [8], 378.3 mg/L [9], 144.37 mg/L [10], 478.8 mg/L [18], 213 mg/L [31] and 283 mg/L [32].

### TABLE 2
According to the sampling months data from laboratory analysis in Karasu stream (mg/L)

<table>
<thead>
<tr>
<th>Month</th>
<th>Cl</th>
<th>Ca</th>
<th>Mg</th>
<th>TH</th>
<th>HCO₃⁻</th>
<th>TA</th>
<th>NO₃⁻</th>
<th>NO₂⁻</th>
<th>NH₄⁺</th>
<th>NH₃</th>
<th>H₂PO₄⁻</th>
<th>P</th>
<th>P₂O₅</th>
<th>SO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov.09</td>
<td>47.33</td>
<td>19.2</td>
<td>118.4</td>
<td>535</td>
<td>527.6</td>
<td>432.5</td>
<td>5.7</td>
<td>0.029</td>
<td>0.24</td>
<td>0.31</td>
<td>0.12</td>
<td>0.04</td>
<td>0.09</td>
<td>23.0</td>
</tr>
<tr>
<td>Dec.09</td>
<td>63.10</td>
<td>43.7</td>
<td>108.8</td>
<td>537</td>
<td>545.9</td>
<td>447.5</td>
<td>4.8</td>
<td>0.013</td>
<td>0.05</td>
<td>0.04</td>
<td>0.10</td>
<td>0.03</td>
<td>0.08</td>
<td>21.0</td>
</tr>
<tr>
<td>Jan.10</td>
<td>71.00</td>
<td>19.6</td>
<td>149.9</td>
<td>666</td>
<td>492.5</td>
<td>403.5</td>
<td>5.8</td>
<td>0.026</td>
<td>0.44</td>
<td>0.42</td>
<td>0.15</td>
<td>0.04</td>
<td>0.15</td>
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</tr>
<tr>
<td>Feb.10</td>
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<td>16.4</td>
<td>119.3</td>
<td>532</td>
<td>508.5</td>
<td>416.8</td>
<td>5.9</td>
<td>0.013</td>
<td>1.03</td>
<td>0.97</td>
<td>0.50</td>
<td>0.16</td>
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<td>46.4</td>
<td>78.5</td>
<td>439</td>
<td>352.2</td>
<td>288.7</td>
<td>5.8</td>
<td>0.000</td>
<td>2.24</td>
<td>2.12</td>
<td>1.18</td>
<td>0.38</td>
<td>0.81</td>
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</tr>
<tr>
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<td>16.4</td>
<td>112.3</td>
<td>503</td>
<td>376.6</td>
<td>308.7</td>
<td>3.6</td>
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<td>0.14</td>
<td>0.13</td>
<td>0.02</td>
<td>0.05</td>
<td>0.02</td>
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<tr>
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<td>489</td>
<td>386.5</td>
<td>316.8</td>
<td>2.4</td>
<td>0.006</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
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</tr>
<tr>
<td>June10</td>
<td>53.24</td>
<td>42.0</td>
<td>91.4</td>
<td>481</td>
<td>460.5</td>
<td>377.5</td>
<td>2.9</td>
<td>0.004</td>
<td>0.20</td>
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<tr>
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<td>47.33</td>
<td>16.4</td>
<td>104.5</td>
<td>471</td>
<td>418.6</td>
<td>343.1</td>
<td>3.1</td>
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<td>0.12</td>
<td>0.12</td>
<td>0.14</td>
<td>0.04</td>
<td>0.11</td>
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<tr>
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<td>95.3</td>
<td>538</td>
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<td>353.7</td>
<td>1.9</td>
<td>0.015</td>
<td>0.22</td>
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<td>0.03</td>
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<tr>
<td>Sept.10</td>
<td>27.36</td>
<td>49.9</td>
<td>110.8</td>
<td>588</td>
<td>442.2</td>
<td>362.5</td>
<td>2.8</td>
<td>0.029</td>
<td>0.12</td>
<td>0.14</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>18.0</td>
</tr>
<tr>
<td>Oct.10</td>
<td>33.27</td>
<td>25.6</td>
<td>132.7</td>
<td>610</td>
<td>484.9</td>
<td>397.5</td>
<td>4.2</td>
<td>0.038</td>
<td>0.12</td>
<td>0.11</td>
<td>0.05</td>
<td>0.015</td>
<td>0.04</td>
<td>24.5</td>
</tr>
</tbody>
</table>

**Mean** 47.66 31.0 111.2 536 452.2 370.3 4.1 0.017 0.41 0.40 0.19 0.06 0.16 19.5
Nitrate is produced by nitrification process that occurs after decomposition of nitrogenous substances by bacteria in natural waters. If there is excessively nitrate, algae and aquatic plants increase. As a result of this, DO decrease in the water [1]. It is known that if there is more than 50 mg/L of nitrate in drinking waters of the infants, it causes cyanosis disease causing weakness [34]. In Karasu, mean value was found as 4.1 mg/L (Table 2), in Turkish, WHO and EU drinking and fisheries standards, our samples is first class [21, 22, 23, 24, 25, 26, 33]. Nitrate is among the nutrients of plants and so it is absolutely necessary substance to find in irrigation waters [27].

Nitrate can absolutely be unwilling to be found in drinking and fisheries waters. It is toxic to aquatic organisms. In soft water, it has more toxicity than hard water. It arises in water which has low DO and more organic matter problems [1]. In this study, average nitrate value was determined as 0.017 mg/L (Table 2), this value is appropriate both fisheries and drinking according to Turkish standards, WHO and EU directives [21, 22, 23, 24, 26, 33]. Nitrate was reported as 0.018 mg/L [8], 0.0214 mg/L [9], 0.023 mg/L [10], 0.0239 mg/L [18], 0.012 mg/L [31] and 0.0517 mg/L [32]. Excessive nitrite in drinking causes the blue baby disease. It is both carcinogenic to itself and effective in formation carcinogenic nitrosamines [34]. Ammonia is dangerous because of organic based and toxic to all living organisms both aquatic and terrestrial. It leads to impairments in taste and causes bad smelling problems. If there is ammonia in water, it indicates that there is an infiltration of domestic waste water [1, 28]. In our samples, NH₃ was determined as 0.40 mg/L and NH₄ as 0.41 mg/L (Table 2). The values are second class according to some Turkish standards [23, 24, 25], and near the upper limit drinking water standard of Turkish, WHO and EU [21, 22, 26]. It is also high for fisheries [1, 26, 33].

Phosphorus has a role in energy transfer and due to that in nucleic acid, it is required for biological activity. It is one of the necessary nutrients for the growth and proliferation of macrophyte and aquatic algae. If there is excessive amount in water, it is a pollution indicator. In fertilizers, foods and detergents, there is excess. It is carried to water resources via runoff, irrigation drainage, and industrial waste waters [1]. It is found at high levels in volcanic origin areas. Lake Van basin has been surrounded by volcanoes that are in the west with Süphan, in the south-west with Bilican and Nemrut and in the north-east with Tendürek. Therefore, the majority of soil is volcanic origin. Sulfates are major pollutants due to taste, smell, toxicity and corrosions [28]. Average value in our samples was found as 19.5 mg/L (Table 2). According to Turkish, WHO and EU standards, our value is in the first class [21, 22, 23, 24, 25, 26]. It was reported as 8.6 mg/L [8], 18.8 mg/L [9], 6.72 mg/L [10], 21.3 mg/L [18], 57.6 mg/L [30], 5.94 mg/L [31], 28.7 mg/L [32].

CONCLUSION

Karasu stream can be considered to be appropriate for human consumption, fisheries and irrigation in terms of analyzed criteria that water temperature, DO, DOS%, NO₃, NO₂, TP and SO₄ are the first quality class and in terms of EC, Cl, Mg and TH are the second quality class water.

Disclosure. This study was produced from the data of Master Thesis conducted by Ibrahim ŞEKERCI in the supervisor of Dr. Fazıl ŞEN.

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THE PARTITION PATTERN OF GLYCOGEN AND EXTRACELLULAR POLYSACCHARIDES IN TWO FILAMENTOUS CYANOBACTERIA FROM DESERT SOIL CRUSTS

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ABSTRACT

Cyanobacteria often store excessive organic carbon in the form of glycogen and extracellular polysaccharides (EPS) under higher light intensities. However, their partition pattern and relation has long been overlooked. In this study, the partition and possible relationship between extracellular EPS and intracellular glycogen under different light intensities (4, 40, 80 μE m⁻² s⁻¹) were explored; with the exception of glycogen and EPS, the intracellular total sugar, water soluble sugar and sucrose of two species cyanobacteria, *Microcoleus vaginatus* FACHB 896 and *Nostoc* sp. FACHB 892 isolated from biological soil crusts, were also synchronously analyzed. The results showed that, the production of EPS (including released polysaccharides, RPS and capsular polysaccharides, CPS) in the two species all began from the exponential phase, and increased with the increasing light intensities, but glycogen content did not increase until the increment of light intensities was enough strong. Even though, their competition was obvious at the optimal light intensity. In pattern, even at the exponential phase, the two species only partitioned a little organic carbon in glycogen, more than 10 times in EPS. In EPS, CPS generally accounted for higher proportion than RPS, but the increase of RPS was much higher than that of CPS with the increasing light intensity and culture time. These results suggested that the partition of extracellular EPS and intracellular glycogen was closely related to acclimation to light intensity.

KEYWORDS:
Light intensity, glycogen, extracellular polysaccharides (EPS), carbon partition, cyanobacteria

INTRODUCTION

Cyanobacteria are photosynthetic organisms that utilize light energy to fix carbon dioxide. The assimilated carbon can mainly store in the form of glycogen and except provision growth, also secrete to the outside of cell to form extracellular polysaccharides (EPS), including loosely combined released polysaccharides (RPS) and tightly combined capsular polysaccharides (CPS). And because their accumulation levels can be further enhanced in the presence/absence of combined nitrogen, sulfur, glycerol and aeration [1-5], temperature, light, and NaCl stresses [6-11], they are regarded to act important role in withstanding starvation and many stresses [5, 8, 12-14].

As photosynthetic autotrophic organisms, light energy is actually the fundamental driving force of cyanobacterial carbon assimilation. It is also indeed found light intensity obviously affected on the accumulation of EPS and glycogen [2, 15-16], even the allocation of fixed carbon and carbohydrate profiles [17]. However, the partition pattern between the intracellular glycogen and the extracellular EPS has long been neglected. No information is available on the effects of light intensity on cyanobacterial polysaccharide partition.

Based on the little information on cyanobacterial glycogen and EPS biosynthesis pathways, it is known that the synthesis of EPS and glycogen all utilizes ADP-glucose as the glucosyl donor, and what the glucose-P and UDP-glucose from degraded glycogen are all feedstock of EPS synthesis [12, 18, 19]. So EPS and glycogen should be competitive in accumulation. In addition, sucrose can be converted into glycogen [18, 19] and polysaccharide in filamentous nitrogen-fixing cyanobacteria [19]. Yet the possible metabolic link on glycogen accumulation and EPS fractionation has never been explored. *Microcoleus vaginatus* and *Nostoc* dominate global biological soil crusts (BSC) [20]. Although they can vertically move, *Nostoc* often directly distributes on the top most of BSC, *M.vaginatus* in the slightly deeper layer under dry natural conditions [20]. In order to further comprehend cyanobacterial polysaccharide partition, in this paper, *M.vaginatus*...
FACHB 896 and Nostoc sp. FACHB 892 isolated from BSC of Tengger Desert were selected, their carbon allocation in EPS and glycogen under different light intensities were studied, the correlations with intracellular total sugar, water soluble sugar and sucrose were explored. Increased knowledge of these issues could provide critical insights into the role of cyanobacterial EPS and glycogen in adapting light intensity.

**METHODS**

**Species and growth conditions.** *M. vaginatus* FACHB 896 and *Nostoc* sp. FACHB 892 isolated from biological soil crusts of Tengger Desert (China) were preserved in the FACHB-collection. The cultures were axenically prepared in 250 ml glass flasks containing 200 ml BG-11 medium [21] in the presence of nitrate at 25 °C ± 1 °C, and they were continuously illuminated laterally on one side by cool white light [16]. In order to differentiate the effect in polysaccharide accumulation, the light intensity was chose at 4, 40, 80 μE m⁻²s⁻¹. The light intensity was maintained using a quantitherm light meter thermometer (Hansatech, London). The cultures were inoculated at a chlorophyll a concentration of 1.4 μg ml⁻¹. All tests were carried out in duplicate. Each bottle was shaken four times per day.

**Biomass analysis.** The cell suspensions were harvested by 4 min of centrifugation at 8000 xg. The cells were washed three times with distilled water and then freeze-dried (Christ ALPHA 1-2 LD plus, German). The biomass was measured in terms of dry cell weight. Sampling for the assay was performed at 4-day intervals.

**EPS assays.** The RPS contents were determined according to Su [22]. The CPS was prepared using the method by Ge [16] with modifications. The cells (dry weight) were added into 5 ml of distilled water, maintained at 80 °C for 6 h, and then centrifuged at 3,000 xg for 15 min. Microscopic observation was conducted by staining with India ink to ensure the extract has not been contaminated from intracellular substances (data not shown). The supernatant was dialyzed with distilled water in a Spectra/Por dialysis tube (3500 Da) for 2 days, and the polysaccharide content was determined. The total EPS refers to the sum of the amounts of RPS and CPS. Polysaccharides were quantified with the phenol–sulfuric acid method using glucose as the standard [23].

**The intracellular sugar components.** After CPS extraction, 6 M HCl and distilled water were added to the remaining cells. The mixtures were then boiled for 30 min. After cooling to room temperature, the extracts were neutralized to pH 7.0 using 6 M NaOH. The extracts were then centrifuged, and the supernatant fractions were stored for future analysis [24]. Soluble sugar was extracted with distilled water at 80 °C for 40 min, and then the extracts were centrifuged and the supernatant fractions were stored for future analysis [25]. The total intracellular carbohydrate, and soluble sugar extracts were quantified using the phenol–sulphuric acid method [23]. The soluble sugar extraction was also used to analyse the sucrose concentration. Sucrose was determined by adding 2 M NaOH at 100 °C for 5 min, followed by 30% HCl and 0.1% resorcin at 80 °C for 10 min. Sucrose was quantified by measuring the absorbance at 480 nm [25].

**Glycogen.** For glycogen extraction, the cells were washed with 80% ethanol, resuspended in 400 μl of H₂O, and autoclaved at 120 °C for 1 h [19]. Then, amyloglucosidase was added and the sample was incubated at 55 °C for approximately 14 h to allow the glycogen to be hydrolyzed into glucose. The concentration of glucose released by acid hydrolysis was determined according to the method by Frederick [26].

**Statistical analysis.** SPSS software (ver. 18.0) was used for the statistical analyses. ANOVA was used to determine the differences among the treatments. Bivariate correlations were used to analyze the correlation. *P* values < 0.05 were considered to
indicate statistical significance. Values are expressed as the mean±standard deviation (n=3).

RESULTS

Growth. The growth of *M. vaginatus* FACHB 896 [16] and *Nostoc* sp. FACHB 892 was similar (Fig. 1), namely their performance at 40 and 80 μE m⁻²s⁻¹ was significantly higher than that of 4 μE m⁻²s⁻¹ (P < 0.01), but there was no difference between 40 and 80 μE m⁻²s⁻¹ (P > 0.05), and no net growth occurred under 4 μE m⁻²s⁻¹.

**FIGURE 2**

EPS changes of *M. vaginatus* FACHB 896 and *Nostoc* sp. FACHB 892 under different light intensities.

**RPS, CPS and the ratios.** Fig. 2 showed the yields of RPS, CPS and EPS of two species all...
increased with the increasing light intensity (P < 0.01), and the higher the light, the higher the ratio of RPS to CPS. However, the ratios of RPS to CPS were most less than 1 (P < 0.01) except the day 16 in Nostoc sp. FACHB 892. After 12 day, RPS of Nostoc sp. FACHB 892 was higher than CPS both under 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1}.

**Glycogen.** The pattern of glycogen contents in M. vaginatus FACHB 896 and Nostoc sp. FACHB 892 was similar during the whole period (Fig. 3). Namely there was an obvious decrease during the initial 4-8 day under the all tested light intensities, then basically kept a stable value. And the contents at 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1} were all significantly higher than that of 4 μE m^{-2}s^{-1} (P < 0.01), but there was no difference between 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1} (P > 0.05).

**Ratio of EPS to glycogen.** The ratios of EPS to glycogen in M. vaginatus FACHB 896 and Nostoc sp. FACHB 892 were depicted in Fig.4. As shown, the ratios all increased with the increasing light intensities and growth time (P < 0.01). And the ratios at 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1} were 4-12 times in M. vaginatus FACHB 896, 3-10 times in Nostoc sp. FACHB 892, the linear relationship between 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1} was obviously.

**Other intracellular components.** The total intracellular carbohydrate, water soluble sugar and sucrose contents of M. vaginatus FACHB 896 and Nostoc sp. FACHB 892 under the different light intensities were shown in Fig. 5. In which, the carbohydrate components showed a similar pattern, namely, there was an obvious decrease during the initial a few days under 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1}, then generally increased. But the contents at 40 μE m^{-2}s^{-1} and 80 μE m^{-2}s^{-1} were the same (P > 0.05), being significantly higher than that of 4 μE m^{-2}s^{-1} (P < 0.05).

**Correlation with intracellular components.** The Pearson’s correlation analysis (Table 1) showed that, glycogen contents under 4 μE m^{-2}s^{-1} were positively correlated with soluble sugar contents in M. vaginatus FACHB 896 (P < 0.01), while positively with CPS and EPS contents in Nostoc sp. FACHB 892 (P < 0.01); EPS contents did not exhibit correlation with all tested intracellular components in M. vaginatus FACHB 896 (P > 0.05), but positively correlated with CPS (P < 0.01) and negatively correlated with RPS in Nostoc sp. FACHB 892 (P < 0.05).

Under 40 m^{-2}s^{-1}, the glycogen and
FIGURE 5
The changes of the intracellular components of *M. vaginatus* FACHB 896 and *Nostoc* sp. FACHB 892 under different light intensities.

EPS contents of two species all significantly negatively correlated (*P* < 0.05), but EPS contents were positively correlated with the contents of both RPS and CPS in *M. vaginatus* FACHB 896 (*P* < 0.01), only with RPS in *Nostoc* sp. FACHB 892 (*P* < 0.01). In the case of 80 μE m⁻²s⁻¹, the situations of two species were quite different, namely, there were no correlation between glycogen and EPS, and any other intracellular components (*P* > 0.05); but EPS contents were positively correlated with RPS and CPS contents in *M. vaginatus* FACHB 896 (*P* < 0.01), only positively with RPS in *Nostoc* sp. FACHB 892 (*P* < 0.05).

**DISCUSSION**

**Growth.** Light is the sole energy source of cyanobacterial growth in our experiments. So like many of other cyanobacteria, the biomasses of *M. vaginatus* FACHB 896 and *Nostoc* sp. FACHB 892 all increased with the increasing light intensities [10, 27]. Despite 4 μE m⁻²s⁻¹ only maintained cellular survival, but the similar growth rates under 40 μE m⁻²s⁻¹ and 80 μE m⁻²s⁻¹ suggested, that a considerable amount of light energy was surplus to the growth of 80 μE m⁻²s⁻¹, whereas the superfluous energy might be both used to the biosynthesis of EPS, and other protective secondary metabolites (i.e. carotenoids content is significantly increased, data did not shown). By contrast, 4 μE m⁻²s⁻¹ was too weak to
support cyanobacterial net growth, thus the limited energy had to be used to maintain cell survival.

**Glycogen.** Glycogen is the primary product of cyanobacterial photosynthesis. It does not only serve as a buffer reservoir in situations of energy surplus, but it may function as well in the supply of organic carbon under low irradiances and acts a respiratory but it may function as well in the supply of organic carbon under periods of darkness [8, 28]. So, the glycogen content is the lowest under 4 μE m⁻² s⁻¹ in the two species of this study. Previous studies have shown that glycogen content is enhanced under higher light intensities [2]. In particular, a rapid shift from low to high irradiance is known to induce glycogen accumulation without interfering with nitrogen metabolism [29]. However, an increase in light intensity from 50 to 270 μE m⁻² s⁻¹ did remarkably influence the glycogen content in *Arthrospira platensis* [2], also from 40 to 80 μE m⁻² s⁻¹ in the two species of this study. Which indicated the augment of glycogen content was different from that of EPS, it only occurred in the enough strong light intensity; otherwise it maintained a relative stable value under some ranges of light intensities. We guessed that glycogen synthesis was finely tuned by the intracellular organic carbon, starting only when the concentration of intracellular carbon accumulated to some thresholds. This aspect need pay much more attention in the future study.

**EPS.** The secretion of EPS, a considerable energy investment, is an inherent feature of cyanobacteria during the long-term evolution. It is attributed to deal with various stresses due to no better interpretation. But it is actually a strategical resource sink. So the amount increase with the increasing light intensities [10, 15, 30]. Under the previous experimental conditions, cyanobacteria can secrete EPS during the whole growth process, only the higher production starts from the late exponential phase or from the stationary phase [10, 31, 32]. In the present study, the EPS production, including CPS and RPS, all began from the exponential phase, and the higher contents occurred at both the higher irradiance. This further demonstrated that EPS production was cyanobacterial resource sink. EPS secretion and cell growth was dependent on the organic carbon partition, especially when glycogen level did not increase with light intensity, the relationship of EPS content and light intensity became obvious linear. While EPS level of *M. vaginatus* FACHB 896 much higher than that of *Nostoc* sp. FACHB 892 at all light intensities, suggesting that *M. vaginatus* FACHB 896 was more more resistant to salt stress and drought stresses than *Nostoc* sp. FACHB 892 [5, 12, 13].

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>The correlation of EPS and intracellular components in <em>Nostoc</em> sp. FACHB 892 (below blank diagonal) and <em>M. vaginatus</em> FACHB 896 (upper blank diagonal) under the different light intensities (μE m⁻² s⁻¹).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Light intensity</th>
<th>RPS</th>
<th>CPS</th>
<th>EPS</th>
<th>TS</th>
<th>SS</th>
<th>Glycogen</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 μE m⁻² s⁻¹</td>
<td>-0.890*</td>
<td>-0.881**</td>
<td>1.000**</td>
<td>-0.562</td>
<td>-0.156</td>
<td>-0.871</td>
<td>-0.134</td>
</tr>
<tr>
<td>EPS</td>
<td>0.547</td>
<td>0.280</td>
<td>0.528</td>
<td>0.300</td>
<td>0.304</td>
<td>0.997**</td>
<td>0.392</td>
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<tr>
<td>TS</td>
<td>0.096</td>
<td>0.771</td>
<td>0.524</td>
<td>0.783</td>
<td>0.473</td>
<td>0.947*</td>
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<tr>
<td>SS</td>
<td>0.578</td>
<td>0.817</td>
<td>0.126</td>
<td>0.966**</td>
<td>0.275</td>
<td>0.945*</td>
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<td>Glycogen</td>
<td>0.522</td>
<td>0.107</td>
<td>0.192</td>
<td>0.749</td>
<td>0.325</td>
<td>0.533</td>
<td>0.371</td>
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<tr>
<td>Sucrose</td>
<td>0.324</td>
<td>0.584</td>
<td>0.747</td>
<td>0.539</td>
<td>0.533</td>
<td>0.371</td>
<td>0.324</td>
</tr>
<tr>
<td>40 μE m⁻² s⁻¹</td>
<td>0.548</td>
<td>0.981**</td>
<td>0.835</td>
<td>0.749</td>
<td>0.789</td>
<td>0.149</td>
<td>0.400</td>
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<tr>
<td>EPS</td>
<td>-0.902*</td>
<td>-0.833</td>
<td>-0.873</td>
<td>-0.885*</td>
<td>-0.395</td>
<td>0.149</td>
<td>0.665</td>
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<tr>
<td>TS</td>
<td>0.804</td>
<td>0.376</td>
<td>0.363</td>
<td>0.210</td>
<td>0.077</td>
<td>0.628</td>
<td>0.665</td>
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<tr>
<td>SS</td>
<td>-0.928**</td>
<td>0.628</td>
<td>0.755</td>
<td>0.798</td>
<td>0.850</td>
<td>-0.349</td>
<td>0.077</td>
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<tr>
<td>Glycogen</td>
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<td>-0.349</td>
<td>0.755</td>
<td>0.798</td>
<td>0.850</td>
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<td>0.077</td>
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<tr>
<td>Sucrose</td>
<td>-0.888*</td>
<td>-0.349</td>
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<td>0.798</td>
<td>0.850</td>
<td>-0.349</td>
<td>0.077</td>
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<tr>
<td>80 μE m⁻² s⁻¹</td>
<td>0.580</td>
<td>0.982**</td>
<td>0.652</td>
<td>0.359</td>
<td>0.939*</td>
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<td>0.731</td>
</tr>
<tr>
<td>EPS</td>
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<td>-0.872</td>
<td>0.410</td>
<td>0.149</td>
<td>-0.434</td>
<td>0.731</td>
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<td>0.731</td>
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Notes: TS: total intracellular sugar; SS: Soluble sugar.
* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.
Patterns of RPS and CPS. As we know, cyanobacterial EPS includes RPS and CPS. The yields have obvious variations from one strain to another. Generally RPS is more abundant than CPS [33], but CPS becomes thicker under stress conditions [9, 33, 34], and light wave-lengths can significantly affect their ratios [34]. In the present study, the ratios of RPS to CPS in two species during the exponential phase were most less than 1 both at 40 μE m⁻²s⁻¹ and 80 μE m⁻²s⁻¹. This pattern was similar to those results from stress conditions [9, 33, 34]. However, in our study, the dominant pattern of RPS and CPS were significantly variable. The ratios of RPS/CPS gradually increased despite the ratios less than 1 within 12 days under all the tested light intensities, which meant the increased amount of CPS was much higher than that of RPS with growth time. Afterward, the proportion of RPS in Nostoc sp. FACHB 892 was much higher than CPS, whereas the proportions of CPS in M. vaginatus FACHB 896 were still higher than RPS. Thus, our results strongly suggest that their formation not only related to light intensity, but also related to the culture time. Specifically, the light is preferentially used in CPS synthesis in a relatively short period of time, then, the increasing light energy is use for RPS synthesis along with the gradual formation of CPS. Additionally from the prior formation of CPS to RPS and no RPS synthesis under limited light energy, we inferred that CPS was more important in cellular protection and survival [9, 12], RPS was inclined to a special storage sink of excessive organic carbon. Whereas the variable ratio of RPS / CPS with culture conditions has important instruction in obtaining higher desired EPS.

Partition of both EPS and glycogen. Although cyanobacterial glycogen has been regarded as promising carbohydrate source, the published results mostly in model species [35, 36], and the highest production of natural strains is still ca. 1.03 g L⁻¹ [2]. By contrast, the production of EPS is far higher [10, 22, 32]. In this study, we found that the ratio of EPS/ glycogen were almost 2-12 times in this two species. Thus, it could further determine that glycogen mainly provides the energy required for the essential cellular process in the dark although it is the main reserve product, and the glycogen storage is limited in algae cell, while the reserve of EPS is larger. A previous study has shown that glycogen yield of Nostoc muscorum almost remain constant after 10 days. A similar result was observed in our study, confirming that in a certain condition, the algae cell keep the light energy and carbon to sustain the glycogen content constantly mainly to used as intracellular carbon source for metabolism, and partitioned more light energy and carbon to synthesis EPS mainly to protect cell.

About metabolic link, the significantly negative correlation of glycogen and EPS (RPS) obviously demonstrated they were physiologically competitive under the optimal light intensity, whereas there were no correlation between glycogen and EPS at 80 μE m⁻²s⁻¹, meant that the main pathway from glycogen to EPS by general soluble sugar became more branched due to an increase of carotenoids, mycosporine, scytomin , stress protein and other secondary metabolites during acclimation to high light [8, 37, 38]. As for the positive correlation of the lower level of CPS and glycogen at 4 μE m⁻²s⁻¹ in Nostoc sp. FACHB 892, indicated glycogen and CPS were the fundamental and pivotal for survival, they were not only the storages, but also structured components.

Sucrose is a common transportable sugar, the interconversion between sucrose and glycogen was discovered in the previous studies [18, 19], and was also demonstrated to convert into extracellular insoluble polysaccharides according to nutritional and environmental signals [19]. However, in this study, sucrose content did not show a correlation with the contents of EPS and glycogen. This might be ADP-Glucose and sugar nucleotide of glycogen and polysaccharides biosynthesis were sufficient during the all light intensities, or sucrose cleavage was limited. Anyway, the direct link of sucrose between glycogen and EPS was complex, this need further study.

In addition, EPS positively correlated with RPS and CPS in M. vaginatus FACHB 896 under 80μE m⁻²s⁻¹, only with RPS in Nostoc sp. FACHB 892,which meant the effect of light intensity on RPS production was much obvious than that of CPS under strong light. Glycogen content positively correlated with soluble sugar content in M. vaginatus FACHB 896 at 4 μE m⁻²s⁻¹, indicated glycogen level was regulated by soluble sugar.

CONCLUSION

In conclusion, glycogen and EPS are both the storage of the surplus organic carbon in cyanobacteria, and the amounts all increased with light intensity. But the storage of EPS is larger, glycogen is limited, and the responses of EPS production to light intensities are much more sensitive than that of glycogen accumulation. In addition, although the accumulation of glycogen and EPS productions exhibit obvious competition under the optimal light intensity, the biosynthesis of RPS is more dependent of culture conditions compared with CPS production, the efforts in culture parameters will have huge potential in enhance production of the desired polysaccharide.

ABBREVIATIONS

EPS: extracellular polysaccharides;
RPS: released polysaccharides;
CPS: capsular polysaccharides;
ACKNOWLEDGEMENTS

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THE EFFECTS OF THE HERBICIDE ROUNDUP ON ZEA MAYS L.

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Kocaeli University, Faculty of Arts and Sciences, Department of Biology, 41380, Kocaeli, Turkey

ABSTRACT

Several herbicidal chemicals are available for use in agriculture to increase the yield. Despite their usage commonly, they have impacts on crops. Related with this, in the current study, the effects of a widely used herbicide Roundup, which has glyphosate as the active ingredient. Molecular and spectroscopic approaches were combined together in order to investigate the effects of glyphosate on Zea mays. Depending on the EC50 value that reduce the root growth to 50%, three different concentrations of Roundup were used as 4 ml/L, 8 ml/L and 16 ml/L. They were applied to Z. mays seeds for 48 and 72 hours. For RAPD (Random Amplified Polymorphic DNA) PCR analysis; 8 of 10 RAPD primers produced band patterns and it was found that five RAPD primers among them produced polymorphic band patterns and then were used to produce a total of 61 bands. Value of polymorphisms was 32.78%. Genomic template stability changed in RAPD profiles at various Roundup concentrations. In addition to PCR analysis, the root samples were studied by Fourier transform infrared (FT-IR) spectroscopy in order to obtain the effects of Roundup on the quantity of biomolecules, membrane fluidity, cell wall components, lipid and protein structure of Z. mays. Each concentration of Roundup caused significant effects on Z. mays cells, particularly, the most effective concentration level was found to be 16 ml/L. Our corresponding results revealed that Roundup has prominent impacts on structure and function of biomolecules of Z. mays, all of which might be important for its growth. In conclusion, the combination of RAPD and spectroscopic analysis would offer efficient tool to determine the effects of any pesticides on plant cells.

KEYWORDS:
Zea mays, genotoxicity, genomic template stability, IR-spectrum, primers.

INTRODUCTION

One of the applications for improving agricultural productivity is the use of pesticides in plants against harmful organisms. Although they are beneficial in terms of productivity in agriculture, over-use of these chemicals can lead to serious environmental problems and also are a source of danger for living organisms. Herbicides, an important member of pesticides, have become an indispensable element of agricultural lands for combating weeds and revolutionised weed management strategy in crops. The isopropylamine salt of glyphosate and the surfactant polyethoxylated tallow amine are the main active ingredients of Roundup. Both of them can cause toxicity in wildlife [1]. Polyethoxylated tallow amine increases the penetration of the herbicide in plant and animal cells [2].

Roundup is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitor and it has been the number one selling herbicide worldwide since at least 1980. It has been reported that it shows medium and long term toxicity and had negative effects on reproduction. Roundup formulations and its metabolitic products might cause the death of human embryonic umbilical cells in vitro at low concentrations [3, 4]. Yousef et al. [5] had shown in rabbits exposed to glyphosate that there was a reduction in the production of sperm cells by 50 percent. In another study, with the leakage of intracellular liver enzymes, glyphosate had caused liver damage in rats [6]. This study investigated genotoxicity of the pesticide Roundup in Z. mays root tip cells. The aim of this study is to test the toxicity of a widely used herbicide roundup on sweet corn (Z. mays). Because of Roundup is utilized in agriculture and introduced into the environment in this form the commercial form of the pesticide was directly tested.

MATERIALS AND METHODS

Plant material and growth conditions. The common sweet corn, Zea mays L. var saccharata Sturt cv. Merit was used in this study. The seeds were sterilized with 10% (v/v) NaOCl for 10 min and 75% (v/v) ethanol for 2 minutes then washed with distilled water for 3 times. The seeds were germinated until the primary roots were 3–5 mm long in sterile Petri dishes. Thick tissue papers were used for germination for at 25 ± 2 °C 48 h in dark conditions. The germinated primary roots were treated with the concentrations of 4 ml/L, 8 ml/L and 16 ml/L Roundup for 72 h. [7].
Determination of EC50. We used 6 different concentrations of the herbicide Roundup including the suggested concentration to find out the EC50 (effective concentration that lower the root length 50% of the control). After 48th and 72th hour, the mean value of 100 roots for each concentration, was expressed as a percent of the control value. The EC50 value that is the concentration which reduces the root growth by 50% was determined with this data. The EC50 value of Roundup was determined as 8 ml/L and then the root tips were treated with 4 (EC50/2), 8 (EC50), and 16 (2xEC50) ml/L concentrations.

DNA extraction and RAPD-PCR technique. At first 0.1-0.2 g Z. mays root tissue was powdered in liquid nitrogen and then Qiagen DNaseasy Plant Mini Kit was used for genomic DNA was extraction. Shimadzu UV-mini 1240 spectrophotometer was used in order to determine the quantity and quality of genomic DNA. A set of 10 randomly chosen primers was purchased from Thermo Scientific. A 25 μL reaction mixture was prepared containing 10 ng of template DNA, 1 mM primer, 1 U of Taq polymerase, 1X Taq polymerase buffer, 2.5 mM MgCl2 and 1 μM dNTP for PCR amplifications. TC-3000 Thermal Cycler was programmed included an initial denaturation step at 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 30°C for 1 min, 72°C for 1 min, and a final elongation at 72°C for 5 min. 1 % agarose gel containing red safe (0.5 μg/mL) was used for the separating of the PCR products and the gel was photographed using the UVP GelDoc-It 310 Imaging System. A 1 kb DNA ladder was used as size marker (Fermentas). A negative control with no DNA template was also included in each PCR amplification in order to verify the absence of contamination.

RAPD data analysis. Only strong bands were scored for analysis. The amplified bands were scored as 1 that means “presence” or 0 that means “absence”. The size of each amplification product was automatically estimated using the Vision WorksLS Version 6.8. Images were captured using a high resolution scan and digitalized images were counted directly for RAPD analysis. Genetic similarity coefficients (phenetic numerical analysis) among the roots of untreated control and treated roots were estimated from Nei’s unbiased measure [8] in POPGENE version 1.31.

Estimation of genomic template stability. Genomic template stability (GTS) was calculated as follows: GTS% = (1- a/n) X 100. Where “a” stands for the polymorphic profiles of RAPD detected in each treated sample and “n” stands for the total number of bands in the control group. The appearance of a new band and the disappearance of a normal band showed the polymorphism in RAPD profiles in comparison to the control RAPD profiles [9]. The average was then calculated for each the experimental groups treated with different concentrations of Roundup.

Spectroscopic analysis. For spectroscopic examination routine solid sample preparation was performed as followed previous works [10, 11]. Briefly, roots were dried by tand drier system (CHRIST Alpha 1-2 LD Plus Benchtop Freeze Dry System), and then were ground. Each grounded powder belonging to each sample was mixed with potassium bromide (KBr) at the ratio of 1:100. Later, it was pressed by a pressure of ~100 kg cm⁻² (1300 psi) in order to produce a KBr disk. Infrared spectra of KBr disks were obtained using a Perkin-Elmer Spectrum One FT-IR spectroscopy (Perkin-Elmer Inc., Norwalk, CT, USA) accoutred with a deuterated-triglycine sulfate detector. Since water and carbondioxide molecules in the air affect IR spectrum throughout scanning, spectra of air was recorded and automatically subtracted by using Spectrum One software. All sample spectra were acquired in the region between4000-400 cm⁻¹ wavenumber range. Scan number was 100 at 4 cm⁻¹ resolution to make each interferogram to collect IR. Same conditions were provided for each individual sample. Each sample was scanned for three times in order to get rid of any variations in samples such as thickness of pellets. Three spectra from the same sample represented identical spectra, therefore three replicates were averaged. The subsequent final spectra were evaluated for IR and statistical analysis.

Spectral analysis was performed by means of same software. The spectra were first smoothed with nineteen-point Savitsky-Golay smooth function and baseline corrected. The wavenumber values were determined corresponding to the center of weight of each mode (80%). The bandwidth values were calculated at 0.80 x height of the peak and the width at this point was converted to cm⁻¹. In order to visual demonstration of spectral alterations the spectra were normalized with respect to specific modes.

RESULTS

RAPD-PCR Findings. List of polymorphic and monomorphic RAPD primers was given in Table 1. The number of primers compared between 4 ml/L, 8 ml/L, 16 ml/L treatments of Roundup and the percentage of polymorphism for all primers (Fig.1) were determined after RAPD analysis. Twenty 10-mer oligonucleotide primers of 60–70% GC content were utilized for screening Z. mays of DNA changes, but only 8 primers generated specific and stable results. The total number of bands was 17 (untreated) and 61 (treated with round up)
ranged from 258 to 1170 pb. Three primers pro-
duced the same RAPD profiles and five RAPD
profiles showed substantial differences between
control and treated roots (Fig. 2) in terms of num-
ber and size of amplified DNA fragments for differ-
ent primers.

### TABLE 1
The list of polymorphic and monomorphic RAPD primers compared to 4 ml/L, 8 ml/L and 16 ml/L treat-
ments of Roundup.

<table>
<thead>
<tr>
<th>Concentration (ml/L)</th>
<th>Monomorphic Primers</th>
<th>Polymorphic Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>OPC-5, OPC-6, OPU-5, OPU-7</td>
<td>OPU-1, OPU-2, OPU-3, OPU-6</td>
</tr>
<tr>
<td>8</td>
<td>OPC-6, OPU-5, OPU-7</td>
<td>OPC-5, OPU-1, OPU-2, OPU-3, OPU-6</td>
</tr>
<tr>
<td>16</td>
<td>OPC-6, OPU-5, OPU-7</td>
<td>OPC-5, OPU-1, OPU-2, OPU-3, OPU-6</td>
</tr>
</tbody>
</table>

### FIGURE 1
The percentage of polymorphism for all primers in up treated Z. mays.

### FIGURE 2
Monomorphic and polymorphic RAPD profiles of Z. mays; control (C), 4, 8, and 16 ml/L treatments of Roundup. RAPD profiles were generated using primer OPC-5, OPC-6, OPU-2, OPU-3, OPU-5, OPU-7 M: GeneRuler 100 bp plus DNA Ladder (100–10000 bp).

### TABLE 2
The number of bands in control and molecular sizes (base pair, bp) of disappearance (-) and/or appear-
ance (+) of DNA bands for all primers in treated Z. mays.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Control</th>
<th>Roundup Concentration (ml/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>OPC-5</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>OPC-6</td>
<td>2</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>-</td>
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<tr>
<td>OPU-1</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>OPU-2</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>OPU-3</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>OPU-4</td>
<td>3</td>
<td>+</td>
</tr>
</tbody>
</table>
The observed variations in RAPD profiles were given in Table 2. Polymorphisms were caused by appearance or disappearance of the amplified bands in the treated profiles when compared to control groups. And, polymorphisms P (%) value was obtained as 32.78%. On the other hand, value of polymorphisms P (%) for Roundup treatments: 4 ml/L, 8 ml/L and 16 ml/L: 62%, 48% and 42% respectively. The genomic template stability (GTS, %) parameters that are qualitative evaluations indicating alterations in RAPD profiles was calculated for each primer and illustrated in Table 3. As shown in the table, GTS values declined in 16 ml/L Roundup concentration.

FT-IR Findings. Within the scope of spectral analysis the alterations in the wavenumber and areas of the bands attributed to primary metabolites of Z. mays was investigated and prominent variations in treated groups compared to control one were obtained. Fig. 3 shows normalized infrared spectra of Z. mays treated with 4 ml/L, 8 ml/L and 16 ml/L Roundup concentrations in 4000-900 cm⁻¹ region. The region covering 3050-2800 cm⁻¹ wavenumber range includes the absorptions of olefinic =CH, CH₂ and CH₃ groups assigned to membrane lipids and proteins [12]. Within the region, the mode at 3015 cm⁻¹ is from the HC=CH groups in unsaturated lipids [12, 13], therefore, this band can be used to monitor the level of unsaturation in lipids. As depicted in Fig. 3 the area of such mode significantly increased for Roundup concentration compared to the control group. No significant change in the frequency bandwidth values of the CH₂ asymmetric stretching vibration (2925 cm⁻¹) was observed for any concentration of Roundup (Fig. 3). The band located around 1653 cm⁻¹ is arisen from the C=O stretching of proteins and pectins [10, 11, 14]. As also illustrated in Fig. 3, there was a significant decrease in the area of the peak only for 8 ml/L and 16 ml/L Roundup concentrations. The modes around at 1060 cm⁻¹ and 1030 cm⁻¹ is from cellulose and hemicellulose [15, 16, 17, 18]. For all concentrations of Roundup a significant reduction in these peak areas was found.

**FIGURE 3**
The spectral investigation of the variations in the frequencies and and peak areas of the vibrational modes from primary metabolites of Z. mays.

**TABLE 3**
Genomic template stability (GTS, %) of corn exposed to untreated control, 4, 8 and 16 ml/L treatments of Roundup.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Control</th>
<th>Roundup Concentration (ml/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>OPC-5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>OPU-1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>OPU-2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>OPU-3</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>OPU-6</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>Average</td>
<td>100</td>
<td>62</td>
</tr>
</tbody>
</table>
DISCUSSION

In the current work, the potency of the RAPD assay was used to investigate Roundup-induced effects on DNA on the roots of Z. mays. Additionally, by taking the advantages of FT-IR spectroscopy we have also determined impacts of Roundup with different concentrations on primary metabolites of Z. mays. In spectroscopic findings a significant (p<0.05) increment in the area of the olefinic band with the addition of Roundup may be resulted from the accumulation of lipid peroxidation end-products, such as lipid aldehydes [19, 20, 21]. The presence of such products may be indicative of alterations in the structure and function of cell membrane due to readily undergoing peroxidation of unsaturated lipids. This outcome may be caused by the production of free radicals that are resulted from dysfunction of both mitochondria and chloroplast. These organelles that have electron transport chain considerably produce reactive oxygen species (ROS) and nitric oxide (NO) in plants. And, the attack of ROS to all lipids in the chloroplast and mitochondrial membrane may bring about impaired oxidative phosphorylation. The inhibition of oxidative phosphorylation coupled with the utilization of energy during plant growth interrupts the cell’s capability to maintain energy levels, subsequently causing mitochondrial and chloroplast damage. This result may also indicate that when compared to other techniques, FT-IR spectroscopy offer more advantageous approach to analyse the presence of lipid peroxidation in a simple and sensitive way [20, 22, 23].

Additionally, the frequency of the CH$_2$ asymmetric stretching band did not significantly shifted to any values for all roundup concentrations. This can be used to monitor trans/gauche isomerization in the system and it increases with the introduction of trans conformers in the fatty acyl chains [14, 24, 25]. Since there was no variation in the frequency values of this peak, it might reveal that roundup treatment has no significant effect on the amount of neither trans nor gauche conformer. This might mean that Roundup did not have ability to change order of membrane system, which also might suggest no altered lipid structure along with the formation of cross-linked lipid-protein moieties [12, 24]. This ineffectiveness may refer no modification of membrane lipid structure upon activation of membrane located proteins such as histidine kinases [26]. Besides this analysis, we have alternatively measured the bandwidth value of the mode. No significant alteration for all concentrations reflects that there is not any change in fluidity of membrane [27]. From this point, no change in membrane may further represent any degree of cellular activation upon roundup treatment. On the other hand, the band located around 1650 cm$^{-1}$ is arisen from the C=O stretching of proteins and pectins [10, 14]. The decrement in the area might reveal low protein content [17] potentially due to degradation, which might be due to attraction of free radicals, and downregulation of various proteins. Here, it should be mentioned that FT-IR spectroscopy cannot distinguish specific proteins, but only information concerning entire whole protein profile of Z. mays including extracellular, plasma membrane, cytoplasm and nuclear proteins. However, the increase in Amide I mode could be assigned to the high content of some proteins depending on the literature. It should be also mentioned that pectin molecule contributes to this mode [10, 15, 16]. The same trend is valid for pectin decrement in the system directly owing to the production of low degree of pectin polysaccharides. It is known that during plant cell growth for the preparation of mitotic division middle lamella is formed, thereby generating pectic substances [15], which was found the lowering ratio for Roundup with rising concentration. With the increasing concentration of roundup the obvious decrease in the peaks (1030 and 1060 cm$^{-1}$) might be resulted from decrement in the content of hemicellulose and cellulose. According to the literature, growing plant cell structure stimulate cellulose to hemicellulose conversion in order to promote rapid enlargement. This enables the cell wall more flexible to expand and the increase in the peak may further demonstrate the conversion of hemicellulose from cellulose during cell growth. The mode at 1030 cm$^{-1}$ is from cellulose and hemicellulose [16, 18]. For all concentration of Roundup a decrease in the peak area was found, which might elicit significant decrease in both cellulose and hemicellulose content. But, as mentioned before, the incident of hemicellulose is expected during cell growth brought about conversion from cellulose. But in our case, the decrement in these peaks might reveal that there is no stimulated plant cell growth upon Roundup treatment. Abiotic and biotic stress factors can cause DNA changes as a result of physiological signals, their effects on protein synthesis and the direct toxic effects of certain chemicals.

DNA changes can be monitored at both the biochemical and the molecular levels using different biomarkers [28, 29]. In earlier studies, the RAPD profiles generated from Nasturtium officinale (under arsenic stress), bean (under 2–4 D and Dicamba stresses) and Hordeum vulgare (under Cd stress) resulted in different patterns of the disappearance of normal band(s) and/or appearance of new band(s) relative to the control RAPD profiles [30, 31, 32]. Similarly, in this study, DNA changes induced by Roundup treated Z. mays exhibited variation in the band size and in the disappearance or appearance of band(s) in the experimental groups in comparison with the control group on RAPD profiles. The changes in the RAPD bands may be due to changes of the initiation sites of the oligonucleotides caused by genome rearrangement. The appearance of new
bands may also be due to DNA damage induced by herbicide toxicity. However, the mechanisms for such changes require further study [28, 31, 33]. The appearance of new bands showed that the effect can be involved in DNA repair and replication mechanisms or may be the result of genomic template instability connected with the level of DNA damage and the effectiveness of DNA repair and replication [33]. The obvious appearance of new bands and disappearance of normal bands in genomic DNA of the roots treated with different concentrations of Roundup in comparison to the control shows us that there are changes between these two groups. Mutations can only be responsible for the appearance of new bands if they occur at the same locus in a sufficient number of cells to be amplified by PCR [34]. The new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage [35]. RAPD-PCR was used to detect DNA damage in the roots of *Z. mays* and the value of polymorphisms P (%) were decreased with increasing up concentration. On the other hand, GTS values decreased obviously in 16 ml/L up concentration. In another study, treated wastewater caused 16 new bands and the loss of 17 bands in *Avena sativa*. RAPD profiles obtained showed that both treated and raw wastewater were having genotoxic effects on oat plants [36].

**CONCLUSION**

Pesticides cause many alterations in the morphological (such as necrosis, colony disintegration, root break-up) and physiological (such as photosynthesis, pigment synthesis and enzyme activity) events of plants. Considering that alterations in the contents of biomolecules can be used as biomarkers to determine the impact of herbicides on plants, we have investigated the effects of herbicide Roundup by using FT-IR spectroscopy and combine the obtained results provided by DNA analyses. Infrared data revealed that exposing to Roundup may have negative effect on growth of *Z. mays* obtained from a decrement in protein, pectin, cellulose and hemicellulose, an increase in lipid peroxidation end products as well as no change in lipid order and fluidity. Additionally, the modifications in genomic DNA were detected by RAPD profiles through the randomly primed PCR reactions. As a conclusion, the present results suggested that RAPD analysis in conjunction with spectroscopic analysis would prove a powerful ecotoxicological tool.

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EFFECTS OF DIRECT SELECTION FOR PLANT YIELD ON SOME OTHER TRAITS IN
G. HIRSUTUM X G. BARBADENSE CROSS

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Ege University, Faculty of Agriculture, Department of Field Crops, Izmir, Turkey

ABSTRACT

The two generations (F2 and F3) derived from interspecific hybridization between Carmen (G. hirsutum) and Giza-45 (G. barbadense) were used to evaluate yield and quality characteristics. The effects of direct selection based on the yield were determined on other agronomic and quality parameters. Seed cotton yield per plant, plant height, length of node first fruiting branch, fruit branches per plant, total bolls per plant, seed cotton weight per boll, seed weight per plant and ginning out turn were measured in the plants of F2 and F3 generations. Additionally, fiber quality characteristics such as micronaire, fiber strength, fiber length, uniformity ratio, short fiber index, fiber elongation traits were measured. The relative selection efficiency of seed cotton per plant could be effective for seed weight per plant, fiber branches per plant, plant height, fiber length and uniformity ratio. The effective direct selection for higher plant yield caused the positive changes in some fiber quality traits as well as the yield for this interspecific hybridization.

KEYWORDS:
Inter-specific hybridization, heritability, selection efficiency, cotton

INTRODUCTION

Cotton is one of the most important fiber crops with 26 million tons of lint production worldwide. Turkey ranks 8th with 0.85 million tons of cotton fiber production [1]. Cotton fiber is widely used as the raw material for the textile industry. The cotton seed obtained from the ginning process is used to produce oil and animal food. G. hirsutum L. and G. barbadense L. are two of the widely grown tetraploid (2n=4x=52) cotton species [2]. Gossypium hirsutum L. (Upland Cotton) is known as high yielding cotton species with significant adaptation capability. Upland cotton contributes 90-95% in total world fiber production [2-4]. However, the inadequate quality characters are major bottleneck for G. hirsutum production. High-quality properties are attributed to Gossypium barbadense L. (Pima, Egyptian, Sea Island). Meanwhile, both low yield and narrow range adaptation are main drawbacks for this species. Thus, only 5% of the total cotton fiber production worldwide is derived from G. barbadense [2, 4].

There is a trade-off between fiber yield and quality characteristics in cotton production. Negative and strong correlations between yield and quality characteristics in cotton plant have been reported in various investigations [5-7]. In two different studies, a negative correlation between fiber length and seed cotton yield has been reported [8, 9]. Moreover, lint yield has negative correlation with fiber strength whereas it is positively associated with micronaire value [10]. High-quality cotton fiber is preferable for weaving factories. However, the farmers generally prefer to cultivate high yielding cultivars. To overcome this issue, plant breeders are attempting to transfer high-quality characteristics of G. barbadense into high yielding and adaptive G. hirsutum cultivars through interspecific hybridization. The interspecific hybridization in cotton (Gossypium hirsutum L. x Gossypium barbadense L.) is a very promising tool to increase the effectiveness of cotton breeding programs. In this process, the aim is to develop new cultivars with desirable characteristics such as high adaptability capacity and yield potential, resistance to biotic and abiotic stresses factors and high-quality traits. Many researchers have worked on interspecific hybridization in the cotton plant [11-13, 4]. This method is still considered as a promising approach among the cotton breeders for developing new gene combinations.

In view of aforementioned observations, the objectives of this study were (i) to combine desired quality and yield characteristics in new cotton breeding lines developed through interspecific hybridization and (ii) to determine the indirect effects of yield based direct selection on other agronomic and quality parameters.
The plant material consisted of F2 and F3 populations was obtained from the crosses between Carmen (G. hirsutum L.) and Giza-45 (G. barbadense L.) varieties. The variety Carmen originated from Australia has high yield potential and adaptation capacity for diverse climatic conditions. It is also resistant to the shedding of the flowers and the squares caused by drought. Carmen has the fiber quality on Fibermax standards and being the prior choice for textile industry. Giza-45 is known as “Queen” of all Egyptian cotton. It is renowned for the fineness and resistance of its extra-long fibers, it is possible to reach incredibly fine yarn counts [14].

The first crosses were made between the parents in 2007 and the F2 and F3 generations were grown during the 2009 and 2011 cotton growing seasons. The experiments were conducted at Ege University, Field Crops Department, İzmir, Turkey. The seeds of the generations were sown by hand then thinned to 20 cm in three meters long and 70 cm wide rows. The fertilization was applied as 80 kg/ha nitrogen and P2O5 before sowing, then 80 kg/ha nitrogen was given with both first and second irrigation. The experiments were irrigated 5 times after flowering stage by furrow irrigation and the plants were hoed two times for weed control.

Plant yield, the yield components, and the fiber quality properties were measured in individual plants of F2 and F3 generations. The selection was performed with the 11% selection intensity depending on seed cotton yield per plant in the F2 generation. Besides seed cotton yield, plant height, the height of node first fruiting branch, fruit branches per plant, total bolls per plant, seed cotton weight per boll, seed weight per plant and ginning out turn were also measured in the F2 and F3 generations. The fiber quality properties of the hand-harvested and selected 14 individual plants and their F3 progenies were measured by using of USTER HVI 900 A (High Volume Instrument). Measured fiber quality traits were micronaire, fiber strength (g/tex), fiber length (mm), uniformity ratio (%), short fiber index (%), fiber elongation (%).

### RESULTS AND DISCUSSION

The mean values of some yield attributes of the parents, F2, F2 (selected) and F3 generations of Carmen × Giza-45 cross are presented in Table 1. It was observed that parent Carmen had higher values than Giza-45 in terms of almost all yield components except plant height and height of node first fruiting branch. The mean values of F2 population for many yield traits were beyond the the values of parents except fruit branches and total bolls per plant. The mean values of plant height and height of node first fruiting branch of F2 population were higher than

### MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Generation</th>
<th>Plant height</th>
<th>Length of node first fruiting branch</th>
<th>Fruit branches plant</th>
<th>Total bolls plant</th>
<th>Seed cotton weight boll</th>
<th>Seed cotton weight plant</th>
<th>Seed weight plant</th>
<th>Ginning turn out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmen</td>
<td>75.9±3.49</td>
<td>11.4±1.09</td>
<td>12.9±0.78</td>
<td>20.3±1.96</td>
<td>5.3±0.31</td>
<td>91.1±7.26</td>
<td>45.4±3.94</td>
<td>50.2±1.63</td>
</tr>
<tr>
<td>Giza-45</td>
<td>117.9±5.36</td>
<td>19.0±1.83</td>
<td>11.5±0.65</td>
<td>18.9±1.64</td>
<td>3.3±0.17</td>
<td>39.0±5.74</td>
<td>22.4±3.46</td>
<td>42.6±1.62</td>
</tr>
<tr>
<td>F2</td>
<td>122.8±3.28</td>
<td>19.7±0.82</td>
<td>10.8±0.39</td>
<td>19.5±1.18</td>
<td>3.0±0.09</td>
<td>33.8±3.14</td>
<td>18.7±1.54</td>
<td>42.3±0.99</td>
</tr>
<tr>
<td>F2 (selected)</td>
<td>127.8±1.16</td>
<td>16.7±2.37</td>
<td>13.2±1.53</td>
<td>35.7±6.69</td>
<td>3.3±0.25</td>
<td>81.4±8.80</td>
<td>42.4±3.25</td>
<td>45.9±2.14</td>
</tr>
<tr>
<td>F3</td>
<td>99.7±2.59</td>
<td>19.1±1.51</td>
<td>8.6±0.38</td>
<td>20.2±1.18</td>
<td>2.9±0.31</td>
<td>43.8±4.91</td>
<td>26.1±2.81</td>
<td>34.3±1.82</td>
</tr>
</tbody>
</table>

The narrow sense heritability ($h^2$) was estimated from parent-offspring regression according to the formula [15]:

$$h^2 = \frac{b}{2\theta}$$

where $b$ is the regression coefficient between parent and offspring and $\theta$ is the coefficient of relatedness between parent and offspring.

The expected direct genetic gain was calculated for all traits using the formula of Falconer [16].

$$R = \frac{i}{2\sigma_p} h^2$$

where $i$ is the selection differential for selection intensity ($i = 1.71$ for 11% selection intensity) and $\sigma_p$ is the phenotypic standard deviation of the trait. The genetic correlations ($r_{g}$) between seed cotton yield and other yield and quality traits were estimated using the covariance method [17].

$$r_{g} = \frac{Cov_{XY}}{\sqrt{\text{Var}X \times \text{Var}Y}}$$

where Var and Cov, respectively, refer to the components of variance and covariance.

The relative effects of direct selection for seed cotton yield component on indirect selection to other traits (CR) were calculated as formula given below [18, 19]:

$$CR = \frac{h_X h_Y r_{g}}{\sigma_Y}$$

where $h_X$ and $h_Y$ are the square root of the heritability of trait X and Y, respectively; $r_{g}$ is the genetic correlation between trait X and Y, and $\sigma_Y$ is the phenotypic standard deviation for trait Y.

### TABLE 1

**Mean values and standard errors for some yield traits in different generations of Carmen × Giza-45 cross**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Plant height</th>
<th>Length of node first fruiting branch</th>
<th>Fruit branches plant</th>
<th>Total bolls plant</th>
<th>Seed cotton weight boll</th>
<th>Seed cotton weight plant</th>
<th>Seed weight plant</th>
<th>Ginning turn out</th>
</tr>
</thead>
</table>
Giza-45 the parent with the highest value. Conversely, the mean values of seed cotton weight per boll, seed cotton yield per plant, seed weight per plant and ginning out turn of F2 population indicated lower values than Giza-45 the parent with the lowest value. These results demonstrate that there are both positive and negative transgressive segregations for these yield components (Table 1). In a previous research, both positive and negative transgressive segregations have been observed for all the yield and yield components, except the boll weight in an interspecific backcross inbred line (BIL) population developed from *G. hirsutum* × *G. barbadense* cross [20].

The fourteen plants having the highest plant yield were selected from the F2 population consisting of 128 single plants (11% selection intensity). The F3 progenies of these 14 plants were grown and same yield and quality traits were observed in individual plants. The mean values of selected single F2 plants with respect to yield traits are given in Table 1. The selected plants showed higher mean values for all traits except the height of node first fruiting branch when compared with the mean values of the F2 generation. However, the mean values of the progenies of these plants (F3 population) showed reduced plant height, the height of node first fruiting branch, fruit branches per plant, seed cotton weight per boll and ginning out turn. Conversely, the mean values of total bolls, seed cotton and seed weight per plant increased in F3 population comparison to F2 generation (Table 1). Despite yielding lower seed cotton weight per boll, an increased seed cotton yield per plant can be explained by the presence of smaller and more bolls per plants in the F3 generation. A similar result in cotton has also been reported by other researchers [21]. Besides, the variations among the mean values of yield traits in F2 and F3 populations could have resulted from homozogyosity of the genes controlling the examined traits. The increases in values of yield traits by the advancement of generation have also been reported in F3 generation of *G. hirsutum* × *G. barbadense* cross [22].

The mean values of fiber quality traits of the parents, F2 (selected) and F3 generations of Carmen × Giza-45 cross are shown in Table 2. The parent Giza-45, used as a male in the crossing, had higher mean values than Carmen for fiber strength, fiber length, uniformity ratio, and fiber elongation. In contrast to these quality traits, the mean values of micronaire and short fiber index were observed as lower in Giza-45 than Carmen. The fiber quality traits of the selected plants possessed the mean values between two parents for micronaire, fiber length, uniformity ratio, and short fiber index. Only the mean values of fiber strength and fiber elongation of selected F2 plants were over the mean values of parents. Similarly, for some yield components, positive transgressive segregations arose for fiber strength and fiber elongation in the F3 population (Table 2). Contrary to our results, negative transgressive segregations have been reported for fiber length, strength, micronaire, and uniformity, while positive transgressive segregation have also been examined for fiber elongation in backcross inbred lines of SG 747 × Giza 75 [20]. However, no transgressive segregation was obtained for micronaire, fiber length and fiber strength in the F2 population of Nazilli-84 × Giza-45 cross (*G. hirsutum* × *G. barbadense*) [23]. The F3 progenies possessed higher values than selected F2 plants in terms of micronaire and short fiber index. The mean values of all fiber quality traits of F3 population had the mean values between two parents (Table 2).

The seed cotton per plant of Carmen × Giza-45 cross was aimed to be increased through selection for higher plant yield in this study. The values of narrow sense heritability, used to estimate the genetic advance and indicate the proportion of additive variance were identified as positive for almost all yield traits except total bolls per plant and ginning out turn (Table 3). In other words, the selection for higher plant yield produced a reversed response for total bolls per plant and ginning out turn [24]. The highest values of narrow sense heritability were obtained for seed weight and seed cotton yield per plant (Table 3). Similar to our results, the narrow sense heritability values of yield and yield components calculated in the F2 generation of *G. hirsutum* × *G. barbadense* cross and all heritability values estimated lower than 0.20 [22]. In our study, for fiber quality traits, the values of narrow sense heritability were positive for micronaire, fiber strength and fiber elongation, negative for other quality traits (Table 4).

### Table 2

<table>
<thead>
<tr>
<th>Generation</th>
<th>Micronaire (g/tex)</th>
<th>Fiber Strength (g/tex)</th>
<th>Fiber Length (mm)</th>
<th>Uniformity Ratio (%)</th>
<th>Short Fiber Index (%)</th>
<th>Fiber Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmen</td>
<td>5.15±0.20</td>
<td>34.20±2.83</td>
<td>29.50±0.52</td>
<td>84.93±0.90</td>
<td>6.58±0.72</td>
<td>5.48±0.49</td>
</tr>
<tr>
<td>Giza-45</td>
<td>4.28±0.16</td>
<td>47.98±4.21</td>
<td>36.28±0.71</td>
<td>88.53±0.29</td>
<td>3.38±0.03</td>
<td>7.88±0.34</td>
</tr>
<tr>
<td>F2 (selected)</td>
<td>4.36±0.15</td>
<td>50.31±1.68</td>
<td>33.71±0.54</td>
<td>87.09±0.38</td>
<td>3.99±0.20</td>
<td>7.90±0.29</td>
</tr>
<tr>
<td>F3</td>
<td>4.39±0.08</td>
<td>37.15±0.26</td>
<td>33.38±0.52</td>
<td>86.91±0.45</td>
<td>4.43±0.30</td>
<td>7.37±0.15</td>
</tr>
</tbody>
</table>
Response to selection (R) for higher plant yield in F2 population occurred 100% in F3 population for seed cotton yield per plant (Table 1 and 3). Moreover, the direct response values for plant height and seed weight per plant were observed relatively higher (Table 3). However, similar high values of direct response could not be obtained for fiber quality properties (Table 2). This observation could be due to the negative correlations between yield and fiber quality traits reported in many investigations [8, 10, 25, 26].

The correlated response values of other yield traits were investigated by selecting genotypes having higher plant yield in the study. When the relations between direct and indirect response values are examined, it was observed that these values are very close to each other for the height of node first fruiting branch, fruit branches per plant and seed cotton weight per boll, however quite different for other yield components (Table 3). Similar to yield components, the direct and indirect response values of fiber quality traits were close to each other (Table 4). The genetic correlations between seed cotton yield per plant and plant height, fruit branches, seed weight, and ginning turn out were higher than genetic correlations between seed cotton per plant and other yield traits. It represented a negative value for the height of node first fruiting branch (Table 3). The highest genetic correlation values were estimated for short fiber index and micronaire among the fiber quality traits. The genetic correlations of seed cotton per plant between fiber strength, fiber length and uniformity ratio revealed negative values (Table 4).

The value of the relative selection efficiency (CR/R) depends on the genetic correlation between the direct and indirect traits and heritability of the traits [18]. The relative selection efficiency showing effects of indirect selection on other yield traits of direct selection for seed cotton yield per plant can be effective for seed weight per plant, fruit branches per plant and plant height (Table 3). In other words, the selection for higher plant yield leads to an increase in values of these three traits. Especially, increased values of seed weight and fruit branches per plant should be the reason for the increase in yield. It was emphasized that it might be more profitable to practice direct selection for seed cotton yield compared to selecting for seed cotton yield through any of the other traits [27]. In fiber quality traits, the relative selection efficiency of seed cotton yield per plant was higher on fiber length and uniformity ratio than other quality traits (Table 4). Fiber length is considered one of the most important property of cotton in marketing and yarn processing [28]. Therefore, an increase in the most important quality criteria with

### TABLE 3
Estimation of the genetic parameters for examined yield components

<table>
<thead>
<tr>
<th>Variables</th>
<th>Plant height</th>
<th>Length of node first fruiting branch</th>
<th>Fruit branches plant⁻¹</th>
<th>Total bolls plant⁻¹</th>
<th>Seed cotton weight boll⁻¹</th>
<th>Seed cotton plant⁻¹</th>
<th>Seed weight plant⁻¹</th>
<th>Ginning turn out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrow-sense heritability (h²)</td>
<td>0.071</td>
<td>0.015</td>
<td>0.042</td>
<td>-0.033</td>
<td>0.003</td>
<td>0.116</td>
<td>0.199</td>
<td>-0.147</td>
</tr>
<tr>
<td>Response to selection (R)</td>
<td>4.486</td>
<td>0.245</td>
<td>0.313</td>
<td>-0.760</td>
<td>0.005</td>
<td>7.038</td>
<td>5.911</td>
<td>-2.808</td>
</tr>
<tr>
<td>Correlated response (CR)</td>
<td>1.359</td>
<td>-0.100</td>
<td>0.092</td>
<td>-5.371</td>
<td>0.001</td>
<td>-</td>
<td>1.634</td>
<td>-6.608</td>
</tr>
<tr>
<td>Genetic correlation (rG)</td>
<td>0.338</td>
<td>-0.268</td>
<td>0.284</td>
<td>0.123</td>
<td>0.031</td>
<td>-</td>
<td>0.886</td>
<td>0.643</td>
</tr>
<tr>
<td>Relative selection efficiency (CR/R)</td>
<td>0.433</td>
<td>-0.737</td>
<td>0.474</td>
<td>-0.008</td>
<td>0.191</td>
<td>-</td>
<td>0.676</td>
<td>-0.058</td>
</tr>
</tbody>
</table>

### TABLE 4
Estimation of the genetic parameters for examined fiber quality components

<table>
<thead>
<tr>
<th>Variables</th>
<th>Micronaire</th>
<th>Fiber Strength (g/tex)</th>
<th>Fiber Length (mm)</th>
<th>Uniformity Ratio (%)</th>
<th>Short Fiber Index (%)</th>
<th>Fiber Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrow-sense heritability (h²)</td>
<td>0.151</td>
<td>0.039</td>
<td>-0.041</td>
<td>-0.039</td>
<td>-0.026</td>
<td>0.046</td>
</tr>
<tr>
<td>Response to selection (R)</td>
<td>0.143</td>
<td>0.425</td>
<td>-0.143</td>
<td>-0.096</td>
<td>-0.033</td>
<td>0.086</td>
</tr>
<tr>
<td>Correlated response (CR)</td>
<td>0.021</td>
<td>-0.020</td>
<td>0.044</td>
<td>0.032</td>
<td>-0.023</td>
<td>0.012</td>
</tr>
<tr>
<td>Genetic correlation (rG)</td>
<td>0.287</td>
<td>-0.047</td>
<td>-0.318</td>
<td>-0.330</td>
<td>0.581</td>
<td>0.155</td>
</tr>
<tr>
<td>Relative selection efficiency (CR/R)</td>
<td>0.251</td>
<td>-0.081</td>
<td>0.536</td>
<td>0.561</td>
<td>-1.228</td>
<td>0.247</td>
</tr>
</tbody>
</table>
the increase in the yield indicates the success of the selection.

The first and crucial criterion of evaluating a population is to constitute a great of genetic variation for the trait of interest in plant breeding. In cotton breeding, G. hirsutum x G. barbadense crosses and their segregation populations are commonly preferred to improve yield and fiber quality traits [20, 22, 23, 29]. In this study, we utilized the F2 population of the crosses of Carmen (G. hirsutum L.) and Giza-45 (G. barbadense L.) varieties to select high yielding plants. The transgressive segregations were identified for some of the yield and quality traits in yielding plants. The transgressive segregations were found to be effective for seed weight per plant, fruit branches per plant, plant height, fiber length and uniformity ratio as well. In conclusion, the effective direct selection for higher plant yield resulted in positive changes fiber quality traits as well as yield traits.

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RELATIONSHIP BETWEEN PLANT STEROLS (B-SITOSITEROL, CAMPESTEROL, STIGMASTEROL) AND NUTRIENTS OF BREAD WHEAT CULTIVARS

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2Gaziosmanpasa University, Faculty of Agriculture, Soil Science and Plant Nutrition Department, Tokat, Turkey
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ABSTRACT

Plant sterols are a group of steroid alcohols with various bioactive characteristics for human health. Phytosterols inhibit cholesterol absorption in intestines and thus reduce blood cholesterol levels. In present study, grain Campesterol, Stigmasterol and Betasitosterol concentrations of 20 different bread wheat cultivars were determined and the relationships between phytosterols and grain mineral nutrients were identified. Correlations analysis revealed that Campesterol had significant positive correlations with nitrogen (r=0.356 **) and manganese (r=0.327) and significant negative correlations with calcium (r= -0.432 **) and zinc (r= -0.424 **). Stigmasterol had significant negative correlations with calcium (r= -0.291*) and zinc (r= -0.466**) and significant positive correlations with manganese (0.256*). Betasitosterol had significant positive correlations with nitrogen (0.342**) and manganese (0.323*) and significant negative correlations with calcium (-0.387**), zinc (-0.468**) and phosphorus (-0.284**). Principle component analysis (PCA) for grain mineral nutrient elements, Campesterol, Stigmasterol and Betasitosterol concentrations revealed that there were 3 traits (Campesterol, Betasitosterol and nitrogen) under PC1 with a high eigenvalue (>0.70). While phosphorus and zinc had high eigenvalue s under PC2, there were not any traits under PC3 with a high eigenvalue. Only boron had a high eigenvalue under PC4.

KEYWORDS:
B-Sitositerol, Campesterol, Stigmasterol, macronutrients, micronutrients, wheat

INTRODUCTION

Nutrients supply the substances required for basic metabolic functions of the organisms. Besides providing macro and micro nutrients required metabolic activities, nutrients also contain various compounds with positive impacts on human health [1]. It was scientifically proved that some nutrients are naturally effective in prevention and treatment of some diseases. Therefore, nutritional support in health preservation has become a significant issue. The carotenoids, antioxidant vitamins, phenolic compounds, terpenoids, sterols, indols and fibers of plants play important roles in prevention of health risks [2]. Plant sterols are also called as phytosterols. Phytosterols are steroid alcohols of triterpene family. Their structure is quite similar with cholesterol, but they include extra ethyl or methyl group and double bond in side chain [3]. Phytosterols naturally exist in plant products, especially in vegetable oils. They have cholesterol-lowering effect in humans. They are also anti-inflammatory, anti-atherogenic, antioxidant and anti-carcinogenic impacts, in brief they have several positive health impact [4]. In recent studies, Daily 2-3 g phytosterol consumption was recommended to keep cholesterol-lowering impact at upper most level [5]. Such phytosterol consumption levels is possible only with the foodstuff enriched in phytosterols. Mathur [6] indicated that plant sterols were not synthesized in animal and human bodies. Therefore, plant sterols are significant agricultural products used in health and catering industry [7]. Cereals and grain products contain less amount of phytosterols than oil seeds, but they are more placed in diets than oil crops. It was reported in a study carried out in Holland that humans get 37% of phytosterols from cereals and 26% from oils of a Daily diet [8]. Wheat, maize and paddy are the most common cereals used in daily diets [9]. Of these cereals, wheat is the most common one placed in human nutrition worldwide [10]. The primary sterols of wheat were reported as Betasitosterol, Campesterol and Stigmasterol [11]. This study was conducted to investigate the relationships of Campesterol, Stigmasterol and Betasitosterol concentrations with mineral nutrients in grains of 20 different bread wheat cultivars.
TABLE 1
Bread wheat cultivars (Triticum aestivum L.) of the study [12]

<table>
<thead>
<tr>
<th>Name</th>
<th>Harvest Year</th>
<th>Harvest (kg/da)</th>
<th>Recommend Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adana 99</td>
<td>2015</td>
<td>665-735</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Altınbaşak</td>
<td>2015</td>
<td>703-941</td>
<td>Coastal Sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Bayraktar</td>
<td>2015</td>
<td>300-400</td>
<td>Central Anatolia</td>
</tr>
<tr>
<td>Bezostaja</td>
<td>2015</td>
<td>200-650</td>
<td>Central Anatolia</td>
</tr>
<tr>
<td>Canlık 2003</td>
<td>2015</td>
<td>250-850</td>
<td>Black Sea Coast and North of Marmara Region</td>
</tr>
<tr>
<td>Es 26</td>
<td>2015-2016</td>
<td>410-550</td>
<td>Coastal sections and Northeastern Anatolia</td>
</tr>
<tr>
<td>Flamura 85</td>
<td>2015</td>
<td>350-600</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Gökkan</td>
<td>2015</td>
<td>699-955</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Harmankaya</td>
<td>2015</td>
<td>700-750</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Karatoprak</td>
<td>2015</td>
<td>665-865</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Kır aç 66</td>
<td>2015</td>
<td>300-500</td>
<td>Central and Eastern Anatolia</td>
</tr>
<tr>
<td>Mesut</td>
<td>2015</td>
<td>240-818</td>
<td>Central Anatolia</td>
</tr>
<tr>
<td>Osmaniye m</td>
<td>2015</td>
<td>632-846</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Pandas</td>
<td>2015</td>
<td>533-709</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Sakın</td>
<td>2015</td>
<td>600-750</td>
<td>Black Sea coast and South of Marmara region</td>
</tr>
<tr>
<td>Seri</td>
<td>2015</td>
<td>715-1023</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Soyer</td>
<td>2015</td>
<td>240-560</td>
<td>Coastal sections and Southeastern Anatolia</td>
</tr>
<tr>
<td>Sönmez</td>
<td>2015</td>
<td>250-600</td>
<td>Central and Eastern Anatolia</td>
</tr>
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<td>Tahiroya</td>
<td>2015</td>
<td>400-900</td>
<td>Marmara coastal sections</td>
</tr>
<tr>
<td>Yunus</td>
<td>2015</td>
<td>550-820</td>
<td>Central Anatolia</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Material. As the plant material of the study, 20 different bread wheat cultivars commonly grown in different regions of Turkey were used. The cultivars and their common regions are provided in Table 1 [12].

Methods. Mineral element analyses. Grain samples were subjected to wet-digestion with H2O2-HNO3 acid mixture in a microwave oven. Then, P, K, S, Mg, Ca, Fe, Zn, Mn, Cu and B concentrations were determined in an ICP-OES (Varian Vista) device [13]. N analysis of grain samples were performed with Kjeldahl distillation method [14].

Phytosterol (Campesterol, Stigmasterol ve Betasitosterol) analyses. Phytosterol composition (Campesterol, Stigmasterol and Betasitosterol) of grain samples were analyzed with gas chromatography–mass spectrometry (GC-MS) device in accordance with “994.10” method of Association of Official Analytical Chemists Official [15]. Grain samples were ground and packed in 25 g polyethylene bags. Samples were kept dark at -20°C until the analyses. Alkaline hydrolysis method was used for oil extraction from ground grain samples [16]. Phytosterol extraction from bread wheat cultivars was performed as specified in Toivo, Lampi [17] with slight modifications. Ground 1 g grain sample was supplemented with standard 5α-cholestan-3β-ol (200 μg) and then with 40 ml ethanol and 10 ml aq-KOH (500 g/l) and saponified in a reflux. Non-saponified portion was extracted in two phases (twice with 20 ml diethyl ether in the first phase and again twice with 20 ml n-hexane in the second phase). Following the compound of organic portion (hexane and diethyl ether), samples were treated with 20 ml 5% NaCl three times. The organic portion was dried with Na2SO4 and then dried with a rotary evaporator at 45°C. Concentrated portion was dissolved in 10 ml chloroform and filtered through 0.48 μm PTFE (polytetrafluoroethylene) syringe filter and transferred to 950 μL vials. The samples were supplemented with 50 μL BSTFA (containing 1% TMCS). In this way, final concentration was completed to 1 ml. The mixture was vortexed for a minute, incubated at 60°C for 60 minutes, cooled down and analyzed in GC-MS [18, 19]. Sterol (Campesterol, Stigmasterol and Betasitosterol) analyses were performed in a gas chromatography-mass spectrometer device. Real-time analysis software was used in all analyses. Capillary column (5% diphenyl- 95% dimethylpolysiloxane) 60 m × 0.25 mm i.d.×0.25 μm was used in analyses. Solvent cutoff time was 16.5 minutes and total analysis duration was 25 minutes. Then, Selected Ion Mode (SIM) was used. Peaks were defined through comparison of standard retention times and mass spectrum fragments.

Statistical Analyses. Correlation tests were carried out to determine the levels of relationships between phytosterols and the other nutrients. Arithmetic mean, standard deviation and variation coefficients were calculated for each traits and normality tests were performed. Correlations among plant traits, the nutrients with the greatest impacts on plant sterols and the level of these impacts was determined. Differences in treatment means were assessed with Duncan’s multiple range test. Statistical analyses were performed with SPSS 21 statistical software was used.
RESULTS AND DISCUSSION

Mineral element concentration of bread wheat cultivars. Grain macro (N, P, K, S, Mg and Ca) nutrient concentrations of 20 different bread cultivars are provided in Table 2 and 3. Average N concentration of bread wheat cultivars was 2.07% with the lowest value in Bayraktar (1.49%) and the greatest value in Mesut (2.545) cultivar. N concentration of the present genotypes were higher than deficiency limit value of 0.13% specified by Reuter and Robinson [20] for wheat grains. Average sulphur (S) concentration of the cultivars was 0.16% with the lowest value in Bayraktar (0.11%) and the greatest value in Harmankaya (0.19%) cultivar (Table 2). S concentrations of the present cultivars were higher than the deficiency limit value of 0.12% specified by Kalo and Kuuranne [16] for wheat grains. Zhao, Hawkesford [22] carried out a survey study in 1992 and 1993 with 793 different wheat grains and reported average S concentration as 0.13%. Present S concentrations comply with those earlier ones. Average magnesium (Mg) concentration of bread wheat cultivars was 0.13% and calcium (Ca) concentration was 0.05%. These values were higher than the critical value 0.1% for magnesium and 0.03% for calcium specified by Reuter and Robinson [20] (Table 2).

Grain micro (Fe, Zn, Mn, Cu and B) nutrient concentrations (mg kg⁻¹) of 20 different bread cultivars are provided in Table 3. Average iron (Fe) concentration of the cultivars was 33.8 mg kg⁻¹ with the lowest value in Bayraktar (21.9 mg kg⁻¹) and the greatest value in Flamura 85 (45.5 mg kg⁻¹) cultivars. Average zinc (Zn) concentration of the cultivars was 22.5 mg kg⁻¹ with the lowest value in Kırcaş (11.7 mg kg⁻¹) and the greatest value in Bezostaja (45.5 mg kg⁻¹) cultivars.

CIMMYT (International Maize and Wheat Improvement Center) used 66 wheat genotypes (summer and winter) in Central Asia breeding programs and reported grain Fe concentrations as between 25 - 56 mg kg⁻¹ (with an average value of 38 mg kg⁻¹), Zn concentrations as between 20 - 39 mg kg⁻¹ (with an average value of 28 mg kg⁻¹). Present findings on Fe and Zn concentrations were lower than those previous ones, probably because of different locations of the experiments [23]. In another study carries out in Turkey, Zn concentrations of

### TABLE 2

Grain macro element (N, P, K, S, Mg and Ca) concentrations of bread wheat cultivars (%)

<table>
<thead>
<tr>
<th>CULTIVARS</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>S</th>
<th>Mg</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmaniyem</td>
<td>1.97±0.16</td>
<td>0.37±0.03</td>
<td>0.43±0.04</td>
<td>0.18±0.00</td>
<td>0.15±0.01</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Bayraktar</td>
<td>1.49±0.07</td>
<td>0.28±0.01</td>
<td>0.41±0.01</td>
<td>0.11±0.01</td>
<td>0.13±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Gökhan</td>
<td>2.12±0.03</td>
<td>0.32±0.02</td>
<td>0.45±0.01</td>
<td>0.15±0.01</td>
<td>0.13±0.00</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>Mesut</td>
<td>2.54±0.06</td>
<td>0.27±0.02</td>
<td>0.38±0.00</td>
<td>0.18±0.00</td>
<td>0.12±0.01</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Kırcaş</td>
<td>2.50±0.23</td>
<td>0.24±0.02</td>
<td>0.44±0.00</td>
<td>0.18±0.02</td>
<td>0.12±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Harmankaya</td>
<td>2.00±0.08</td>
<td>0.32±0.03</td>
<td>0.41±0.02</td>
<td>0.19±0.00</td>
<td>0.13±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Panda</td>
<td>2.11±0.08</td>
<td>0.31±0.02</td>
<td>0.42±0.00</td>
<td>0.15±0.01</td>
<td>0.12±0.01</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Es 26</td>
<td>1.81±0.03</td>
<td>0.36±0.06</td>
<td>0.46±0.05</td>
<td>0.15±0.01</td>
<td>0.15±0.01</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Adana 99</td>
<td>1.88±0.08</td>
<td>0.30±0.01</td>
<td>0.40±0.02</td>
<td>0.14±0.01</td>
<td>0.12±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Sönmez</td>
<td>1.94±0.03</td>
<td>0.36±0.01</td>
<td>0.41±0.00</td>
<td>0.15±0.01</td>
<td>0.14±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Seri 2013</td>
<td>2.09±0.04</td>
<td>0.29±0.00</td>
<td>0.39±0.01</td>
<td>0.16±0.00</td>
<td>0.13±0.00</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>Sakin</td>
<td>2.08±0.02</td>
<td>0.32±0.04</td>
<td>0.40±0.01</td>
<td>0.15±0.02</td>
<td>0.12±0.01</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Flamura 85</td>
<td>2.36±0.26</td>
<td>0.33±0.01</td>
<td>0.38±0.00</td>
<td>0.16±0.01</td>
<td>0.12±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Soyer</td>
<td>1.78±0.02</td>
<td>0.34±0.01</td>
<td>0.46±0.02</td>
<td>0.13±0.02</td>
<td>0.13±0.01</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Tahirova</td>
<td>2.17±0.16</td>
<td>0.32±0.01</td>
<td>0.40±0.01</td>
<td>0.16±0.00</td>
<td>0.11±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Bezostaja</td>
<td>2.21±0.03</td>
<td>0.40±0.02</td>
<td>0.45±0.00</td>
<td>0.18±0.00</td>
<td>0.14±0.01</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Yunus</td>
<td>2.28±0.07</td>
<td>0.33±0.02</td>
<td>0.42±0.03</td>
<td>0.17±0.00</td>
<td>0.15±0.01</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Karatoprak</td>
<td>2.23±0.16</td>
<td>0.29±0.02</td>
<td>0.40±0.02</td>
<td>0.16±0.01</td>
<td>0.12±0.01</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Canik 2003</td>
<td>1.76±0.02</td>
<td>0.33±0.02</td>
<td>0.42±0.02</td>
<td>0.15±0.00</td>
<td>0.11±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Altınbaşak</td>
<td>2.07±0.05</td>
<td>0.32±0.02</td>
<td>0.41±0.01</td>
<td>0.14±0.01</td>
<td>0.12±0.01</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Min.</td>
<td>1.49</td>
<td>0.24</td>
<td>0.38</td>
<td>0.11</td>
<td>0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>Max.</td>
<td>2.54</td>
<td>0.40</td>
<td>0.46</td>
<td>0.19</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean</td>
<td>2.07</td>
<td>0.32</td>
<td>0.42</td>
<td>0.16</td>
<td>0.13</td>
<td>0.05</td>
</tr>
</tbody>
</table>
wheat grains were reported as between 20-30 mg kg\(^{-1}\) for soils with sufficient Zn levels and as between 5-12 mg kg\(^{-1}\) for soils with deficit Zn conditions [24]. Average manganese [14] concentration of the cultivars was 40.8 mg kg\(^{-1}\), copper (Cu) concentration was 5.54 mg kg\(^{-1}\) and boron (B) concentration was 2.05 mg kg\(^{-1}\) (Table 3). These values were above the limit values of 20 mg kg\(^{-1}\) for Mn, 2.5 mg kg\(^{-1}\) for Cu and 2.0 mg kg\(^{-1}\) B specified by Reuter [20]. Grain macro and micro nutrient levels of 20 different bread wheat cultivars were sufficient in some cultivars and insufficient in some others. Such variations in nutrient concentrations were resulted from cultivars, environmental, soil and climate conditions and cultural practices [25, 26].

**TABLE 3**

<table>
<thead>
<tr>
<th>Grain micro element (Fe, Zn, Mn, Cu and B) concentrations (mg kg(^{-1})) of bread wheat cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td>CULTIVARS</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Osmaniyem</td>
</tr>
<tr>
<td>Bayraktar</td>
</tr>
<tr>
<td>Gökkan</td>
</tr>
<tr>
<td>Mesut</td>
</tr>
<tr>
<td>Kiraç</td>
</tr>
<tr>
<td>Harmankaya</td>
</tr>
<tr>
<td>Panda</td>
</tr>
<tr>
<td>Es 26</td>
</tr>
<tr>
<td>Adana 99</td>
</tr>
<tr>
<td>Sömmez</td>
</tr>
<tr>
<td>Seri 2013</td>
</tr>
<tr>
<td>Sakin</td>
</tr>
<tr>
<td>Flamura 85</td>
</tr>
<tr>
<td>Soyer</td>
</tr>
<tr>
<td>Tahirova</td>
</tr>
<tr>
<td>Bezostaja</td>
</tr>
<tr>
<td>Yunus</td>
</tr>
<tr>
<td>Karatopراك</td>
</tr>
<tr>
<td>Canik 2003</td>
</tr>
<tr>
<td>Altunbaşak</td>
</tr>
<tr>
<td>Min.</td>
</tr>
<tr>
<td>Max.</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>

**TABLE 4**

Grain Campesterol, Stigmasterol and Betasitosterol concentrations of bread wheat cultivars (SIM, mg kg\(^{-1}\))

<table>
<thead>
<tr>
<th>CULTIVARS</th>
<th>Campesterol (SIM, mg kg(^{-1}))</th>
<th>Stigmasterol (SIM, mg kg(^{-1}))</th>
<th>Betasitosterol (SIM, mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmaniyem</td>
<td>17.5±0.05</td>
<td>4.6±0.06</td>
<td>304.8±0.49</td>
</tr>
<tr>
<td>Bayraktar</td>
<td>18.2±0.03</td>
<td>8.5±0.08</td>
<td>335.6±0.15</td>
</tr>
<tr>
<td>Gökkan</td>
<td>28.0±0.09</td>
<td>9.4±0.03</td>
<td>409.9±2.07</td>
</tr>
<tr>
<td>Mesut</td>
<td>49.9±0.44</td>
<td>14.1±0.05</td>
<td>590.5±1.33</td>
</tr>
<tr>
<td>Kiraç</td>
<td>36.8±0.08</td>
<td>8.7±0.07</td>
<td>552.0±3.99</td>
</tr>
<tr>
<td>Harmankaya</td>
<td>27.5±0.16</td>
<td>8.6±0.02</td>
<td>451.6±0.88</td>
</tr>
<tr>
<td>Panda's</td>
<td>35.8±0.18</td>
<td>7.5±0.08</td>
<td>362.5±2.59</td>
</tr>
<tr>
<td>Es 26</td>
<td>38.5±0.34</td>
<td>10.1±0.09</td>
<td>422.4±1.59</td>
</tr>
<tr>
<td>Adana 99</td>
<td>22.8±0.53</td>
<td>9.5±0.04</td>
<td>582.3±1.04</td>
</tr>
<tr>
<td>Sömmez</td>
<td>20.0±0.25</td>
<td>6.2±0.11</td>
<td>387.8±5.10</td>
</tr>
<tr>
<td>Seri 2013</td>
<td>20.8±0.07</td>
<td>7.5±0.17</td>
<td>354.5±3.63</td>
</tr>
<tr>
<td>Sakin</td>
<td>50.8±2.24</td>
<td>14.7±0.13</td>
<td>613.4±0.83</td>
</tr>
<tr>
<td>Flamura 85</td>
<td>23.8±0.16</td>
<td>5.5±0.12</td>
<td>379.8±1.56</td>
</tr>
<tr>
<td>Soyer</td>
<td>36.5±0.26</td>
<td>9.5±0.15</td>
<td>493.8±1.13</td>
</tr>
<tr>
<td>Tahirova</td>
<td>34.8±0.12</td>
<td>7.6±0.09</td>
<td>510.1±2.02</td>
</tr>
<tr>
<td>Bezostaja</td>
<td>25.1±0.18</td>
<td>5.4±0.09</td>
<td>359.5±0.16</td>
</tr>
<tr>
<td>Yunus</td>
<td>75.3±0.68</td>
<td>23.1±0.14</td>
<td>681.8±1.71</td>
</tr>
<tr>
<td>Karatopрак</td>
<td>17.0±0.57</td>
<td>4.9±0.19</td>
<td>347.7±1.00</td>
</tr>
<tr>
<td>Canik 2003</td>
<td>29.0±0.25</td>
<td>9.9±0.02</td>
<td>395.4±1.05</td>
</tr>
<tr>
<td>Altunbaşak</td>
<td>23.5±0.09</td>
<td>6.7±0.05</td>
<td>383.2±1.43</td>
</tr>
<tr>
<td>Min.</td>
<td>17.0</td>
<td>4.6</td>
<td>304.8</td>
</tr>
<tr>
<td>Max.</td>
<td>75.3</td>
<td>23.1</td>
<td>681.8</td>
</tr>
<tr>
<td>Mean</td>
<td>31.5</td>
<td>9.1</td>
<td>420.9</td>
</tr>
</tbody>
</table>
and the greatest value in Yunus (23.1 mg kg$^{-1}$). Grain average Betasitosterol concentration of the cultivars was 420.9 mg kg$^{-1}$ with the lowest value in Osmaniyem (304.8 mg kg$^{-1}$) and the greatest value in Yunus (681.8 mg kg$^{-1}$) cultivars. Among 20 different bread cultivars, the greatest plant sterol concentrations were observed in Yunus and the least sterol concentrations were observed in Osmaniyem cultivars (Table 4). Wheat is the greatest source of nutrient worldwide. It was reported in previous studies that about 763 - 818 $\mu$g g$^{-1}$ of grain dry matter was constituted by phytosterols [27, 28]. Of the phytosterols of wheat grains (Campesterol, Stigmasterol, Sitostanol, Betasitosterol and etc.), Betasitosterol constitutes about 51-54% of total phytosterols. [10]. Previous researchers working on barley, rye and wheat indicated that genetic factors had great impacts on phytosterol composition and concentration of the grains [27, 28]. In another field study carried out with 24 different wheat genotypes at different years and locations, phytosterol concentrations of the genotypes were reported as between 700 - 928 $\mu$g g$^{-1}$ and researchers indicated the reason of such variations as the locations (environment) and thus reported insignificant effects of the years on phytosterol concentrations [29].

Correlations between Betasitosterol, Campesterol and Stigmasterol concentrations and mineral element concentrations of bread wheat cultivars. The correlations between Campesterol, Stigmasterol and Betasitosterol concentrations and mineral nutrient concentrations of wheat cultivars are provided in Table 5. Campesterol concentrations had significant positive correlations with N (p<0.01) and Mn (p<0.05) concentrations of the grains. Campesterol concentrations had significant negative correlations with Ca and Zn concentrations (p<0.01). Stigmasterol concentrations had significant negative correlations with Ca and Zn concentrations of the grains (p<0.01). Stigmasterol concentrations had positive significant correlations with N (p<0.01) and Mn (p<0.05) concentrations of the grains. Betasitosterol concentrations had positive significant correlations with N (p<0.01) and Mn (p<0.05) concentrations of the grains and significant negative correlations with Ca (p<0.01), Zn (p<0.01) and P (p<0.05) concentrations of the grains (Table 5).

### TABLE 5
Correlations between Campesterol, Stigmasterol and Betasitosterol concentrations and mineral nutrient concentrations of wheat cultivars

<table>
<thead>
<tr>
<th></th>
<th>Campesterol</th>
<th>Stigmasterol</th>
<th>$\beta$-Sitosterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol</td>
<td>1</td>
<td>0.918**</td>
<td>0.813**</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0.918**</td>
<td>1</td>
<td>0.356*</td>
</tr>
<tr>
<td>$\beta$-Sitosterol</td>
<td>0.813**</td>
<td>0.356*</td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>0.356*</td>
<td>-0.079</td>
<td>0.062</td>
</tr>
<tr>
<td>P</td>
<td>-0.079</td>
<td>-0.157</td>
<td>-0.229</td>
</tr>
<tr>
<td>K</td>
<td>0.062</td>
<td>0.025</td>
<td>0.229</td>
</tr>
<tr>
<td>S</td>
<td>0.229</td>
<td>0.074</td>
<td>0.153</td>
</tr>
<tr>
<td>Mg</td>
<td>0.153</td>
<td>0.164</td>
<td>0.221</td>
</tr>
<tr>
<td>Ca</td>
<td>-0.432**</td>
<td>0.0804</td>
<td>0.221</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.424**</td>
<td>0.066**</td>
<td>0.221</td>
</tr>
<tr>
<td>Zn</td>
<td>0.327*</td>
<td>0.256*</td>
<td>0.021</td>
</tr>
<tr>
<td>Mn</td>
<td>0.217</td>
<td>0.146</td>
<td>0.217</td>
</tr>
<tr>
<td>Cu</td>
<td>-0.048</td>
<td>-0.046</td>
<td>0.160</td>
</tr>
</tbody>
</table>

Significant at **: p<0.01; *: p<0.05

### TABLE 6
Principle component analysis (PCA) for investigated traits of bread wheat cultivars

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>4.087</td>
<td>3.0319</td>
<td>2.3861</td>
<td>1.3682</td>
</tr>
<tr>
<td>Diversity (%)</td>
<td>29.1928</td>
<td>21.6568</td>
<td>17.0433</td>
<td>9.7731</td>
</tr>
<tr>
<td>Cumulative Variance</td>
<td>29.1928</td>
<td>50.8496</td>
<td>67.8929</td>
<td>77.6659</td>
</tr>
<tr>
<td>Campesterol</td>
<td>0.7846*</td>
<td>-0.2243</td>
<td>-0.4611</td>
<td>-0.2632</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0.7846*</td>
<td>0.3239</td>
<td>-0.5291</td>
<td>-0.2197</td>
</tr>
<tr>
<td>N</td>
<td>-0.1488</td>
<td>0.5755*</td>
<td>-0.1806</td>
<td>-0.5376</td>
</tr>
<tr>
<td>P</td>
<td>-0.2343</td>
<td>0.4779</td>
<td>-0.5833</td>
<td>-0.1275</td>
</tr>
<tr>
<td>K</td>
<td>0.6751</td>
<td>0.4644</td>
<td>0.2563</td>
<td>0.3287</td>
</tr>
<tr>
<td>Mg</td>
<td>0.0496</td>
<td>0.6684</td>
<td>-0.5744</td>
<td>0.0180</td>
</tr>
<tr>
<td>Ca</td>
<td>-0.4130</td>
<td>0.0964</td>
<td>0.2283</td>
<td>0.0494</td>
</tr>
<tr>
<td>Fe</td>
<td>0.5459</td>
<td>0.2222</td>
<td>0.6105</td>
<td>-0.4009</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.3172</td>
<td>0.7274</td>
<td>0.1673</td>
<td>0.0189</td>
</tr>
<tr>
<td>Mn</td>
<td>0.6103</td>
<td>0.5121</td>
<td>-0.1525</td>
<td>0.1424</td>
</tr>
<tr>
<td>Cu</td>
<td>0.6629</td>
<td>0.4879</td>
<td>0.3851</td>
<td>0.1100</td>
</tr>
<tr>
<td>B</td>
<td>0.0733</td>
<td>0.4240</td>
<td>-0.3499</td>
<td>0.7400</td>
</tr>
</tbody>
</table>

*: underlined eigenvalues were used as >0.70.
Principle component analysis (PCA) was performed for mineral element concentrations and Campesterol, Stigmasterol and Betasitosterol concentrations of the wheat grains (Table 6). As it was seen in Table 6, 4 principle components (PC1, PC2, PC3 and PC4) with an eigenvalue over 0.70 were obtained. These 4 principle components were able to explain 77.67% of total variation. Three traits (Campesterol, Betasitosterol and Nitrogen) had high eigenvalue (>0.70) under PC 1. While phosphorus and zinc had high eigenvalue under PC2, traits did not have high eigenvalue under PC3. Only boron had high eigenvalue under PC4 (Table 6, Figure 1).

Both the correlation analysis (Table 5) and principle component analysis (Table 6) revealed significant positive relationships between Campesterol, Stigmasterol, Betasitosterol and nitrogen. The greatest sterol quantities were observed in Yunus and the least sterol quantities were observed in Osmaniyem cultivars. Present findings indicated a strong relationship between plant sterols and nitrogen. Gul, Egesel [30], investigated the variations in phytosterol concentrations of 19 different canola (Brassicanapus L.) genotypes under two different nitrogen doses (0 and 130 kg N/ha). Researchers reported that Campesterol concentration of 1292 mg kg\(^{-1}\) in control treatments (N 0) decreased to 1246 mg kg\(^{-1}\) in 130 kg N/ha treatment; Stigmasterol concentration of 4.62 mg kg\(^{-1}\) in N0 treatments increased to 4.97 mg kg\(^{-1}\) in N130 treatments; Sitosterol concentration of 1769 mg kg\(^{-1}\) in N0 treatments decreased to 1495 mg kg\(^{-1}\) in N130 treatments. Pavlik et al. (2010) investigated the effects of different nitrogen doses (2 gr N/pot (control); 4 g N/pot) applied in different forms (ammonium nitrate, urea ammonium nitrate) on β-Sitosterol concentrations of maize kernels. Researchers reported as compared to ammonium nitrate treatments that urea ammonium nitrate treatments increased β-Sitosterol concentrations by 94%. In another study, green herbage phytosterol, protein, phenol, alkaloid, total sugar, total P, Ca, Na, K and Fe concentrations of common mallow (Corchorus depressus) plants collected from five different environments were determined and the correlations among these traits were investigated with principle component analysis (PCA). Researchers obtained 4 principle components (PC1, PC2, PC3 and PC4) with an eigenvalue of greater than 1. There was a high eigenvalue among phytosterol, protein (nitrogen), phenol and plant K concentration under PC1. Therefore, they indicated a correlation among the traits gathered under PC1 (phytosterol, protein nitrogen, phenol and plant K concentration) [6].

![Factor loads of principle component analysis](image)
ACKNOWLEDGEMENTS

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PACKED WATER CONSUMPTION PREFERENCES OF URBAN RESIDENTS AND FACTORS AFFECTING BOTTLED WATER MARKET: AN EXAMPLE FROM TURKEY

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ABSTRACT

Since 1990s, packed water demand is rising and is preferred instead of tap water by the consumers in the world. Modern marketing requires understanding and identifying consumer needs and to meet these needs with a higher level of satisfaction than the competitor. Public and private companies have been operating in the water market and the sector demonstrates a rapid development in Turkey as well as in the world. Accordingly, the determination and marketing of consumer-oriented products is important. This study is focused on the plastic carboy bottled water - demijohn (19 litres) sector which constitutes an important segment of the packed water market. In this context, a field survey was conducted in the Mediterranean region of Turkey to determine optimum product patterns providing the highest benefit to the demijohn packed water consumers. Conjoint analysis was to determine the optimum product set. Price, origin of the water, HACCP certification, production mode of water (natural or mineral water) and taste (soft or hard) were found out as affecting factors that maximize consumer benefits in demijohn packed water preferences respectively. The recent findings of the surveys conducted in the world demonstrated significance of price sensitivity of consumers and quality expectations from packed water as well. This similarity indicated that the marketing strategies of the market and demijohn segment should focus on cost reduction and product differentiation with regards to taste and meeting quality standards. Product differentiation should consider local brand development or improvement of existing brands.

KEYWORDS:
Consumer preferences, conjoint analysis, demijohn bottled water, Turkey.

INTRODUCTION

Urbanisation phenomenon has been bringing many problems to especially metropolitan cities all over the world. This situation is definitely valid for Turkey as well. Significant problems had emerged regarding the water market are adequate water supply and tap water safety in this urbanisation process. After 1990s, intensive immigration of rural population to metropolitan cities has improved the development of packaged water market accordingly in Turkey. As a substitute to tap water, there is a rapid increase in demijohn bottled water demand.

Turkey packed water market is composed of almost 300 brands and 40-50 of these brands are regional that are provided to the market during summer time [1]. Packed water market demonstrates a growing trend in Turkey. Due to high average temperatures and decreasing confidence to tap water, the value of demijohn and carboy plastic bottle water markets, which was 600 million Dollars in 2003, had reached to 1.2 billion Dollars in 2006. Annual return of this market had reached to 2 billion dollars by the end of 2008. According to this, water market in Turkey has grown by 233 % between these five years. In accordance with the growth in water market, packaged water consumption per capita has been increasing in Turkey as well. While it was 81 litres in 2003, it rose to 128 litres in 2010 recorded as 139 litres for 2015 [1]. 79 litres of these 139 litres were consumed as demijohn bottled water. Domestically fast growing water market has improved in exports market as well. While in 2001 approximately 4 % of bottled water was exported, in 2014 this ratio had become more than 15 % and had reached to 42.2 million Dollars export volume in 2015 [1].

Packaged water sector constitutes a big share in Turkey’s non-alcoholic beverages market. Among these beverages, packed (bottled) water has a correspondence in the market with 70 % consumption volume. Demijohn water segment refers to 69 % of this packed water sector and remaining 31 % refers to the rest of plastic bottled water. Therefore, 19 litre dem-
Demijohn water products have the highest share in packaged water consumption [1]. The per year potential of 19 litres HOD-Demijohn water market is 6 billion litres by the end of 2015 and the capacity for the remaining products is over 1 billion litres [2]. The amount of total bottled water production had reached to 11 billion litres in 2015 from 1 billion litres in 2003 [1].

Contemporary marketing requires the determination of consumer needs and requests to meet these needs and requests with a higher satisfaction level than the competitors. Public and private companies are operating in the water market and the sector demonstrates a rapid development in the world. The success rates of new products are 20% and 40% in the consumer market and industrial markets respectively. Accordingly, determination and promotion of the consumer-oriented products is important. Departing from these facts, this study focused on the demijohn water (19 litres) segment of the sector. In this context, a field research was conducted in the Western Mediterranean region of Turkey in 2009 in order to determine optimum product patterns promising the highest level of satisfaction to consumers. The findings were interpreted with regards to the recent challenges in the market to conclude whether right marketing strategies were pointed out or not.

MATERIALS AND METHODS

In order to determine factors affecting demijohn bottled water consumption, a field survey was conducted through face-to-face interviews with 962 households in Antalya, Adana and Hatay city centres in the Mediterranean region of Turkey in 2009. The data retrieved was analysed with conjoint analysis in order to determine optimum product patterns.

Conjoint analysis is a multivariable analysis methodology used to analyse consumer preferences reflecting features required for a product/service or a market mix and to enable policy making in marketing. Conjoint analysis, which enables the realistic reflection of consumer decisions, is applied nowadays to measure afore mentioned consumer perceptions of product or service and for market segmentation, profitability analysis, optimum product design and development of efficient product design [3, 4, 5]. In this study, market segmentation in packed water sector (demijohn and bottled) was made in addition to measurement of consumer perceptions for optimum product design with conjoint analysis.

Conjoint analysis encompasses of three core processes. Firstly, features of the ideal product providing maximum satisfaction level are determined or estimated. Secondly, the connection between the product/service and the concerned features are determined in terms of utilities [6]. Thirdly, the analyses are accompanied with market share simulation, profitability analyses and market segmentation analyses to set forward the optimal product concept. The onset of the conjoint analysis is based on the total utility theory. According to this, total utility can be expressed as a function of utilities of price and product quality [6].

\[
\text{Total utility} = f (\text{price utility + quality utility})
\]

Part-worths supplement model to the conjoint analysis suggests that part-worths of every single feature of the product is independent from one another and total of mentioned feature levels’ part worths brings out the total worth. Contribution of every feature level to the general assessment of consumer on product or service and accordingly to the consumer preference is determined with part-worths.

Two different calculation methods are used in conjoint analysis to determine the importance levels of product’s features. First one is the calculation of the difference in part-worth values for every feature. The other one is the calculation of relative importance levels of combinations. Variance between part-worth values of features refers to the difference between the feature with the highest part value and the feature with the lowest part value. This variation shows the importance level of every combination within itself. In calculation of relative importance between combinations, part-worth conversion value calculated for every combination is proportioned to the total part-worth conversion value [7]. Accordingly [6];

\[
\text{Conversion} = (\text{highest part-worth value} - \text{lowest part-worth value})
\]

\[
\text{Relative importance} = \frac{\text{Conversion} \times 100}{\sum \text{Conversion}}
\]

Two different data collection methods are used in conjoint analysis. These are full concept method and paired comparison methods [6, 8]. Full concept method, which is preferred in most of the researches [6], was utilised in this research as well. Subsequently, key feature set for potable water (demijohn and bottled) has been created (water source, mode of production, taste-consistence, price, quality management system-ISO, food safety management system-HACCP). Question cards including the levels of every feature were prepared in SPSS 13.0 statistical program accordingly and presented to consumers for scoring. Yet, demijohn bottled water preferences were analysed in the scope of this study.

Departing from the significant findings of the survey and analysis conducted, recent challenges and future potential of the market were evaluated. The aim of the comparison is to bring out a discussion whether the right marketing preferences were chosen or not to extend the market referring to the field survey results.
RESULTS AND DISCUSSION

Demijohn Water Purchasing Preferences. The elementary findings of the research demonstrated that 55.8% of households in the sample cities had been using demijohn water regularly. Monthly demijohn water consumption for these households was estimated as 5.63 dispensers. The households seemed to buy approximately 3-4 demijohn bottled water (34.1%) with the highest share. The population share buying 5-8 bottles per month was 30.6%. Less than 35% used to purchase less than 3 demijohns per month. Besides, the timing of demijohn using attitude was questioned as well. It was found out that 74.2% of the households, which constituted a significant majority, had been using demijohns for more than 1 year. This result indicated that demijohn water creates consumption dependence to a certain degree. Mean consumption duration was estimated as 5.08 years for households using demijohn water more than 1 year.

Within the scope of this study, brand loyalty level for demijohn water was surveyed. Targeted households were examined under 4 groups according to their brand loyalty degrees. Brand loyalty degree of households, who always purchase the same brand was defined as absolute, the ones who choose over 2 or 3 brands were defined as strong, the ones who are used to prefer certain brands for certain periods were defined as medium and the population who do not have any specific brand choice and change their brand often was defined as the independent population [9]. When brand loyalty levels of households were examined accordingly, it can be said that this loyalty level for demijohn water was absolute for this study. In fact, ratio of households who always purchase the same brand was 47.9%. Ratio of the households with strong (2 or 3 brands) brand loyalty was 22.9%.

When competition structure of packaged water market in Turkey was examined, it was observed that total share of top 5 companies is 45% in the market, in which over 300 companies operated by the year of 2015. Within this study conducted in the Mediterranean region as well, market share of the relevant top 5 companies in demijohn water market was determined as 47.5%. This ratio indicated that competition in demijohn water sector in the region is relatively high but close to oligopoly. Purchased number of brands in demijohn water market was estimated approximately as 2.03 for the surveyed households.

Conjoint Analysis for Demijohn Water and Optimum Product Pattern. Optimum product pattern that provides maximum utility to consumer or maximizes consumer utility in demijohn water market was determined with conjoint analysis. Factors and factor analysis composed in conjoint analysis for demijohn water were presented in Table 1. Four fundamental factors affecting consumer preferences in demijohn water purchases were determined as origin, production mode, taste (consistence) and price. These factors are accompanied with existence of two safety related certifications as ISO Certificate (Quality Management System Certificate) and HACCP Certificate (Food Safety Management System Certificate). Factor levels were determined for indices that determine the saturation degree in the market in order to measure this size [11]. Saturation is controlling or possessing a large percentage of economic resources and activities (labour utilised, sales, generated income and assets owned by firms) by a small percentage or few units. In other words, saturation degree of a structure depends on numbers of the units in that structure and distribution of economic resources and activities among these units. There is a negative correlation between number of firms in the market and saturation degree [12, 13]. When saturation index values are high, the assumption is that price and output decisions are determined by fewer firms in the market. Low values, on the other hand, indicate that price and outcome decisions are determined by many firms in the market [14, 15].

In a study conducted by State Institute of Statistics on saturation degrees of manufacturing industry in Turkey between 1980 and 1994, four categories for market structures were determined due to CR-4 saturation degree [16]. When the degree is lower than 30 it is low (there is competition), moderate level if it is between 31 and 50 (competition is decreasing, a competition close to oligopoly). The saturation is high when the score is between 51 and 70 (competition decreasing widely and oligopolistic structure is arising) and very high between 71 and 100 (monopolistic structure is arising) [17]. Also some practices about saturation show that 50% level in CR-4 analysis is equal to 70% in CR-8 analysis [12].

When competition structure of packaged water sector in Turkey is examined, it was observed that total share of top 5 companies is 45% in the market, in which over 300 companies operated by the year of 2015. Within this study conducted in the Mediterranean region as well, market share of the relevant top 5 companies in demijohn water market was determined as 47.5%. This ratio indicated that competition in demijohn water sector in the region is relatively high but close to oligopoly. Purchased number of brands in demijohn water market was estimated approximately as 2.03 for the surveyed households.
origin as Region-1 (National Brand), Region-2 (Regional Brand) and Region-3 (Local Brand). Discrimination for production mode was natural spring water, natural mineral water and processed water and hard and soft for taste. The scaling for price was as low-medium-high, while for ISO and HACCP Certificates the factors were determined depending on the existence or inexistence of the certificates.

Considering the factors and factor levels Orthogonal Design, in other words consumer question cards were created in SPSS program. Accordingly, interpretations of households for every product set were received through face to face survey. Consumer assessment was measured with a 10-points scale. Point scoring system was based on the principle that consumers give the highest point (10) to their primarily chosen alternative and lowest (0) to the newer preferred alternative. Subsequently, conjoint questions cards presented to the consumers during the survey were created for demijohn packed water (Table 2).

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>Product Feature Set Composed for Demijohn Water</strong></td>
</tr>
<tr>
<td><strong>Factor</strong></td>
</tr>
<tr>
<td>1. Origin</td>
</tr>
<tr>
<td>2. Production Mode</td>
</tr>
<tr>
<td>5. ISO Certificate (Quality Management System Certificate)</td>
</tr>
<tr>
<td>6. HACCP Certificate (Food Safety Management System Certificate)</td>
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</tbody>
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<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td><strong>Conjoint Cards Developed for Demijohn Water</strong></td>
</tr>
<tr>
<td><strong>Card 1</strong></td>
</tr>
<tr>
<td>Origin: Region-3</td>
</tr>
<tr>
<td>Production: Natural mineral water</td>
</tr>
<tr>
<td>Price: Low</td>
</tr>
</tbody>
</table>

| **Card 5** | **Card 6** | **Card 7** | **Card 8** |
| --- |
| Origin: Region-2 | Origin: Region-2 | Origin: Region-1 | Origin: Region-1 |
| Production: Natural spring water | Production: Processed water | Production: Natural spring water | Production: Natural spring water |

| **Card 9** | **Card 10** | **Card 11** | **Card 12** |
| --- |
| Origin: Region-3 | Origin: Region-2 | Origin: Region-1 | Origin: Region-2 |
| Production: Natural spring water | Production: Processed water | Production: Natural spring water | Production: Natural spring water |
| Price: Low | Price: High | Price: High | Price: Middle |

| **Card 13** | **Card 14** | **Card 15** | **Card 16** |
| --- |
| Origin: Region-1 | Origin: Region-1 | Origin: Region-3 | Origin: Region-1 |
| Production: Natural spring water | Production: Natural spring water | Production: Processed water | Production: Natural spring water |
| Price: High | Price: High | Price: Middle | Price: High |
When results obtained were examined from Table 3, the most important factor affecting the demijohn water preference and purchasing decision of consumers had appeared to be ‘Price’. Level of price influence on consumer purchasing decisions was estimated as 28.04%. Second important factor on consumer purchasing decision after price was ‘Origin’. Origin of the water resource affected consumer purchasing decision for demijohn packed water with 21.36%. Third factor affecting the purchasing decision was ‘HACCP certificate’. According to this, existence of HACCP certificate affected the surveyed consumers’ decisions with a rate of 19.07%. Fourth effective factor was ‘production mode’ with 13.38%.

Water taste was the fifth important factor in demijohn water purchases with 12.11%. Sixth factor was the existence of an ISO certificate. Existence of the ISO certificate affected consumers’ decisions with 6.04%. Due to these findings, it can be said that the top three features in optimum product compound that provides the maximum total utility or satisfaction to consumers in demijohn water market and affect consumer preference are price, water origin and existence of HACCP certificate. Therefore, both product knowledge and quality safety play determinative roles in consumer purchasing decision as well as affordability.

Part worth values of every factor demonstrated the effects of relevant factors on consumer preferences. Factor level with the highest part worth value is considered as the option most commonly preferred by consumers [15]. Factor level with the highest part-worth value in price factor was the lowest price level with 1.818 worth value. Factor level with the lowest part worth value was the highest price level with -2.036 worth value. This result indicated that consumers tended to prefer the most affordable (economic) price.

When part-worth values of the second most effective factor, the origin were examined, the regional discrepancies became visible as well. Factor level with the highest part-worth value was Region-3 (local brand) with 0.0840. Factor level with the lowest part worth value was Region-2 (regional brand) with the value of -1.349. From this point of view, we can say that consumers tended to purchase from locally to regionally known water brands and national brand stand in between. When part-worth values of the third most effective factor, the HACCP certificate, were examined existence and inexistence of the certificate again made a difference. Factor level with the highest part worth value was looking for existence of a food security certificate with the value of 1.413. Accordingly, it can be said that consumers care about the safety of water production process.

Then part-worth values of fourth factor, the production mode were examined. Factor level with the highest part-worth value was natural spring water with the value of 0.821. Part worth value of natural mineral water was 0.211. Processed water provided the lowest value with -1.032. Therefore, primary choice of consumers in demijohn water purchasing was natural spring water. Fifth important factor in households’ demijohn water purchasing decisions was taste and consistence. Factor level with the highest part-worth value was soft water with the value of 0.955 for this factor. Finally, the sixth effective factor, existence of ISO certificate was examined. Consumers’ primary choice on demijohn water purchasing was the products with ISO certificate. In as much, part worth value of ISO certificate existence was relatively high (Table 3).

In conjoint analysis, as much as part-worth values of every factor level, difference between these factor levels referring to the significance level of relevant factors concerned on consumer choices are important. When analysis results were examined, the highest difference between part-worth values was respectively on price, origin of water and HACCP certificate. Subsequently, it can be said that the participating consumers tended to purchase water with affordable price, trustable production mode and known origin referring to the regional orientation in demijohn water market. In other words, consumers wanted to purchase familiar, trustable product/brand presenting the highest value to them in the lowest price level.

Total value of question cards, combinations presented to costumers within the scope of the conjoint analysis, was formed by total of factor level values. Combination with the highest total worth value is acknowledged as the product feature set providing the optimum utility to consumer and exists as the primarily preferred option. On the other hand, product feature set with the lowest total worth value provides the minimum utility and refers to the least preferred option [15,16].

According to results of this conjoint analysis applied to around 1.000 households in the target region of Turkey, optimum product pattern (compound) maximizing the consumer utility for demijohn water was card number 9 with the total worth value of 6.277 (Table 4). This feature combination refers to lowest priced, soft, locally known branded natural spring water containing demijohn brand having both HACCP and ISO certificates.

**DISCUSSION**

Afore mentioned growth of packed water market was confirmed globally in the recent years as well. Yet, most of the studies referring to consumer preferences were not classified as PET or demijohn water consumption preferences. Therefore, considering plastic bottle as the main correspondent, it is possible to refer the challenges in comparison with our study.
A recent survey conducted in Australia and New Zealand with 192 households in 2013, reported the relationship between bottled water consumption and both features and perceptions of the target group [18]. While most of the consumers considered packed water as ‘a waste of money’ with 63 %, they also found it convenient with 21 %. Although there appeared a health consciousness, considering packed water as a necessity or trusted product was not found as a significantly perceived factor. Mainly plastic packing is perceived considerably negative due to environmental consciousness. However, qualitative assessment of the target group revealed that even if the majority finds packed water as an additional cost, 64 % indicated that they maintain consuming it. Yet, health risk considerations, which can be combined with the certification processes in our study, were found almost negligible. While 32 % considered tap water as unhealthy, they did not accept packed water as a good alternative mainly due to plastic packaging. 11 % seemed to prefer bottled water due to its taste even though only 28 % found it tastier than tap water. One of the most important findings of the research is that even if people continuously consume packed water, they have ambiguous considerations and they do not consider packed water as a product to be promoted via adverts.

In a study conducted in Northern California and Nevada of the United States, the effect of price and non-price factors on demand for bottled water consumption both referring to 16 ounce (480 millilitres) and 1 gallon (3.79 litres) bottles in the grocery stores between 2001 and 2005 [19]. The effect of health consciousness, which again can be attributed to certification and trust in the contaminants, is not related significantly with the willingness to pay. This confirms with the dependence on price-efficiency of our study.
In a survey conducted in rural Guatemala in 2012 with 500 households, number of carboy bottled water consumed per week was estimated against socio-demographic factors as well as perceived health risks and qualities of tap water. The findings indicated that, health-sensitivity is very low in the low-income district, where 26% of the population buys at least one demijohn of water. Only 29% of the households perceived tap water as unhealthy. The price and income sensitivity of demijohn demand was confirmed in the study. Therefore, price was the main destructing factor and consumers pay the highest level of attention on price in their consumption preferences [20]. The low health risk perceptions in the target rural areas were in line with Gleick’s proposition that tap water unreliability is a major consideration for urban rather than rural settings [21].

A survey was conducted in England and Wales for tap water demand with relevance to hardness and aesthetic features like taste and smell. Even for tap water, unit price and service maintenance and improvement were considered as essential for not to shift plastic bottle use [22]. This at the same time means that price-efficiency and taste might be considered as important factors to promote packed water.

For saturated markets, the consumer responsiveness needs to be considered in improvement of marketing strategies for bottled water. The Italian hedonic price assessment conducted via the retrieved consumer data from sales markets in 2015 [23], demonstrated the market preferences of 374 water brand examples. The price of packed water seemed to vary among brands and both packaging type and aesthetic features like taste and smell. Even for tap water, unit price and service maintenance and improvement were considered as essential for not to shift plastic bottle use [22]. This at the same time means that price-efficiency and taste might be considered as important factors to promote packed water.

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One of the countries that occupy the packed water market with significant demand of 40 gallons (151 litres) per capita per year is the United Arab Emirates [24]. In a survey conducted with 891 households, high per capita income of the country revealed that price has a very limited effect on packed water consumption [25]. Even though the households assume tap water as a quality potable water source, they prefer packed water due to its taste and ambiguous beliefs on contamination of the tap water. So, standardised, tasty and packed water seemed to be preferred by the households due to the findings of the study.

CONCLUSION

Reducing water resources and rising in environmental pollution accompanied with weak infrastructure are significant considerations in the management of drinking water supplies in the world. These facts affect the tendency to shift from tap water to bottled water. This tendency is in the same direction for drinking water consumption even with a more powerful orientation. While, public resources are devoted on development of tap water infrastructure and increasing hygiene standards in the management of the network, the private sector is focused on enlarging packed water market under public control.

In this process, consumer perceptions and preferences also play important roles for both tap water and bottled water supply and management. As in study findings refer, directing consumer-oriented drinking water services is expected to provide efficiency in the resource utilisation.

Conjoint analysis helps those concerned in consumer choice determination by considering the relevant product’s features in product design. This description is made for demijohn water in this study and findings suggest that consumers tended to purchase water with affordable price, safe production system and known origin. Therefore, consumers want to purchase safe products with the lowest price level.

Considering the recent studies, price and safety concerns continue to be the main drivers of packed water consumption. Mostly people attribute relative importance on affordability and health consciousness in making their choices. There appeared limited information on whether the preference towards natural spring water or processed water. This can be attributed to limited natural water resources and getting used to processed water in many developed or developing countries.

Furthermore, even with limited detailed information, the health consciousness of consumers in urban areas in Turkey, leads requirements on product (HACCP) and process (ISO) qualities. Departing from our study, it can be noted that cost reduction of packed (demijohn) water and watching over quality standards are the main prerequisites of the consumers. Yet, producers can mainly make product differentiation towards extending natural spring water production as it is the most preferred water type and soften the taste without intervening in quality or chemical standards. Besides, preferences towards local brands and trust in national brands should be considered in brand development and market strategy implementation processes. Finally, it can be concluded that, the results of our field survey maintains its validity and can provide insights to producer and suppliers of the sector regarding consumer needs.

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REMOVAL OF REACTIVE RED 45 USING ATERMIT FACTORY SOLID WASTES BY ADSORPTION

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2Department of Environmental Engineering, Faculty of Engineering, University of Mersin, Mersin, 33000, Turkey

ABSTRACT

This study, atermit factory solid wastes were used as an adsorbent to remove Reactive Red 45 (RR45) from aqueous solution by batch method. Effects of pH, initial dye concentration and time were studied. It was found that the removal of RR45 increased with increasing initial dye concentration. Adsorption isotherms were analyzed by Langmuir and Freundlich models. Moreover, kinetic of adsorption were studied. Pseudo second order is the best model for the removal of RR45 from aqueous solution. Thermodynamic parameters were also studied. Gibbs free energy value was found to be -5.320 kJ/mol and indicating the spontaneity of the system. Atermit factory solid wastes were characterized by SEM. As a result, atermit factory wastes could be employed as no-cost and effective adsorbent for the removal of RR45 from aqueous solution.

KEYWORDS: Adsorption, RR45, environment, SEM, thermodynamic, isotherm.

INTRODUCTION

Today, studies have been conducted to investigate the use conditions of waste materials for different purposes in order to ensure the availability of natural resources more efficiently. For this reason, reuse of waste is of great importance in terms of environmental and economic values. It is important to carry out studies to characterize of wastes from industrial facilities as raw material for another industry. In this study, the efficiency of solid wastes of the atermit production plant in adsorption of dyestuffs in the textile industry wastewater has been investigated.

Atermit is a roofing material produced using cement containing asbestos [1]. The use of asbestos in the production of roofing materials is forbidden after the adverse health effects have been demonstrated. Atermit first factory in Turkey was established in 1956. After that, the roofing materials were named as the aterit with the same name as the founding firm [2]. Turkey is completely prohibited the use of asbestos in the Official Gazette published the law on 2010, 31 December [3]. After this date, all Atermit producer companies are closed except Atermit firm which was changed their technology. In the new technology, raw cellulose and waste cardboard packaging, which provide a flexible feature to the final product instead of asbestos, are used in the production of atermit [4, 5, 6]. In the production of atermit; the main raw materials (cement and unbleached cellulose fibers) and auxiliary raw materials (waste cardboard packaging, anti-foam and flocculants) are used [7, 8, 9, 10, 11]. Unbleached cellulose fibers are made into paper clay by the help of hot water. Unbleached cellulose fibers are made into paper clay with the help of hot water and then sent to the production band by adding other raw materials. The wastewaters formed during the atermit production are collected in the sedimentation basin. The water formed after precipitation is reused in the system and the sediment is stored as non-hazardous waste in the municipal landfill [10, 11].

The textile industry is one of the most important industries in the world. Textile dyes produced by natural and synthetic routes that form a large market all over the world are one of the most important raw materials of this industry. The textile paint industry, which has an economic volume of $ 5,625 billion in 2017, is expected to reach $ 7.982 billion by 2020. Textile dyes are used to color textile products such as fibers, yarns and fabrics [12]. Generally, 125-150 L of water is consumed in the dyeing of 1 kg fabric [13]. Textile wastewaters have high chemical oxygen demand (COD) due to dense color, suspended solids, cellulose fibers, dyes and solvents [14]. The wastewater of this industry is responsible for the coloring of the water, the inhibition of photosynthesis, affect the lifespan of living things due to toxic content and the aesthetic worry in the receiving environment [15]. The dyestuff Reactive Red 45 (RR45) is a commonly used in the textile industry. Since it has a potential to cause pollution in the water environment, it must be purified before discharged [16]. Azo dyes constitute about 70% of reactive dyes [17]. Reactive azo dyes are produced to resist biodegradation [18]. For this reason, biological treatment methods do not provide
FIGURE 1
Sem image without dye (a) with dye (b)
the desired efficiency in the treatment of such contaminated water.

Adsorption is one of the advanced treatment techniques used in waste water treatment. In recent years, studies have been made to use solid wastes such as olive [19] and plastic industries [20] and also egg shells [21], plastic bottle [22], bentonite [23-24], peanut husk [25] as adsorbents.

In this study, atermit factory solid wastes were utilized as an adsorbent to remove Reactive Red 45 (RR45) from aqueous solution by batch method. Also, effects of pH, initial dye concentration and time were studied. Surface of atermit factory solid wastes characterized by SEM.

**MATERIALS AND METHODS**

**Preparation of RR45.** Reactive dye, RR45, was obtained from Merck and used without further purification. pH values of the medium were adjusted by addition of 0.1 M HCl or NaOH. Properties of RR45 described Table 1.

**Preparation of Atermit Factory solid wastes.** Firstly, atermit factory solid wastes were washed pure water, secondly dried oven at 100 °C for two days and finally screen (100 mesh) before using.

**Experiments.** Experiments were performed in 750 mL erlenmeyer flasks including 1.5 g of atermit factory wastes with 150 mL of RR45 solution. All the adsorption experiments were performed at room temperature (25 °C) via batch method and four set (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L). The solution was shaken by a mechanical shaker (Edmund Bühler GmbH) at the constant agitation time (100 rpm) during 120 min. Then the supernatant was centrifuged at 6000 rpm and 10 minutes in a centrifuge (Hettich Zentrifugen) after the batch tests. The absorbance of RR45 was measured at maximum wavelength (\( \lambda_{\text{max}} \): 505 nm) by UV–VIS Spectrophotometer (T 90). The incubation time was tested in a time from 5 to 120 min. All experiments were repeated three times. The removal efficiency of Malachite Green dye was calculated as follows, Eq. 1:

\[
\text{Dye Removal (\%) = } \left( \frac{C_o - C_t}{C_o} \right) \times 100
\]

where \( C_o \) is the initial dye concentration (mg/L) whereas \( C_t \) is the dye concentration after sorption time t (mg/L) [27].

**RESULTS AND DISCUSSION**

**Sem Images.** Sem photo of atermit factory solid wastes presented Figure 1. The atermit factory solid wastes have rough structure. After adsorption, waste surfaces loaded dye.

**Effect of Contact Time on RR45.** First of all, adsorption experiments were done for different contact time (10, 20, 30, 60, 90, 120, 150, 180 minutes) and different conditions (600, 700, 800, 900, 1000 mg/L). Figure 2 is shown the effect of contact time on RR45.

**Effect of Initial Dye Concentration.** The effects of initial concentration on the removal of RR45 by atermit factory solid wastes were studied at room temperature (20 °C) and pH 6.0 at equilibrium times, and the results were graphed in Figure 3. According to Figure 3 the equilibrium sorption capacities of the sorbents increase with an increase in initial dye concentration and then stable. Huang et al., (2017) studied adsorption of Rhodamine B (RhB) and Acid red 1 by bentonite [28]. They found that similar results. At first, both the initial dye concentration from 50 to 300 mg/L to increased and amount of adsorbent increased. After that, the initial dyes concentration were 300 to 350 mg/L, adsorption process reached steady state.

**Effect of pH.** The pH is the one of the most important parameters to the adsorption because of the charge of sorbent surface. Figure 4 is shown that effect of pH for RR45. The removal of the

---

**TABLE 1**

Properties of RR45 [26]

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Molecular weight (g/mol)</td>
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<tr>
<td>Color</td>
<td>Red</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
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</tr>
<tr>
<td>Dye purity</td>
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<tr>
<td>Chemical formula</td>
<td>C27H19ClN7Na3O10S3</td>
</tr>
</tbody>
</table>

---

\[
\text{Dye Removal (\%) = } \left( \frac{C_o - C_t}{C_o} \right) \times 100
\]
RR45 increases with increasing initial pH from 4 to 6. The maximum dye removal shows pH 6.

**Adsorption Isotherm.** Langmuir model which describes the monolayer adsorption of dye molecules on a homogenous surface with a limited number of identical sites is given by Eq. 2 [29]:

\[
\frac{C_e}{q_e} = \frac{1}{K_L} + \frac{1}{K_L a_L} C_e
\]

where; \(C_e\) is the equilibrium concentration of adsorbate in solution after adsorption (mg/L), \(q_e\) is the equilibrium solid phase concentration (mg/g), as well as \(K_L\) (L/g) and \(a_L\) (L/mg) are the Langmuir constants.

**FIGURE 2**
Effect of contact time on RR45

**FIGURE 3**
Effect of initial dye concentration
FIGURE 4
Effect of pH for Reactive Red 45

However, the Freundlich isotherm supposes a heterogeneous surface with a nonuniform distribution and can be expressed by Eq. 3:

\[ \log q_e = \log K_F + \frac{1}{n} \log C_e \]  

(3)

where \( K_F \) (L/g) is the adsorption capacity at unit concentration and \( 1/n \) is adsorption intensity.

Isotherm models also studied for adsorption of RR45. Langmuir and Freundlich isotherm models applied for removal of RR45 by adsorption. Freundlich isotherm is better than Langmuir isotherm. Because of high correlation coefficient (\( R^2 = 0.9335 \)). Table 2 demonstrates isotherm constants.

**Kinetic Study.** Second order rate equation can be written as:

\[ \frac{1}{(q_e - q_t)} = \frac{1}{q_e} + kt \]  

(4)

\( k \) (g/mg•min): The rate constant for second order

\( q_e \) and \( q_t \) are the amount of adsorbed on the atermite factory solid wastes (mg/g) at equilibrium and at time \( t \) (min), respectively, and \( k_{ad} \) (1/min) is the rate constant of pseudo-second-order kinetics.

Dye concentration values were in the range of 600-1000 mg/L. For understand the reaction mechanism, kinetic models are applied kinetic data. The pseudo first order and pseudo second order kinetic models are explained the adsorption kinetic. The results of the pseudo second order kinetic experiments graphics were given in Figure 5. Moreover, the data fitted to pseudo second order kinetic model (Table 3).

**TABLE 2**
Isotherm parameters for the adsorption of RR45 onto atermite factory wastes

<table>
<thead>
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<th>Isotherm constants</th>
<th>RR45</th>
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<td><strong>Langmuir</strong></td>
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<tr>
<td>( q_{max} ) (mg/g)</td>
<td>35.771</td>
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<tr>
<td>( K_L ) (L/mg)</td>
<td>0.0125</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.464</td>
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<tr>
<td><strong>Freundlich</strong></td>
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</tr>
<tr>
<td>( K_F ) (L/g)</td>
<td>0.0315</td>
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<tr>
<td>( n )</td>
<td>1.211</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.9335</td>
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TABLE 3
Comparison of adsorption capacity of RR45 on atermit factory solid wastes in literature

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<th>Dye</th>
<th>Q_max (mg/g)</th>
<th>References</th>
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<td>Si-APTES-BP</td>
<td>Reactive blue 19</td>
<td>37.45</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>Reactive yellow 84</td>
<td>32.36</td>
<td></td>
</tr>
<tr>
<td>Humin immobilize silica</td>
<td>Reactive Orange 16</td>
<td>19.45</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>Reactive Red 120</td>
<td>2.29</td>
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</tr>
<tr>
<td>Clinoptilolite</td>
<td>Reactive Blue 21</td>
<td>9.652</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>Reactive Red 195</td>
<td>3.186</td>
<td></td>
</tr>
<tr>
<td>Industrial Sludge</td>
<td>Remazol Brilliant Blue R</td>
<td>33.47</td>
<td>[38]</td>
</tr>
<tr>
<td>Activated red mud</td>
<td>Reactive black 5</td>
<td>35.58</td>
<td>[39]</td>
</tr>
<tr>
<td>Atermit factory solid wastes</td>
<td>Reactive Red 45</td>
<td>35.771</td>
<td>This study</td>
</tr>
</tbody>
</table>

FIGURE 5
Pseudo Second Order Kinetic

TABLE 3
Values of Pseudo Second Order Kinetic Model

<table>
<thead>
<tr>
<th>Initial Dye Concentration (mg/L)</th>
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<th>k_2ad</th>
<th>R^2</th>
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<td>600</td>
<td>54.644</td>
<td>0.30400</td>
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<tr>
<td>700</td>
<td>64.516</td>
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<tr>
<td>800</td>
<td>75.187</td>
<td>0.02940</td>
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<tr>
<td>900</td>
<td>83.333</td>
<td>0.02570</td>
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<tr>
<td>1000</td>
<td>87.719</td>
<td>0.00386</td>
<td>0.999</td>
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</table>

TABLE 4
Gibbs Free Energy Values For Systems

<table>
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<th>Sorbent</th>
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<th>ΔG° (kJ/mol)</th>
<th>T (K)</th>
<th>References</th>
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<tr>
<td>Atermit Factory Solid Wastes</td>
<td>RR45</td>
<td>-5.320</td>
<td>298</td>
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</tr>
<tr>
<td>Banana peel powder</td>
<td>RB5</td>
<td>-0.198</td>
<td>298</td>
<td>[31]</td>
</tr>
<tr>
<td>Prawn shells</td>
<td>Reactive Red</td>
<td>-2.5616</td>
<td>303</td>
<td>[32]</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>Bentonite</td>
<td>-17.46</td>
<td>303</td>
<td>[33]</td>
</tr>
<tr>
<td>Ash</td>
<td>Reactive blue</td>
<td>-2.979</td>
<td>293</td>
<td>[34]</td>
</tr>
</tbody>
</table>
Thermodynamic Studies. The thermodynamic parameters for the adsorption process of RR45 onto the atermit factory solid wastes can be determined using the experimental data in the following equations Eq. 5-7: Gibbs free energy values are shown in Table 4.

\[ 
\Delta G = -RT \ln K_c^0, \quad (K_c = C_o/C_a) \]  
\[ \ln K_c = \frac{\Delta H}{R} - \frac{\Delta S}{R} \]  
\[ \Delta G = \Delta H - T \Delta S \]

\( \Delta S \): The changes of entropy  
\( \Delta H \): the changes of enthalpy  
\( \Delta G \): Gibbs free energy  
\( K_c \): The equilibrium constant  
\( T \): Temperature (K)  
\( R \): The ideal gas constant (8.314 J/(mol K)).  
\( C_a \): The solid phase concentration in equilibrium (mg/L).

CONCLUSION

In this study, atermit factory wastes were used as an adsorbent to remove Reactive Red 45 (RR45) from aqueous solution by adsorption. Isotherm and kinetic parameters were studied for the adsorption. Freundlich isotherm model is more suitable than Langmuir model. Because of high correlation coefficient (R²=0.9335). Adsorption kinetics also studied. The data fitted to pseudo second order kinetic model. Thermodynamic parameters were also studied. Gibbs free energy value was found to be -5.320 kJ/mol and indicating the spontaneity of the system. Properties of surface characterized by SEM. As a result, atermit factory wastes could be employed as no-cost and effective adsorbent for the removal of RR45 from aqueous solution.

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REFERENCES


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PHYLOGENETIC AND MORPHOLOGICAL CHARACTERIZATION OF *FUSARIAUM OXYSPORUM* F. SP. *CEPAE* THE CAUSAL AGENT OF BASAL ROT ON ONION ISOLATED FROM TURKEY

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¹Amasya University, Suluova Vocational School, Department of Plant Protection, 05100 Amasya, Turkey
²Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Plant Protection, 046100 Kahramanmaras, Turkey

ABSTRACT

*Fusarium oxysporum* f. sp. *cepa* (FOC) is a serious pathogen, which causes basal rot on onion. In the present study, a total number of 152 *Fusarium* strains were isolated from infected onions picked from middle black sea region of Turkey. Based on the mean disease severity (DS %), the virulence of each isolate was recorded as highly virulent (70-100% DS), moderately virulent (20-69% DS), low virulent (10-19% DS). At the morphological characterization all of high virulence *Fusarium* strains was identified as *Fusarium oxysporum* which based on structures of microconidia (2.5–15 μm×2–3 μm and 0-septate), macroconidia (15–20 μm × 2.5–3 μm and 3-septate), chlamydospores and other morphological characters. Phylogenetically characterization of strains used primers ITS1 and ITS4. These primers have been successfully amplified a specific 330-bp PCR product the internal transcribed spacer (ITS1-5.8S-ITS2) region of FOC strains. At the phylogenetic classification of high virulence FOC strains Neighbor-joining method was used. There was very little genetic differences (99% similarity) between strains and reference FOC (HQ658965) NCBI Gen Bank due to environmental conditions. This isolates probably from the same clonal lineage. On the other hand, at the phylogenetic characterization, FOC strains formed major genetic differences with other pathogens on onion. Thus, the results indicate that molecular profiling using ITS is a valid method for phylogenetic characterization of FOC.

KEYWORDS:
Phylogenetic characterization, *Fusarium oxysporum* f. sp. *cepa*, ITS, Onion

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most widely cultivated commercial crops in Turkey. In the many onion growing areas, various harmful and disease agents pathogenic fungi, bacteria, viruses and nematodes cause important yield losses. FOC causing basal rot disease is the most destructive for all growing areas of onion [1]. The pathogen FOC is from species (f.sp.) in the *Fusarium*. Fungicide treatments at the seeds such as methoxymethyl, mercury chloride, carbendazim, maneb, carboxin, prochloraz, tebuconazole, benzylam and soil solarisation in the fields usually fail to control the pathogen. But use of resistant cultivars is the most effective and economical strategy to control basal rot disease prevention [2-3]. The agent of basal rot infects the onion root epidermis and then extends into the vascular tissue at the basal region areas. The characteristic symptoms of the rot disease include onions leaf curving, drooping, turning pale and discoloring of onion and then the contaminated tissue gets brown and hydrated. As a result of these serious infections, the plant eventually dies [4].

Control of plant diseases requires identification of pathogens. Especially, genetic characterization of pathogenic agents on the plant is required for increased crop productivity. FOC is one of more than 120 strain species of *F. oxysporum*; for this reason this strain complex also contains nonpathogenic strains. The diverse formae speciales on host specificity of *F. oxysporum* show major genetic variety with polyphyletic origin [5-6-7]. Various methods have been used to identify genetic variation on populations of FOC. Such as random amplified polymorphism DNA (RAPD), amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphism (RFLP), intergenic spacer (IGS) regions of rDNA, intersimple sequence repeats (ISSR) and vegetative compatibility grouping (VCGs) [9].
ITS (Internal transcribed spacer) region is a fragment of non-functional RNA situated between structural RNA on a common precursor transcript. rDNA region consists of multiple copies arranged in repetitions including the 18S Small SubUnit-coding, the 5.8S, and the 28S Large SubUnit-coding genes separated by internal transcribed spacer regions (ITS1 and ITS2) [10-11]. Since the 1990s Internal Transcribed Spacer (ITS) region have been widely used as a DNA barcoding marker to identify the variety and composition of a unknown fungal group [12]. Because, it is simple and has the broadest range for fungi [13]. One advantage of using the ITS region as a standard marker is that most fungal species have been identified based on this genomic region [14]. Therefore, we used ITS markers to analyze the FOC isolates in this study. This present study, has characterized different pathogenic race of FOC strains using pathogenicity test, morphological and molecular methods isolated from onion.

### MATERIALS AND METHODS

#### Isolation of fungal cultures. One hundred and fifty two strains of *Fusarium* spp. were isolated from naturally infected onion (*Allium cepa* L.) basals plates. The highly virulent FOC strains are listed in Table 1. Infected stem samples were cut with a sterile blade (ca. 6x6 mm) onion bottom and sterilized by dipping in 5% (w/v) sodium hypochlorite solution. Stem samples were incubated on potato dextrose agar (PDA; Merck, Darmstadt, Germany) including with 40.0 mg streptomycin sulphate per liter at 25±2 °C. The pure cultures of the strains were obtained using a single-spore culture technique [15]. *Fusarium* spp. strains were stored on solid PDA at 4°C and in 35% sterile glycerol at −80°C.

#### Pathogenicity tests. *Fusarium* spp. strains were inoculated with potato dextrose broth in an Erlenmeyer and then it was shaken at 150 rpm 7 days at 25±2°C in dark conditions. After one week, *Fusarium* mycelials were filtrated. Each conidia concentrations were adjusted to 1x10³ conidia/ml. In the pot experiment, autoclave sterilized soil mixture (field soil, sand and manure (v/v 3:1:1, pH = 7.2) was used in 250 ml plastic pots. Each experiment pot was inoculated with 100 ml spore solution one week before seeding [16]. As negative control, the same amount of water was added. In pathogenicity tests Kantartopu onion seeds, which are susceptible to FOC [17-18], was used. The onion seeds were surface sterilized with 0.1% (v/v) of sodium hypochlorite for 3 min. Three onions were cultivated in each pot. The experiment was conducted in five repetitions in controlled greenhouse with an average temperature of 25±2°C. Three months later, each plant was graded on a scale of 0-4 as follows 0 = no symptoms, 1 = discoloration in leaves and root development deceleration, 2 = discoloration up to 50% in plant and growth deceleration, 3 = discoloration all plant and stained, 4 = root completely decayed and dead plant [19].

<table>
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<tr>
<th>No</th>
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<th>Species</th>
<th>Locations</th>
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<td><em>Fusarium</em> spp</td>
<td>Amasya/Göynücek</td>
</tr>
<tr>
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<td>Amasya/Tasova</td>
<td>36</td>
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<td>Amasya/Suluova</td>
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<td>Amasya/Hamamözü</td>
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<td><em>Fusarium</em> spp</td>
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<td>19</td>
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<td><em>Fusarium</em> spp</td>
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</tbody>
</table>

*Amasya located middle black sea region of Turkey
The disease severity (DS %) was calculated for each isolate in all experiments. DS = (Σ Vxn) / (N x Z) X 100 [n: Number of plants in each scale value, V: Scale value, Z: Maximum scale value, N: Total number of plants observed] as described by formula Tawssend-Heuberger [20]. The *Fusarium* spp. strains were further subdivided into highly virulent (70-100% DS), moderately virulent (20-69% DS), low virulent (10-19% DS). Strains causing no or less than 9% DS were considered non-virulent [21]. From the artificially inoculated plants, strains of fungus with high virulence were re-isolated and pathogenicity test was carried out on previously grown Kantartopu onion bulbs.

**Morphological identification of *Fusarium* isolates.** For morphological identification of high virulence *Fusarium* spp, single spore strains were grown on PDA medium [22]. *Fusarium oxysporum* strains were identified on the basis of colony morphology, morphological characteristic of macro and micro-conidia and conidial measurement was used as the identification methodology [23].

**DNA extraction and molecular identification of *Fusarium oxysporum*.** Each highly virulent *F. oxysporum* mycelial plugs (15 mm in diameter) were transferred to 150 ml of potato dextrose broth (PDB) (Difco) and were grown for 5 days in an orbital shaker (130 rpm) at 25 ±2°C in dark. Fungal mycelia from cultures were harvested by filtration through a sterile filter paper and was frozen in liquid nitrogen in 2 ml Eppendorf tube. The cell walls of the frozen cells were broken using a steel ball with 0.5 mm diameter and was added 700 μl extraction buffer (0.1 M Tris HCl, 1.4 M NaCl; 0.02 M EDTA; 2% CTAB; pH 8.0). The mixture was vortexed for 40 s and incubated at 65°C until complete breakdown of cells (about 50 min). Then was added 500 μl of SEVAG solution (chloroform: isoamylalcohol 24:1) and vortexed. This mixtures was centrifuged at 5000 min and than centrifuged again at 10000 rpm for 12 min. The supernatant was removed and the DNA pellet, was washed twice in 70% cold ethanol, then dried in air and added 50 μl sterile TE (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). It's stored at -20 °C [24]. Identification of *F. oxysporum* isolates was confirmed by PCR using universal primers, ITS1F (ACATACACCCTTTGGCCCTCG) and ITS4R (CGCCAATCATCTTGAGGAACG) [25]. Amplification of ITS1-5.8S-ITS2 rDNA region was performed on a Thermal Cycler (Applied Biosystems 9700) with 20 μl reaction mixtures containing 2 μl of 10X buffer (10 mM Tris–HCl, pH 8.8); 2.5 μl MgCl₂ (25 mM); 1 μl each of dNTP ((5 mM); 1 μl each of ITS1 and ITS4 primer (10 pmol); 0.5 μl of Taq DNA Polymerase; 1 μl genomic DNA (10 ng) and 11 μl dH₂O. The amplification cycle consists of an initial denaturation at 94 °C for 2 min followed by 30 cycles at 94 °C for 0.5 min, 57 °C for 0.5 min, and 72 °C for 1 min and a final extension at 72 °C for 10 min. Amplified PCR products were separated on an agarose gel (1.5% w/v) in 1X TAE buffer at 65 V for 150 min. Amplified products were observed with ethidium bromide in Tris-acetate EDTA (TAE) buffer and photographed under transmitted ultraviolet light, using an Alpha Imager 2000 (AlphaInnotech, San Leandro, CA, USA) [26].

**ITS data sequences and phylogenetic analyses.** *Fusarium oxysporum* ITS1-5.8S-ITS2 rDNA amplification products observed single only bright band under UV light were sequenced using an automatic sequencer 3730XL from Macro Gen Inc (Korea). The ITS1-5.8S-ITS2 nucleotide sequences for each isolate were compared to databases NCBI using Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN). These sequences were compared to ITS1-5.8S-ITS2 region of FOC (HQ658965 gene sequences and different *Fusarium* spp. (*F. proliferatum* HQ332533.1, *F. longipes* KF918596.1, *F. brachygibbosum* KJ541486.1, *F. phyllophilum* AB587006.1, *F. equiseti* KU366254.1, *F. fujikuroi* HF679024.1, *F. oxysporum* KC119203.1, *F. subglutinans* JX960431.1, *F. verticilloides* FJ867932.1) and with other onion pathogens (*F. acuminatum* KU886148.1-Leveillula taurica, *F. equiseti* KP257580.1-Sclerotium cepivorum, *F. redolens* JX475897.1-Setophoma terrestris, *F. solani* KX343007.1-*F. oxysporum*, LC193726.1-*F. acuminatum*, IQ693101.1-*F. proliferatum* and KP055057.1-*F. redolens*) at the database of NCBI gen bank. All ITS DNA sequences were aligned using CLUSTALW program [27]. Phylogenetic analyses of ITS1-5.8S-ITS2 data sequence were conducted using both distance and parsimony techniques [28]. For parsimony and distance analyses, the heuristic search option and the Neighbor-joining method of PAUP* 4.0 were used. Alignment gaps were treated as missing data. Strengths of internal branches of resulting tree were statistically tested by the bootstrap analysis of 1000 replications [29].

**Statistical analysis.** The results of disease severity (%) were subjected to statistical analysis SPSS 20 program. Multiple comparisons of the means were conducted according to the Duncan test at p < 0.05.
FIGURE 1
Inoculated to onion bulbs with Fusarium species and negative control
(a: water, b: ib-124, c: ib-49, d: ib-150, e: ib-57

FIGURE 2
Images of Fusarium oxysporum strains in PDA medium a) structure of mycelia floccose b) mycelia color from white to pale violet c,d ) macroconidia structures e) septate of macroconidia f) microconidia structures g) phialit simple and bulging h) chlamydospores

RESULTS AND DISCUSSION

Fungal strains and pathogenicity tests. Typical symptoms of basal rot disease were first observed 20–25 days after inoculation. Most of Fusarium strains were determined to be pathogenic with pathogenicity test. According to the disease severity (%), existence of 41 strains was identified as being highly virulent, 68 strains as being moderately virulent and 8 strains as low virulent. These strains caused basal rot disease on onion plant; however inoculation of 36 strains did not cause basal rot disease on Kantartopu onion cultivars. In the negative control inoculum, no disease was observed. Results of the pathogenicity test showed that ib-107, ib-57, ib-61, ib-84, ib-109, ib-56 strains were most aggressive (DS=78.3%). DS (%) of highly virulent Fusarium spp. when compared are statistically between DS (%) is not significant according to the Duncan test (p < 0.05) (Table 2). These highly virulent FOC isolates were successfully re-isolated from disease affected onions and subjected to a pathogenicity test on the Kantartopu onion bulbs. All re-isolated strains were found to be created by most aggressive rot on the onion bulb (Figure 1).

Morphological identification of Fusarium isolates. Highly virulent strains were identified by morphological characteristics. Based on colony morphology and characteristic features of macro and microconidia, all highly virulent fungal strains were identified as F. oxysporum according to iden-
**Molecular identification based on ITS1-5.8S-ITS2 rDNA sequence data analysis.** In this study, the ITS1-5.8S-ITS2 gene region was successfully amplified for all highly virulent strains by the fungal universal ITS1 and ITS4 primers. The size of single fragment 330 bp was clearly observed at the capillary gel electrophoresis under UV (Figure 3). For each strain, the ITS1-5.8S-ITS2 gene sequences were compared with public domain databases NCBI using BLAST 2.0 (http://www.ncbi.nlm.nih.gov/BLAST). These sequences were identified as having the closest similarity (99–100%) with the *Fusarium oxysporum* in the NCBI GenBank (Table 2). The BLAST analysis in this present study and the ITS rDNA sequence data supported morphological identification.

**TABLE 2**

<table>
<thead>
<tr>
<th>Isolate codes</th>
<th>Disease severity (%)</th>
<th>Morphological identification</th>
<th>ITS identification</th>
<th>Reference Gen Bank Accession No</th>
</tr>
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*Highly virulent strains and disease severity (%) do not differ significantly according to the Duncan test at p<0.05, Host: Allium cepa and rDNA ITS size:330 bp*
FIGURE 3
Amplification of ITS1-5.8S-ITS2 gen region of *Fusarium oxysporum* using the primers ITS-1 and ITS-4. ‘M’ – 100 bp DNA ladder

FIGURE 4
Majority rule and strict neighbor-joining consensus tree for Phylogenetic analysis of strains *F. oxysporum f. sp. cepae* and other *Fusarium* spp.
FIGURE 5
Phylogenetic tree using the neighbor joining method generated nucleotide sequence information of the ITS1-5.8S-ITS2 sequences region of the 41 *Fusarium oxysporum* isolates and other pathogens on onion

For ITS sequences of *Fusarium oxysporum* 41 strains were aligned with the consensus region using CLUSTAL W program. 1000 bootstrap replicates were phylogenetic tree based on sequence analysis of 41 strains of *Fusarium oxysporum* were given on the tree’s internal nodes. According to sequences ITS1-5.8S-ITS2 gen region of *Fusarium oxysporum*, it was observed that the genetic difference between all strains originating from the same population of distinction was very low in this area. This similarity was found to be supported by 99% bootstrap value. When all *Fusarium oxysporum* strains were compared to the reference FOC strain (HQ658965) high degree of phylogenetic similarity was detected. Among these strains, isolate ib-57 in the phylogenetic classification was identified as the closest isolate to the reference FOC isolate in terms of the ITS gen locus. The ib-57 strain isolated from Suluova region, was found to be quite highly (78.3%) virulent. It was determined that these strains were separated into a different arm and formed a different clade compared to different ITS gen region of the NCBI database from other species of *Fusarium* spp. (*Fusarium proliferatum* HQ332533.1, *Fusarium longipes* KF918596.1, *Fusarium brachygibbosum* KJ541486.1, *Fusarium phyllophilum* AB587006.1, *Fusarium equiseti* KY365254.1, *Fusarium fujikuroi* HF679024.1,
Fusarium oxysporum KC119203.1, Fusarium subglutinans JX960431.1, Fusarium verticillioides FJ867932.1) (Figure 4).

When the strains were phylogenetically compared with other disease factors on onion, a different clade has been observed (Figure 5). Fusarium basal rot has been reported in Amasya until the late 1980’s but so far there has been no wide study on the genetic variety of the agent, which occurs actually in all production fields of the Turkey. The present study isolated forty one highly virulent Fusarium oxysporum isolates from the rhizosphere of onion in Amasya. Identification of Fusarium species has been difficult as it relies on minor differences in morphology, and as diverse cultural conditions can cause the same species vary [30]. All Fusarium oxysporum isolates were identified to morphological species level. Then, at the molecular characterisation all forty one isolates were found positive by PCR amplification of ITS region using species universal primers of F. oxysporum. The ITS rDNA gene sequence is most frequently used analysis for phylogenetic studies in the F. oxysporum species because of Fusarium species specificity of this region and they are known to provide better distinction at the sub-species level [31]. The amplification of ITS region of all isolates of F. oxysporum isolates indicate that pathogenic forms of this fungus may have evolved from single ancestry. In this study molecular identification of F. oxysporum was supported with morphological identification and it has been proved that PCR analysis is an effective and rapid way to identify F. oxysporum strains using primer sets FOF1 and FOR4. This present study shows, for the first time, the genetic variety at ITS region of F. oxysporum f.sp. cepae isolates obtained from Amasya in Turkey. The acknowledged DNA barcode for Fungi is the rDNA ITS regions. ITS is accepted as a fungi barcode, because it is the most sequenced area of fungi and it is always used for phylogenetic systematics and identification method [32].

In a characterization study conducted by Morales-Barrera et al. [33] isolated pathogen fungi from the soil was identified as Fusarium lichenicola by the D1/D2 domain sequence of its according the 28S rDNA gene. In another study, Bailey et al. [34] have identified 73 Fusarium solani species according to sequences of fungal internal transcribed spacer region of the nuclear ribosomal DNA (ITS nrDNA). Due to the fact that this disease agent has been identified in different regions of the world, there is limited information in Turkey with respect to the genetic and pathogenic variability of FOC. Study on virulent diversity of FOC by Southwood et al. [21] showed that at the positive control experiments, isolates of F. oxysporum f.sp. cepae, caused 80 to 100% highly virulent disease in all inoculated bulbs. Malathi and Mohan [35] used RAPD markers to characterize 12 F. oxysporum f. sp. cepae isolates collected from different geographical regions; reported that RAPD fingerprints showed genetic specificity and diversity (range of 14–85%) among the isolates. The genetic diversity among the strains of FOC in Turkey was revealed by RAPD analysis. Bayraktar et al. [36] at an PCR–RFLP analysis study has revealed low level of pathogenic and genetic diversity among isolates of F. oxysporum f. sp. cepae in Turkey. Similar results have been obtained recently in our study. Esfehani [37] showed during pathogenicity tests that F. oxysporum f. sp. cepae strains were pathogenic but virulence variability among the isolates in onion in Iran. At the same time their research randomly amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR) used for analyses 26 F. oxysporum f. sp. cepae isolates obtained from the main onion- growing regions of Iran. These results are in parallel with those of ours studies, which indicated variability in the virulence and genetic of F. oxysporum f. sp. cepae isolates obtained from onion.

CONCLUSIONS

The conclusion obtained in this research define degree of genetic variability within populations of FOC in Amasya. This study showed that FOC were pathogenic on onion but virulence varied. The phylogenetic analysis based on the fingerprints obtained through ITS analysis shows the presence of low genetic variety among FOC isolates in Amasya. At the same time the strains showed rarely genetic difference in terms of ITS gen region from the reference isolate F. oxysporum f. sp. cepae (Acc.No.HQ 658965). This low genetic diversity is thought to be caused by temperature, humidity and various environmental conditions. All FOC isolates come together into one origin in the dendrogram. Also, the ITS technique has been successfully used to identify variability within FOC. In addition, the present study contributed to the knowledge on the diversity of FOC on onion.

ACKNOWLEDGEMENTS

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FOREST ROAD DESIGN WITH CAD SOFTWARE: A CASE STUDY IN THE WESTERN BLACK SEA REGION OF TURKEY

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ABSTRACT

It is easier to make the projects and applications of forest roads on the terrain through developed technological tools in today. Road planning and designing by computer aided software is become very demanding task for road engineers. In this study, a total of 14 forest roads within the boundaries of the Bolu Forest Regional Directorate (BFRD) were designed with CAD-based Civil 3D and Plateia software. The suitability of the software was investigated and the obtained field measurements and software results were compared. In addition, the design speed and quality of the forest road-design stages of the software were revealed. With them, the design of a 1000-m section for each of the 14 forest roads was carried out. The style database (GDF) was prepared according to the geometric standards of forest road and the drawing style was used during the design stages of the roads. The field Excavation measurements and software excavation values for the 14 forest roads were compared in order to determine the suitability of the Civil 3D and Plateia software. There was no statistically significant difference between the field excavation measurements and software excavation volume values, therefore indicating the suitability of the software for forest road design.

KEYWORDS:
Civil 3D, Plateia, road design, Turkey

INTRODUCTION

Forest roads are the most important infrastructural facilities for the utilization of renewable natural forest resources. In order to achieve the goal of planning sustainable forestry activities, a road network needs to be established. In addition to forestry services, forest roads provide economic benefits for the rural population by enabling them to market their products and helping them meet their healthcare, educational and other social needs. Forest roads interact with many technical, economic, environmental and social factors to render these services [1]. Although roads are the first step in the development of forestry resources [2, 3], they are also infamous for bringing about erosion and sedimentation [4, 5, 6, 7] and for adversely impacting wildlife and water resources, all of which devastate the forests in terms of production and other significant forestry activities. Therefore, the implementation of environmentally friendly as well as economically and technically adequate road plans is required. In many countries, forest road networks are realized as a part of the land planning. When a forest road system is planned, many factors must be taken into account, including the condition of the forest, the land structure, climate data, environmental factors, infrastructures, non-wood forest products and services, road-user groups, the value of forest access and national policies [2, 3].

Forest roads are invaluable for providing transportation from the establishment stage of the forest to the production stage. Road design and production studies contribute significantly to forestry activities [8, 9]. The planning and design of forest roads is essential in order for production processes to be carried out efficiently, safely, comfortably and economically [10, 11].

For many years, the economic feasibility of forestry production work has served as the main goal for the establishment and maintenance of forest road networks. However, recently, the utilization of new techniques has paved the way for research evaluating additional factors such as falling considerations, transportation costs, distances, maintenance costs, vehicle types, road categories, friction distances and costs, road surfaces and road space and density values [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23]. New dynamic software has been developed by different companies to provide quick and easy access to and utilization of the findings from these studies. The use of the CAD-based Civil 3D and Plateia software in forest road design was examined in the present study. Among the advantageous features of this combination of GIS and CAD software are its wide use in all engineering branches, its capability of creating geometric standards for a variety of applications and its user-friendly interface. This study the obtained field measurements and software results were compared and investigat-
ed the suitability of the area data measurements obtained by the software. In addition, the designing speed and quality of the forest road-design stages of the software were revealed.

**Design of forest roads.** Today, forest road projects and applications are more easily carried out on the terrain through the use of developed technological tools. Road planning and design via computer-aided software has become a very demanding task for road engineers. Various software products used in the design stage of forest roads include Advanced Road Design, Allplan, AnadeltaTessera, AutoCAD Civil 3D, AutoRoads, Bentley In Roads, Bentley MXROAD, CAD&PILLAR, Carlson Civil, Civil Designer, DIGICORP CIVIL Design, Diolkos, HEADS Pro, HighRoad, HY-SZDL, ISTRAM ISPOL, KeyTERRA-FIRMA, LISCAD, NetCAD, Novapoint, NoviTDM, Optimum Road Design Model, PDS, Plateia, RoadEng, SierraSoftProSt, and VESTRA Roadare. Apart from these, there is RoadEng software, which was developed exclusively for highway design [24].

Although a forest road is a type highway, the technical and economic standards of forest roads differ from highways in terms of the transported forest products [25, 26]. This requires the software to be used in forest road design to have specific geometric standards. However, there is no commercially available software that is widely used exclusively for forest road planning and design.

The Civil 3D and Plateia software was developed for road design and reconstruction and has been used in highway design. This software has dynamic data processing capability which enables planners to make auto-updating revisions on any part of a project, and thus achieve more effective design of roads. With the dynamic data processing, optimal decisions can be made from one stage of design to another in a project [11]. In the literature, dynamic programming has been frequently used in practice in forest road design for the optimization of vertical alignment [27, 28]. Moreover, Akgul and Esin [24] used the dynamic programming to plan an exemplary forest road.

Civil 3D and Plateia software includes a "style" function that allows the user to instantly change the appearance of model objects. With its dynamic modelling capabilities, the software allows the designer to find alternative and improved solutions in a shorter amount of time. By changing any of the properties of objects or groups of objects defined in the design stage process, it is possible to see the simultaneous changes in the values of these objects as well as their respective labels and tables, with no further action necessary.

**Forest roads in Turkey.** The forest area in Turkey covers 21.7 million ha and 53.3% of the total area is productive. The Turkish government owns almost all forests in Turkey and they are managed by Government Forest Enterprises [29]. Forest road construction is more expensive in mountainous areas such as those found in Turkey. Systematic forest-road network planning studies by the General Directorate of Forestry were started in 1964 and completed in 1974. In these studies, a total of 144,425 km of forest roads were planned for productive forest areas. These plans, however, had to be revised due to the development of forestry technology, the requirements of the national forestry enterprises and actual forestry practice. According to the revisions, the total length of planned forest roads amounted to 201,810 km. By 2011, 73.56% of these forest roads, a total of 148,451 km, had been constructed, with the planned forest road density being 20 m/ha. Forest road design is conducted in accordance with Communiqué no. 292 of the Directorate General of Forests in Turkey. Location, route, slope, width and curves of the roads already built in the forests were examined for evaluation. The roads meeting the current forest road standards were included in the new plan while those impossible to re-build even through extensive repair were left out of the road plan [30]. Forest roads in Turkey are divided into three main groups with respect to the load to be transported over them, the objective of their construction and traffic density and loads. These three groups consist of primary forest roads (Type A), secondary forest roads (Type B) and tractor roads.

**MATERIALS AND METHODS**

**Study area.** A total of 14 forest roads within the borders of the Bolu Forest Regional Directorate (BFRD) were planned with CAD-based Civil 3D and Plateia software. The suitability of the software was investigated and the obtained field measurements and software results were compared. The BFRD has a total forest area of 1,036,829 ha, including 504,468 ha of normal forest, 125,284 ha of degraded forest and 406,466 ha of unforested land. The BFRD is located on broken and rough terrain in the Western Black Sea Region of Turkey. The BFRD consists of 12 forest enterprise directorates and covers the whole of Bolu and Duzce provinces. In this study, the construction costs and excavation volumes of the forest roads in Akcakoca, Aladag, Bolu, Duzce, Golyaka and Yigilca Forest Directorates were examined. The lengths of forest roads in the study area range from 1600 to 7800 m. Figure 1 shows the roads within BFRD borders in Turkey.

**Field measurements.** Excavation work contributes the most to increasing the cost of forest road construction. Excavation and fill volumes in levelling work along the road alignment in forest
road projects are taken and their expenses are calculated [31]. Since excavation affects road construction costs the most, the excavation quantities were examined in this study. The excavation volume of the first 1000-m section of each road in the study was found in order to assess the suitability of the Civil 3D and Plateia software. To find the excavation volume, the excavation areas between the profile points (20 m) on each road were measured. The average excavation area between the profiles was calculated by multiplying the distance between the profiles (20 m) by the excavation volume between the two profiles this volume was found with a laser distance measurer, which automatically calculates the triangle via three-edge recognition methods. When the laser distance meter is aimed at the two edges, i.e., the excavation width and excavation slope length, the device automatically finds the other edge of the triangle and calculates the excavation area. Moreover, the road widths and road slopes required during the design of the roads in the software were measured by a laser distance meter at 20 m (the first 1000-m section of each road). In general, excavation volume measurements have been made at 20 m since there is no significant change in road construction [30, 32]. Therefore for each forest road, 51 points (714 points in total) were measured. Coordinates were taken from the differential global positioning system (DGPS: horizontal accuracy: <0.4 m RMS) in order to transfer the location of roads to the Civil 3D and Plateia software for the design of the roads. Similarly, the coordinates were taken on the road axis at a distance of 20 m on a 1000-m section of each road. The coordinates were taken from their beginning, middle and end points of the curves in the roads. In addition, the field and assembly parameters were taken the same.

**Roads design with software.** The design of forest roads was done on 1/25000-scale topographic maps. In order to carry out the Civil 3D and Plateia software design, a digital terrain model (DTM) was created from the contours covering the roads. The points taken from the road alignment were transferred onto the DTM. The points were then merged and the alignment of the roads were defined (Fig. 2). At this stage, the geometric standards used were those of a normal B-type secondary forest road application in Turkey, according to the 292 numbered forest road types listed by the General Directorate of Forestry [30]. Consequently, the average road widths were used to compare the software results with the field results. In the design, a GDF style database was prepared according to the geometrical standards of a normal B-type secondary forest road and the drawing style was then applied. The style database described each of the metric, cross-section and cubic scale phases separately.

**FIGURE 1**
Location map of the study area
First, a GDF (geographic data file) drawing standard (style) was created in the software. The measures and limitations for the metric style, metric spacing, curve radius, etc. were assigned in this GDF style (Fig. 3).

After the horizontal alignment was created in the software, the cross-section was adjusted to closer resemble reality. For this, sample lines (profile points) were created perpendicular to the alignment on the previously prepared DTM and the constraints in the sample line editor of the GDF
style (Fig. 4) were used. According to this, from the beginning, sample lines were defined at 10 m from the right and left of the middle point of the alignment, 20 m at the curves and at the horizontal geometry points.

When forming the assembly, the assembly standards previously prepared for the normal B-type secondary forest roads were used (1:1 in the cut slope, 3:2 in the fill slope, 3:1 in the 1 m-wide ditches [30] (Fig. 5).

According to the defined type of assembly, sample lines were updated at 3 m in curves and 5 m at horizontal alignments in order to increase the sensitivity of the excavation and fill volume calculations. Sample lines were formed after the forest road corridor.

The 1/2000-scale horizontal and 1/200-scale road profiles were created using the GDF style (Fig. 6). The sample lines were updated to increase the sensitivity of the excavation and fill volume calculations, and the forest road corridors and cross sections were thus generated. According to the generated assembly, a standard surface material was defined for the cubic calculations. At this stage, the cubic scale used in highway projects was taken as a basis.

**Statistical Analysis.** The differences between the field excavation volume measurements and the software excavation volume values and between the two software excavation volumes were investigated. The paired samples t-test was applied to determine whether the differences between these values were significant. The paired sample t-test, sometimes called the dependent sample t-test, is a statistical procedure used to determine whether the mean difference between two sets of observations is zero. In a paired sample t-test, each subject or entity is measured twice, resulting in pairs of observations. Common applications of the paired sample t-test include case-control and repeated-measures studies [33]. Normal distribution of the data before analysis was determined to be normal distribution according to One-Sample Kolmogorov-Smirnov Test (P>0.05).

**RESULTS AND DISCUSSION**

**Field measurement results.** The measurement results for the study area forest roads are given in Table 1. A mean road slope of 4.02%, mean road width of 5.40 m and mean unit excavation volume of 8.66 m³ / m were found in the study area. In addition, the average excavation volume in the 1000-m sections was 8661 m³. The highest excavation volume was recorded on the Tatlıdere-33 road, while the lowest was measured on the Karduz-333 road. In a study conducted in the Goller Region (Turkey), Karabacak [34] found a unit excavation volume of 3.91 m³ / m. In another study carried out in the Eastern Black Sea Region (Turkey), Erbas [35] reported the unit excavation volume to be 10.19 m³ / m. In the present study and in the other two studies, the varying unit excavation values can be attributed to the different slope classes of the study areas.

![Figure 5](image5.png)

**FIGURE 5**
Creation of assembly in software: (a) Civil 3D, (b) Plateia

![Figure 6](image6.png)

**FIGURE 6**
Profile of the designed road alignment: (a) Civil 3D, (b) Plateia
**TABLE 1**  
Field measurements for forest roads in the study area

<table>
<thead>
<tr>
<th>Number</th>
<th>Chief - Road Code Number</th>
<th>Mean Road Slope (%)</th>
<th>Mean Road Width (m)</th>
<th>Mean Excavation Area (m²)</th>
<th>Total Excavation Volume (m³) (1000 m)</th>
<th>Unit Excavation Volume (m³/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Caydurt-201</td>
<td>6.33</td>
<td>4.13</td>
<td>10.54</td>
<td>10.057</td>
<td>10.06</td>
</tr>
<tr>
<td>2</td>
<td>Aksu-34</td>
<td>2.23</td>
<td>5.45</td>
<td>9.49</td>
<td>9.233</td>
<td>9.23</td>
</tr>
<tr>
<td>3</td>
<td>Aksu-38</td>
<td>3.68</td>
<td>5.26</td>
<td>8.17</td>
<td>7.966</td>
<td>7.97</td>
</tr>
<tr>
<td>4</td>
<td>Konuralp-296</td>
<td>3.47</td>
<td>6.16</td>
<td>14.91</td>
<td>10.317</td>
<td>10.32</td>
</tr>
<tr>
<td>5</td>
<td>Tatildere-33</td>
<td>6.11</td>
<td>5.08</td>
<td>11.59</td>
<td>11.413</td>
<td>11.41</td>
</tr>
<tr>
<td>6</td>
<td>Aydinpınar-19</td>
<td>5.53</td>
<td>5.18</td>
<td>11.14</td>
<td>10.989</td>
<td>10.99</td>
</tr>
<tr>
<td>7</td>
<td>Aydinpınar-21</td>
<td>2.28</td>
<td>5.99</td>
<td>9.50</td>
<td>9.437</td>
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</tr>
<tr>
<td>8</td>
<td>Balıklı-39</td>
<td>3.94</td>
<td>6.44</td>
<td>9.72</td>
<td>9.542</td>
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</tr>
<tr>
<td>9</td>
<td>Balıklı-67</td>
<td>3.49</td>
<td>6.09</td>
<td>7.55</td>
<td>7.383</td>
<td>7.38</td>
</tr>
<tr>
<td>10</td>
<td>Karduz-329</td>
<td>3.31</td>
<td>5.77</td>
<td>7.31</td>
<td>7.101</td>
<td>7.10</td>
</tr>
<tr>
<td>11</td>
<td>Karduz-333</td>
<td>2.97</td>
<td>4.56</td>
<td>5.28</td>
<td>5.123</td>
<td>5.12</td>
</tr>
<tr>
<td>12</td>
<td>Kargoknar-54</td>
<td>3.21</td>
<td>5.16</td>
<td>7.62</td>
<td>7.491</td>
<td>7.49</td>
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<tr>
<td>13</td>
<td>Karagoknar-56</td>
<td>3.66</td>
<td>6.23</td>
<td>6.81</td>
<td>6.729</td>
<td>6.73</td>
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<tr>
<td>14</td>
<td>Karagoknar-81</td>
<td>6.04</td>
<td>4.07</td>
<td>8.69</td>
<td>8.459</td>
<td>8.46</td>
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</table>

**TABLE 2**  
Forest road excavation volume software results

<table>
<thead>
<tr>
<th>Number</th>
<th>Chief - Road Code Number</th>
<th>Civil 3D Excavation Volume (m³) (1000 m)</th>
<th>(m³/m)</th>
<th>Plateia Excavation Volume (m³) (1000 m)</th>
<th>(m³/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Caydurt-201</td>
<td>1.628</td>
<td>1.63</td>
<td>1.886</td>
<td>1.89</td>
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<td>2</td>
<td>Aksu-34</td>
<td>14.750</td>
<td>14.75</td>
<td>12.397</td>
<td>12.40</td>
</tr>
<tr>
<td>3</td>
<td>Aksu-38</td>
<td>19.573</td>
<td>19.57</td>
<td>20.326</td>
<td>20.33</td>
</tr>
<tr>
<td>4</td>
<td>Konuralp-296</td>
<td>48.564</td>
<td>48.56</td>
<td>50.548</td>
<td>50.55</td>
</tr>
<tr>
<td>7</td>
<td>Aydinpınar-21</td>
<td>4.837</td>
<td>4.84</td>
<td>4.904</td>
<td>4.90</td>
</tr>
<tr>
<td>8</td>
<td>Balıklı-59</td>
<td>4.922</td>
<td>4.92</td>
<td>7.325</td>
<td>7.33</td>
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<tr>
<td>11</td>
<td>Karduz-333</td>
<td>3.955</td>
<td>3.96</td>
<td>3.885</td>
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<tr>
<td>12</td>
<td>Kargoknar-54</td>
<td>19.050</td>
<td>19.05</td>
<td>20.507</td>
<td>20.51</td>
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<tr>
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<td>4.689</td>
<td>4.69</td>
<td>4.379</td>
<td>4.38</td>
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<td>14</td>
<td>Karagoknar-81</td>
<td>2.769</td>
<td>2.77</td>
<td>2.542</td>
<td>2.54</td>
</tr>
</tbody>
</table>

**TABLE 3**  
Paired sample t-test between field results and software results

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>P (&lt; 0.05)</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field Civil 3D</td>
<td>14</td>
<td>8.66</td>
<td>1.789</td>
<td>-1.044</td>
<td>0.316</td>
</tr>
<tr>
<td>Field Plateia</td>
<td>14</td>
<td>11.93</td>
<td>12.195</td>
<td>-1.118</td>
<td>0.284</td>
</tr>
<tr>
<td><strong>Excavation Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civil 3D Plateia</td>
<td>14</td>
<td>8.66</td>
<td>1.789</td>
<td>-0.876</td>
<td>0.397</td>
</tr>
<tr>
<td><strong>Fill Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civil 3D Plateia</td>
<td>14</td>
<td>21.14</td>
<td>24.601</td>
<td>-0.494</td>
<td>0.630</td>
</tr>
</tbody>
</table>

**Software results.** A 1000-m section for each of the 14 forest roads was planned using Civil 3D and Plateia software. All stages of the road design were made in the Civil 3D and Plateia environment. Table 2 gives the 1000-m and 1-m excavation volume values of the roads planned in the software. When Table 2 is examined, it is determined that the software is close to the average unit excavation volume values for 14 roads (Civil 3D: 11.93 m³/m, Plateia: 12.29 m³/m). Akgul and Esin [19] design a sample forest road in the Civil 3D environment using a sample 1/2000-scale topographic map and as a result, they also concluded that Civil 3D software was sufficient for forest road design.
Statistical results. In order to determine whether any difference existed between these values, paired sample t tests were applied. According to test results, there was no statistically significant difference between the Civil 3D excavation and fill volume values and the Plateia values. Moreover, the average software excavation and fill volume values were close to each other, indicating that there was no difference between the Civil 3D and Plateia software performances. There was no statistically significant difference between the field excavation volume measurements and the Civil 3D and Plateia excavation volume values either (Table 3). However, the average values of the Civil 3D and Plateia excavation volumes were found to be 1.38 and 1.42 times higher, respectively, than the average field measurement of the excavation volume. These differences were due to the DTM, which was made up of contour lines derived from the small-scale (1/25000) maps used for the software design of the study area forest roads. As a result of the design of the 14 forest roads, the study found the Civil 3D and Plateia software to be suitable for forest road design.

CONCLUSIONS

A total of 14 forest roads within the borders of the BFRD were planned with Civil 3D and Plateia software and the suitability of the software was investigated in accord with the measurements performed in the field which found a mean road slope of 4.02%, mean road width of 5.40 m and mean unit excavation volume of 8.66 m$^3$/m.

All steps in the design of the roads and drawing styles were done in the Civil 3D and Plateia software environment, using the GDF style database prepared according to the geometrical standards of normal B-type secondary forest roads. The style database designated each of the metric, cross-section and cubic scale phases separately. As a result of the design of the 14 forest roads, the study found the Civil 3D and Plateia software to be suitable for forest road design. Thanks to the dynamic model processing of Civil 3D and Plateia software, changes made at any stage of the project are reflected in all phases of the project simultaneously, even if the project has been completed. Therefore, the time allocated for office and field work is kept at a minimum. In addition, the software alerts users to non-standard drawings and data that they may have erroneously introduced into the database.

Forest road projects employ design and planning processes that are different from those for other roads. While considering the geometrical and economic conditions on the one hand, on the other hand, forest road design needs to comply with the concept of sustainable forestry and the different constraints which emerge as a result. Thus, in order to more successfully and efficiently complete design and planning processes for forest roads, it is necessary to employ software capable of processing dynamic data and meeting different design criteria as well as having the ability to update the effect of changes on any part or all of the design simultaneously for the entire project. Civil 3D and Plateia, having been used effectively in the design of highways, can also be developed for this purpose. The GDF style can also be developed and used effectively for the planning and design of forest roads.

The statistical analysis results indicated no differences between Civil 3D and Plateia software or between the field excavation measurements and the Civil 3D and Plateia excavation volume values. However, the average excavation volume values of the Civil 3D and Plateia software were found to be 1.38 and 1.42 times higher, respectively, than the average field excavation measurement. This difference was attributed to the DTM, which was created using contour lines derived from the small-scale (1/25000) maps used in the software design of the study area forest roads. Generally, maps covering forest areas are produced from large-scale maps. In recent years, unmanned aerial vehicles (UAVs) have been used in the production of forested area DTM s having high-resolution accuracy. With a DTM obtained by this method, it would be possible to determine excavation volume and filling values that are closer to the actual amounts.

The preliminary survey for the road construction was produced without the necessary project preparation, however, the tender dossier included construction excavation volumes and an estimation of the amount of work to be required. Disagreements between the contracting firms and the administration emerged as a result of the tender because the estimated amount of work for a project generally differs from the final amount when the work has been completed. This, in turn, results in contractors sometimes making unfair profits, or building roads that do not meet the standards. In both cases, the administration has difficulty in achieving the desired public benefit. Therefore, forest road construction work should be carried out with tenders based on detailed road projects and not on preliminary field survey reports. Thus, a more rational use of the Forestry Directorate resources can be realized, and the trust level of the contracting firm can be strengthened in the forestry sector. This study showed that by taking advantage of the latest technology, all phases of design studies for forest roads can be made more effective, less time consuming, and at lower cost than with the traditional methods presently employed.
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STUDY OF AN RFID-BASED MEDICAL WASTE CLASSIFIED DISPOSAL MODEL

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ABSTRACT

Medical waste management has strict classified treatment criteria and processes. However, many medical wastes, which are simply disposed of by simple methods like incineration or landfilling, are not classified strictly according to the corresponding standards in reality. Because of the inappropriate classification of medical waste, the bacteria carried by the wastes can lead to cross-infection, resulting in the spread of the disease and environmental pollution. In this paper, we designed the medical waste classified disposal system model based on the existed problems of medical waste classification. This system based on the RFID technology has four modules: inspection and weighing, inbound and temporary storage, classified disposal, and information feedback. There are eight kinds of medical waste disposal methods were compared and analyzed based on the detailed analysis of the system model. At the same time, the benefits evaluation of the system model was presented and the research deficiencies were shown. The application of this system model could strictly regulate the classified disposal of medical waste, effectively reduce the spread of disease and prevent environmental pollution.

KEYWORDS:
RFID, Medical waste, Disposal plant, Classified disposal, Model

INTRODUCTION

Medical waste is a direct or indirect infectious, toxic and other hazardous waste generated by medical treatments, therapies, and other related activities in medical or health institutions [1]. Due to its harmful characteristics, medical waste has a greater social and environmental impact compared to other wastes [1]. As medical wastes may lead to the spread of disease and environmental pollution, there are strict procedures and requirements for its recovery, transportation, and classified treatment. However, in practice, many hospitals and disposal plants do not strictly implement the standards of medical waste management.

Because of the fusion of medical waste classification, insufficient awareness of waste recycling, a large number of medical waste and domestic waste mixed together while at the same time, many medical wastes, which are simply disposed by simple methods like incineration or landfilling, are not classified strictly according to the corresponding standards in reality. The poor management of medical waste is not only baleful for soil and groundwater resources but also the spread of the disease. Additionally, the medical waste, which was originally recyclable, results in a waste of resources for recoverable medical waste. Mixed medical waste and domestic waste also cause cross-contamination of waste, increasing the risk of disease transmission. Medical waste, incinerated without rigorous pre-treatment, lead to significant emissions of toxic and harmful gases such as dioxins, polycyclic aromatic hydrocarbons, hydrogen chloride, and hydrogen fluoride, causing serious environmental pollution.

The problems existed in the classification and disposal of medical wastes have seriously affected the development of national medical and health undertakings, posing a serious security risk to the environment and public health. How to effectively solve the problems existed in the classified treatment of medical waste is exactly significant.

MATERIALS

At present, many scholars are focusing on the treatment of waste. Narayana has identified the most economical and effective way to address the waste management problem by comparing the cost, advantages, and issues of the three main treatment methods of municipal solid waste which is compost, landfill and incineration [2]. Babayemi and Dauda conducted a questionnaire survey to analyze the problems of recycling, treatment, reuse and environment problems of solid waste in Nigeria and found that a large number of solid wastes were not managed by efficient technology [3]. Kumar et al. conducted a survey towards the situations of solid waste management in 59 cities in India. Many existed problems were found including the inadequate manpower, capital, machinery equipment, and corresponding suggestions have been made [4]. Hazardous waste not only poses a serious risk to the
environment but also affects public health. Couto et al. studied the legislative framework, technical application and waste production in Portugal and found that the Portuguese government is currently able to effectively manage and dispose of hazardous wastes, however, more environmentally friendly and efficient methods are needed [5]. Salihoglu critically assessed the problems of current Turkish HWM practices and discussed the challenges to be faced based on the analysis of hazardous wastes in Turkey [6]. A comparative analysis was conducted on the collection, transportation, and treatment of solid waste in eight cities over the past eight years, and Tai et al. pointed out the differences and problems, while at the same time proposed the importance of improving legislation, coordination mechanisms and public education [7]. In recent years, great efforts have been made by the central and local governments to improve the waste management in China. Chen et al. analyzed the current situation of waste management in China, as well as the opportunities and challenges faced, and then proposed some suggestions to integrate the solid waste management framework in order to improve the eco-efficiency of waste system [8]. Zhang et al. analyzed the problems in the collection, separation, recovery, and disposal of solid waste in China and made some suggestions on the improvement of waste management system [9]. There are some differences between the treatment methods of medical wastes and general waste. However, the current research on waste treatment actually provides some ideas for medical waste classified disposal.

In addition, there are many researches on medical waste treatment. Arshad et al. pointed out that there are many problems in the treatment of medical waste, including mixed treatment of medical waste, toxic gases generated during incineration, etc. [10]. In some developing countries, medical waste and hazardous waste are not categorized for disposal. Instead, they are treated together with domestic waste, which posed a significant risk to the municipal workers, the general public, and the environment. Da Silva et al. conducted an investigation and assessment of information on the management, isolation, production, storage, and disposal of medical waste in 91 medical facilities in Brazil, point out the existing problems and give the corresponding suggestions [11]. Marinković et al. proposed an integrated approach to manage medical waste based on a hierarchical structure for the problems from generation to disposal of hazardous medical waste in Croatia, in order to reduce the quantity of medical waste and the potential risks [12]. Abdulla et al. investigated the management of medical waste in hospitals in northern Jordan and found that the most common treatment method for solid medical waste is incineration, for liquid waste, 57% of hospitals being investigated were discharged into the urban sewage system, and the rest is collected by septic tanks [13].

Birpnar et al. conducted a survey on the quantity, collection and temporary storage of medical waste in 192 hospitals in Istanbul, Turkey, they found that 83% of the recyclable materials are separate recycling, and about 25% of hospitals used unsuitable containers for medical waste recycling. 63% of hospitals have the temporary storage room [14]. El-Salam conducted a questionnaire survey on the collection, isolation, temporary storage, transportation, and final disposal of medical waste at 8 hospitals in the Damanhour City of El-Beheira Governorate in Egypt, as same as, the existing problems are analyzed and the corresponding suggestions are given [15]. Patwary et al. adopted a series of sampling strategies and data collection techniques to conduct a medical waste management survey in Dhaka, Bangladesh, in order to assess the status and problems of medical waste treatment [16]. Insa et al. found that there are some differences in classification, collection, storage, transportation, and treatment standards through studying the legislation of medical waste management in 13 regions of Spain. These differences will influence the health, environment, and economy in different degree [17]. Windfeld and Brooks emphasized that unnecessary classification of waste will lead to higher treatment costs and negative impact on the environment. The key way to improve the management level of medical waste is strengthening the education of medical workers and the standardized classification [18]. Savalem et al. conducted research on medical waste disposal procedures, techniques, treatment methods, and current treatment situations in 14 different medical institutions in Libya, then pointed out the problems and gave the corresponding suggestions [19]. Manga et al. evaluated the medical waste management system in Cameroon, the existing problems are pointed out and explored the development of sustainable medical waste management legislation [20]. Zhang et al. analyzed and evaluated the current status of medical waste management in Nanjing through field survey and questionnaire surveys, the corresponding suggestions were put forward according to the investigation to ensure that the harm of medical waste to the environment and health is minimized [21]. The current research has analyzed and evaluated the methods, procedures, and problems of medical waste disposal, which has better realized the classification management of medical waste, and also provided some references and experiences for the research work of this article.

The development of information technology, especially RFID and sensor technology, it provides new ideas and methods for waste disposal. In Germany, RFID systems have been widely used in the recycling of waste in different cities and communities [22]. Wyld combined with the current situation and existing problems of MSW treatment in the United States, then discussed how to use RFID technology to change the way of waste disposal [23].
Waste management in the framework of smart city is studied, and Srikantha et al. put forward a comprehensive waste management model based on the RFID and sensor technology, which involves the collection, transportation, and disposal of waste [24]. Liu and Yao designed a medical waste management system model based on RFID technology and the theory of reverse logistics [25]. Gupta and Kumar developed a waste management system based on RFID and GSM technology to realize real-time monitoring and management of waste information from generation to treatment [26]. Sushma and Sridhar developed smart waste bins using RFID and sensor technology to classify waste and solve problems of waste disposal [27].

Hannan et al. designed the monitoring and management system of solid waste based on RFID and GIS technology to effectively monitor and manage the entire collection and transportation process of solid waste [28]. Cavdar et al. developed a smart garbage collection system with intelligent features and route algorithm optimization, and then demonstrated the system from the perspective of design concepts, feasibility, low-cost and high-efficiency operation [29]. Namen et al. designed the waste management system based on RFID technology in order to ensure that hazardous waste can be accurately delivered to the destination and avoid improper disposal during transportation [30]. Information technology provides new solutions for waste classified disposal, these studies provide experiences for the application of information technology, especially RFID technology, in classifying disposal of medical waste.

In summary, through analysis of current research of solid waste treatment and the application of RFID technology in waste treatment, it is providing some research experiences and theoretical foundation for the research of RFID technology in medical waste treatment.

KEY TECHNOLOGY

The medical waste classified disposal system model takes RFID technology as the core technology and integrates the temperature sensor, humidity sensor, pressure sensor, gas sensor and other environmental monitoring equipment to manage the entire process of medical waste classified treatment.

Radio frequency identification (RFID) technology is a non-contact automatic identification technology. The RFID system is composed of RFID tags, RFID readers and antennas [22] [31]. According to the needs of system model, 13.56MHz high-frequency passive electronic tags are generally used. This kind of RFID tag has the characteristics of fast read-write, lightweight, long life, low cost, various encapsulation, etc., which could be adapted to the complex and bad working environment of medical waste, such as infection, poison, and radiation, etc.

Temperature and humidity sensor is used to monitor the temperature and humidity of medical waste storage areas. The pressure sensor is used to measure the weight of medical waste. Medical waste generates a large amount of toxic gas during the process of treatment. The gas sensor is used to monitor the emission of toxic gas, make it meet the corresponding emission standards and reduce the pollution of the environment.

PROPOSAL OF A RFID-BASED SYSTEM MODEL

Overall System Model. As shown in Fig.1, the medical waste classified disposal system model could be divided into four parts: the first part is the inspection and weighing business, the second part is the temporary storage business, the third part is the classified disposal business and the fourth part is information feedback business. These four business modules constitute the medical waste classified disposal system model.

Part 1. This part is the inspection and weighing business of medical waste in the unloading area of the disposal plant. After the transport vehicle arrives at the disposal plant, the staff of disposal plant will check the information of medical waste and vehicles. It is allowed to unload in the designated loading area if the information is confirmed correct.

Medical waste should be inspected and weighed separately after completion of the unloading process. Within the allowable error range to ensure that the medical waste data information is consistent with the original data. If there is an abnormality, it is necessary to promptly report and identify the cause to ensure the safety of medical waste transportation and treatment.

Part 2. This part is the inbound and temporary storage business of medical waste at the disposal plant. The disposal plant arranges the medical waste disposal schedule depending on the order of “first-in, first-out, last-in and last-out” (FIFO, LILO). Different types of waste may require single or multiple treatment process.

Due to the processing capacity of the disposal plant and the limitation of the operation plan, there is can’t guarantee that all medical waste will be disposed immediately. Medical waste that cannot be disposed immediately needs to be stored in the temporary storage room of the disposal plant according to the category of waste.

Part 3. This part is the classified disposal business of medical waste in the disposal plant. The disposal plant develops the medical waste treatment
plan based on the processing capacity and the time of medical waste entering. Currently, the treatment methods of medical waste mainly include Sanitary landfill, High-temperature incineration, Pressure steam sterilization, Chemical disinfection, Electromagnetic wave sterilization, High-temperature pyrolysis, Dry heat crushing sterilization and Plasma. The disposal plant workers use different treatment methods depending on the type of medical waste. For some special medical wastes, may need multiple treatment methods to ensure that the treatment methods meet the requirements of safety and environmental protection.

Part 4. This part is the treatment information feedback business of medical waste. It is necessary to recover RFID tags after classified disposal the medical waste, at the same time, disposal data of medical waste should be uploaded to the disposal plant data center and regularly report to the government regulatory authorities. The medical waste classified disposal system model based on RFID technology, optimizes the business process from the aspects of inspection and weighing, inbound and temporary storage, classified treatment and information feedback, in order to ensure that medical waste could be treated strictly according to the corresponding standards, and reduce the spread of disease and pollution of the environment.

Medical Waste Inspection and Weighing Model. Medical waste needs to be inspected and weighed after being unloaded to ensure it is consistent with the collection of medical waste from hospitals. If the data of medical waste is inconsistent, the system will automatically issue an alarm, then the staff of the disposal plant can promptly identify the cause and track back the relevant responsible person, this effectively avoids the theft of medical waste during transportation.

1) Weighing. As shown in Fig 2, the forklift carries medical waste to the weighing area according to the instructions. When medical waste passes through zone A of the conveyor belt, the RFID reader installed in zone A will automatically read the information of RFID tag on the waste packaging, and synchronize data to the weighing system and disposal plant data center.

When medical waste passes through the pressure sensor area, the pressure sensor transmits the measured data to the weighing system and calculates the weight of the waste, then uploads the data to the disposal plant data center for comparison. When medical waste arrives in area B, if the comparison result is confirmed within the allowable error range, the RFID reader arranged in area B will write the confirm data into the RFID tag on the packaging, then finished the weighing process. If the results are not consistent, the alarm will be issued, and the staff of the disposal plant will perform the corresponding handle.

2) Inspection. After medical waste is weighed, it needs to be inspected in turn. The medical waste information, including name, weight, category, date of the generation, source, etc., could be inspected by
FIGURE 2
Inspection and weighing model

FIGURE 3
Inbound and temporary storage model
fixed RFID readers or hand-held RFID reader. At the same time, the data should be uploaded to the disposal plant data center for check, and then finished the inspection process after confirmation. If an abnormality occurs, it must be quickly reported and found out the reasons to ensure the safety of medical waste transportation and treatment.

Medical Waste Inbound and Temporary Storage Model. Due to the daily treatment plan and the processing capacity, the disposal plant can’t guarantee to arrange deal with the medical waste immediately. For medical waste that cannot be disposed in time, it should be stored in the temporary warehouse of the disposal plant. As shown in Fig 3, the waste temporary warehouse is mainly classified into infectious, pathological, injury, drug-induced, chemical, and recyclable, then storage of corresponding medical waste respectively.

1) Inbound. For medical waste that cannot be disposed in time after inspection and weighing, it should be stored in the temporary warehouse. When the forklift carries medical waste to the temporary warehouse, RFID readers arranged at the entrance of the warehouse will automatically read the information of RFID tags on the waste packaging and upload it to the disposal plant data center for comparison. The forklift will be allowed to enter the storage area if the data are consistent. At the same time, the disposal plant data center allocates storage room for the wastes according to the summarized data, and the forklift carries medical waste to the corresponding storage room according to the instructions.

2) Entering the storage room. When the forklift carries medical waste to the corresponding storage room, the RFID reader disposed at the entrance of the storage room will automatically read the information of RFID tags on the waste packaging and check the type of medical waste whether conform to the storage room. The medical waste is allowed to enter the storage room if the data are consistent, an alert will be issued to remind that put the waste correctly if the data are not consistent. The disposal plant will complete the data synchronization and updates automatically after the medical waste is put into the storage room.

Medical waste Classified Disposal Model. As shown in Fig 4, the disposal plant processes different kinds of medical waste depending on the daily treatment plan and processing capacity. Since medical waste has the strict temporary storage time limit, the principle of “first in, first out, last in, last out.” (FIFO, LILO) is generally adopted to arrange for the disposal of medical waste. Some medical waste may need to combine with various methods to disposal, so as to ensure that waste disposal meets safety and environmental requirements in order to reduce the spread of disease and environmental pollution. Current treatment methods of medical waste mainly include eight kinds of methods: Sanitary landfill, High-temperature incineration, Pressure steam sterilization, Chemical disinfection, Electromagnetic wave sterilization, Plasma method, Dry heat crushing sterilization, High temperature pyrolysis.
wave sterilization, High-temperature pyrolysis, Dry heat crushing sterilization and Plasma.

1) **Outbound.** The disposal plant processes the medical wastes according to the schedule of treatment and sends out medical waste outbound instructions. The forklift enters the corresponding waste temporary storage room to carry it outbound according to the type of medical waste after received the instructions.

When the forklift carry medical waste away from the temporary storage area, the RFID readers, which are arranged in the storage room and entrance of the warehouse, will automatically read the data of RFID tags on the waste packaging to ensure that it conforms to the principle of “FIFO, LILO”, at the same time, check with the data of disposal plant to confirm is correct, then allow outbound and the disposal plant data center will automatically complete the data update.

2) **Classified disposal.** After medical waste enters the disposal procedure, eight types of treatment method that Sanitary landfill, High-temperature incineration, Pressure steam sterilization, Chemical disinfection, Electromagnetic wave sterilization, High-temperature pyrolysis, Dry heat crushing sterilization and Plasma are used depending on the type of waste. For medical waste that requires multiple treatments, it is should be processed in turn according to the corresponding treatment procedures. The packages need to be opened up before processing, and the server will automatically record the result of medical waste treatment, and match data with the RFID tags on the outer packaging of the waste, then upload the data to the disposal plant data center to complete the process.

The smoke, temperature, humidity, gas, and other sensors arranged in each treatment area could effectively monitor the environmental information to ensure that the disposal environment meets the corresponding standards, if an abnormality occurs, it can be alerted and handled in time. The camera could effectively monitor the situation, ensure the safety of the disposal plant, avoid the theft of the situation, and ensure that the relevant work processes comply with the relevant regulations.

**Medical Waste Information Feedback Model.** As shown in Fig 5, disposal information feedback of medical waste mainly includes data interaction and RFID tags recycling. The disposal data need to summarize and save after the medical wastes completed the classified disposal procedures, at the same time, RFID tags should be recycled in order to be used again.

1) **Data interaction.** After the medical waste is disposed, the server will automatically record the information such as the disposal date, disposal method, and disposal result of the medical waste, and compare with the RFID tags on the waste packaging. At the same time, the result of comparison is uploaded to the disposal plant data center to complete the data update. When the disposal plant data center completes the data update, the system will exchange data with hospital waste management center to ensure the data is consistent on both sides. Medical waste disposal data should report to the government is regularly in order to inquiries and supervision.

2) **RFID tags recycling.** RFID tags need to be recycled after medical waste is classified treatment. In the RFID tags recycling area, the RFID tags information that needs to be recycled is read through the RFID reader, at the same time, it is compared with the disposal plant data center to ensure that the recycled RFID tags are consistent with the RFID tags on the waste packaging. RFID tags information of the disposal plant data center is updated automatically after it is recovered.

This paper studies the medical waste disposal model based on RFID technology from these four key business modules that inspection and weighing, inbound and temporary storage, classified disposal and information feedback, and analyzes the key links in each module, which provides a certain theoretical basis for the practical application of classified disposal model.

**DISCUSSION**

This paper mainly evaluates the system model from 3 aspects of medical waste classified disposal method model, disposal method comparison and benefits evaluation.

**Classified Disposal Methods.** Disposal methods of medical waste are mainly divided into the eight kinds that Sanitary landfill, High-temperature incineration, Pressure steam sterilization, Chemical disinfection, Electromagnetic wave sterilization, High-temperature pyrolysis, Dry heat crushing sterilization and Plasma. The basic principles are shown in Fig 6, different types of medical waste need to be disposed with different methods, for some medical wastes, may need combined with a variety of disposal methods, and in order to reduce the emission of harmful gases and toxic substances such as dioxins and sulfur dioxide, then to reduce the soil, water, and air pollution.
High temperature incineration
Pressure steam sterilization
Chemical disinfection

Sanitary landfill
High temperature pyrolysis
Dry heat crushing sterilization
Plasma method

RFID tag Recycling
Forklift Park Area

Circulation area
RFID reader
Classified disposal

RFID reader
Classified disposal

Electromagnetic wave sterilization

RFID reader
Classified disposal

RFID reader
Classified disposal

RFID reader
Classified disposal

RFID reader
Classified disposal

RFID reader
Classified disposal

FIGURE 5
Information feedback model

Sanitary Landfill
Medical waste is buried underground and converted into harmless substances through the long-term action of microorganisms.

High Temperature Incineration
Medical waste is converted into residues and gases after drying, igniting, incineration.

Pressure Steam Sterilization
The microbial protein of medical waste was coagulated and denatured under the condition of 100 kPa and 121°C.

Chemical Disinfection
The medical waste is mixed with disinfectant after crushed.

Electromagnetic Wave Sterilization
High-frequency oscillations of electromagnetic waves increase the cell membrane energy of micro-organisms, generates high temperature and eventually kills pathogen.

High Temperature Pyrolysis
Heated to 600-900 degrees Celsius in the absence of oxygen or in oxygen-deficient conditions and convert the medical wastes into combustible gas, liquids and coke.

Dry Heat Crushing Sterilization
Far-infrared dry-heat sterilization equipment is used to kill microorganisms.

Plasma
Through ionized inert gas, which causes the organic waste to dehydrate and pyrolyze at high temperature to destroy pathogenic microorganisms.

FIGURE 6
Classified disposal method model [32] [33] [34] [35] [36]
**Classified Disposal Comparison.** As shown in Table 1, eight disposal methods for medical waste have different advantages, disadvantages, and applicable range. In actual situations, we must select the appropriate disposal method according to the type of medical waste, with a low cost, high efficiency, high volume, weight reduction, and low pollution to complete the treatment of medical waste.

**Benefits Evaluation.** As shown in Fig 7, this paper evaluates RFID-based medical waste classified disposal model in terms of cost-benefits, environmental benefits, and social benefits.

1) **Cost-benefits.** In order to convenient for the application of RFID technology, waste disposal plant needs to improve the corresponding software and hardware equipment. RFID tags and reader devices could be used in a rental way, which avoids the financial pressure of one-time purchase for disposal plants, at the same time. RFID tags could be reused, and then reduced the cost of use. The software could be developed according to the module customization, which reduces the cost of system construction and maintenance. All kinds of sensors, video surveillance equipment, computer room and other hardware devices will be put into operation for a long time, with a longer investment return period.

The business that inspection and weighing, inbound and temporary storage, classified disposal, and information feedback can be processed automation. Medical waste information can be read, wrote, queried in real time by RFID readers, real-time monitoring of data, it reduces the inefficiencies and inaccurate data caused by the manual operation, improves the work efficiency of disposal plants, and reduces manpower and material cost expenditures.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages: simple process, low investment, a large amount of medical waste can be disposed, etc.</th>
<th>Disadvantages: need to be disinfected before landfill, less waste reduction, occupy large amounts of land, pollute the soil and ground water, etc.</th>
<th>Disposal Range: Medical waste after strict pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanitary Landfill</strong></td>
<td><strong>Advantages:</strong> the volume and weight of medical waste are significantly reduced, sterilization and pollutant removal effect are good, and the heat energy could be recycled, etc.</td>
<td><strong>Disadvantages:</strong> high cost, serious air pollution, easy to generate toxic gas, and flue gas and residue need to dispose harmlessly, etc.</td>
<td>Suitable for all kinds of infectious medical waste</td>
</tr>
<tr>
<td><strong>High-Temperature Incineration</strong></td>
<td><strong>Advantages:</strong> low investment, easy to detect, low residue risk, good disinfection effect, and a wide range of wastes suitable for disposal, etc.</td>
<td><strong>Disadvantages:</strong> the volume and appearance of waste are essentially unchanged, it is easy to generate odors and pollute the air, etc.</td>
<td>Suitable for disposal of contaminated waste, not suitable for formaldehyde, phenol, mercury and pathological waste</td>
</tr>
<tr>
<td><strong>Pressure Steam Sterilization</strong></td>
<td><strong>Advantages:</strong> simple operation, good deodorization effect, quick disinfection, low investment, low operation cost, etc.</td>
<td><strong>Disadvantages:</strong> dry waste requires high pH value monitoring, and wet waste disposal process generates liquid waste and exhaust gas, etc.</td>
<td>Suitable for disposal of liquid and pathological waste</td>
</tr>
<tr>
<td><strong>Chemical Disinfection</strong></td>
<td><strong>Advantages:</strong> the volume of medical waste is significantly reduced, good destruction effect, small environmental pollution, and easy operation, etc.</td>
<td><strong>Disadvantages:</strong> high cost of construction and operation, poor effect of weight reduction, bad odor, and unsuitable for disposal of blood and hazardous chemicals, etc.</td>
<td>Medical waste other than blood and hazardous chemicals</td>
</tr>
<tr>
<td><strong>Electromagnetic Wave Sterilization</strong></td>
<td><strong>Advantages:</strong> thorough disposal, low generate of hazardous substances, lower disposal cost than conventional incineration, no pretreatment and classification, etc.</td>
<td><strong>Disadvantages:</strong> poor thermal performance, carbon in the residue is not easy to burn out; environmental protection indicators are high, etc.</td>
<td>No obvious selectivity for medical waste</td>
</tr>
<tr>
<td><strong>High-Temperature Pyrolysis</strong></td>
<td><strong>Advantages:</strong> good sterilization effect, low construction and operation cost, and disposed waste can be landfilled or comprehensively utilized, etc.</td>
<td><strong>Disadvantages:</strong> Need pre-treatment, slow heat conduction, easy to generate odor, pollute the air, etc.</td>
<td>No obvious selectivity, no pretreatment and classification required</td>
</tr>
<tr>
<td><strong>Dry Heat Crushing Sterilization</strong></td>
<td><strong>Advantages:</strong> Low exudation, high volume reduction, high strength, high disposal efficiency, no emission of harmful substances, and heat energy can be recycled, etc.</td>
<td><strong>Disadvantages:</strong> high cost of construction and operation, poor system stability, and the reliability need to be verified and improved, etc.</td>
<td>Any kind of medical waste can be disposed</td>
</tr>
</tbody>
</table>

**Table 1:** Disposal Methods Comparison [32] [33] [34] [35] [36]
2) **Environmental benefits.** All kinds of sensors arranged in the disposal plant can effectively monitor the environmental information of the waste, and ensure that the temporary storage and treatment environment meet the corresponding standards. Medical waste is disposed strictly according to the rules of “FIFO, LILO” to avoid bacterial cross-infection due to long storage time, and result in disease transmission and environmental pollution.

RFID technology could achieve medical waste classified disposal, different types of medical waste should adopt different treatment methods, and some medical wastes require multiple methods for joint treatment. Strict classified disposal procedure could effectively reduce the emission of toxic gases, waste water and waste residues, and reduce the pollution to air, water and land.

3) **Social benefits.** The classified disposal model based on RFID technology could treat different types of medical waste separately, reduce the pollution of the environment, and change the public’s negative impression on the treatment of medical waste. The social image of the government, hospitals, and disposal plants has been improved. The level of management and classification of medical waste, the informatization and automation of the disposal plant has been upgraded.

Through analysis of medical waste classified disposal methods, disposal method comparison and benefits evaluation, which illustrates the importance of strengthening medical waste classified disposal, and also confirms the important role of RFID technology in medical waste classified disposal.

### CONCLUSION

According to the problems existing in the classified disposal of medical waste, this paper designed the classified disposal model of medical waste based on RFID technology from four aspects that inspection and weighing, inbound and temporary storage, classified disposal, and information feedback. The business process of medical waste classified disposal is optimized and standardized through technical model design. On this basis, 8 kinds of medical waste disposal methods and the applicable range of each disposal method are analyzed in detail. At the same time, it provides some support for the practical application through analyzed the benefits of RFID technology model.

Through the application of the system model could effectively standardize classified disposal of medical waste, realized the safety, environmental protection and harmless treatment of medical waste, and reduce the situation of environmental pollution and disease transmission caused by improper treatment of medical waste.

There are some limitations in this research: 1) this paper provided the system model, but the corresponding simulation experiments not designed to verify the model.2) There isn’t quantitative analysis in this paper due to lack of detailed data. The insufficiency of this article is also the key research direction in the future.

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FRUIT DEFORMATION IN THE OLIVE GROVES OF AKHISAR-TURKEY: SOIL CHARACTERISTICS AND THE BORON CONTENT OF LEAVES AND FRUITS

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ABSTRACT

In the current study, the relation between boron nutrition of olive trees cv Domat and the boron content of the soils in two olive groves, one grove with deformation in its fruits and the other without any deformation / healthy was examined.

Leaf and fruit flesh were sampled and their boron concentrations were measured. The physical and chemical properties of the soil it is cultivated on was also examined and the relation between the soil characteristics and boron content of the studied plant parts was determined. Domat cv is considered as edible in the region and in this regard, fruit deformation is highly significant. However, in our present study, no statistically significant relationship was determined between deformation of Domat olives and their boron concentration.

KEYWORDS:
Olive cv domat, boron, plant nutrition, soil, micro element

INTRODUCTION

Boron (B) is one of the most important secondary plant nutrients affecting olive yield and quality. Its deficiency not only prevents the formation of buds, flowers and fruits but also can lead to the deformation of fruits. Deformation is a significant problem especially in the olive cultivars for table. In the Akhisar province of Ege region –Turkey, the olive cv. Domat which is for table is believed to be widely affected by B deficiency.

Boron which has many functions in plants plays an important role both in the formation of the cell membrane and cell wall and in the carbohydrate and protein metabolism. There are great differences between plants in the way they forms B complexes with the components of the cell wall structure of the stem, roots and vegetative parts of a tree. These differences result from the size of these complexes and the plant B requirements and uptakes depending on their water consumption [1].

There is a very little difference between B content of the soils which can cause B deficiency or toxicity in plants. Therefore, compared to other plant nutrients, the signs of B deficiency and toxicity may occur more commonly in a narrower range [2]. Boron deficiency or excess B in agricultural soils not only leads to nutritional disorders, reduced yield and lower quality in plants but also can affect animal and human health through plants [3-4]. Boron deficiency is generally related to soil pH and CaCO3. It is stated that B deficiency is common in calcareous soils and drought enhance the deficiency. In such cases, it is recommended to use ‘Sodium tetraborate decahydrate’ [3-4].

B requirement of plants is very low and deficiency can be apparent especially in sensitive species [5]. Symptoms of B deficiency in the leaves are apparent when the B concentration falls below 15 mg kg⁻¹ in the dry matter [6]. Reported that [7] B application (Borax, 11% B) do not affect the N, P, K, Ca and Mg contents of the leaves but has a positive effect on flowering in early spring. B deficiency becomes more serious under drought conditions [8]. In extremely rainy or hot years, or when the soil stays wet for a long time, B deficiency gets worse [9]. To overcome the lack of B, it is recommended to incorporate 40 g of borax to the soil under each tree in the first year and the common annual maintenance dose of borax is 25 g per tree. The other alternative is a foliar application. Foliar fertilization is performed with a 0.5% solution prepared from a chemical containing 20.8 % B [10].

The objective of this study is to determine the relations between B nutrition of olive trees and the B content of the two Domat olive groves, one grove with deformation in its fruits and the other one without any deformation / healthy in fruits.

MATERIALS AND METHODS

The study was conducted in two olive groves in Akhisar, a district of Manisa province in Western Turkey, situated between 38° 48' North latitude and 27° 50’ East longitude. One grove had healthy and the other grove deformed fruits. From each grove, leaf and fruit samples were collected from 10 olive
trees cv Domat. Domat is an edible table variety and generally grown under irrigated conditions. Leaf samples were collected from the leaf pairs in the middle of one-year old shoots from all sides of the trees, dried at 65°C, ground and prepared for analysis. Fruit samples were also collected from all sides of the trees, then flesh was separated from seeds, dried at 65°C, ground and prepared for analysis. Soil samples were taken from two different depths [(0-30cm) and (30-60cm)] in order to represent the nutrient status of the olive groves, then air-dried in the laboratory and sifted through a 2-mm sieve for analysis.

Within the scope of the project, the sampling started in 2011 September and ended in the August of 2013. The periods and dates are given in Table 1.

<table>
<thead>
<tr>
<th>Sampling Dates</th>
<th>Sample Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>September, 2011</td>
<td>Leaf-Fruit</td>
</tr>
<tr>
<td>May, 2012</td>
<td>Soil</td>
</tr>
<tr>
<td>August, 2012</td>
<td>Leaf-Fruit</td>
</tr>
<tr>
<td>September, 2013</td>
<td>Leaf-Fruit</td>
</tr>
</tbody>
</table>

The B concentration of all the samples was determined with the ICP-MS instrument. To determine the B concentration of the soil samples, regular hot water extraction method was used whereas the B content of the plant samples was determined by wet digesting the samples with a mixture of concentrated H₂O₂ + concentrated HNO₃ acid in a microwave oven [11]. In addition to B content, pH levels of the soils [12], lime [13] and calcium [14] contents which are thought to be related to B uptake were determined. Results were statistically analyzed with the SPSS statistical software package [15].

RESULTS

Soil. The pH level of the soils was found 7.84 (slightly alkaline) in the 1st depth (0-30cm) and 8.03 (mild alkaline) in the second depth (30-60cm) of the olive grove where the fruits were not deformed / healthy. The pH levels of the soils collected from the olive grove with deformed fruits were as follows: 7.61 (slightly alkaline) in the 1st depth and 7.89 (mild alkaline) in the second depth (Table 2).

Lime content in the soils of the olive groves with healthy undeformed fruits was 3.51% in the first depth (calcareous) and 5.85% in the second depth (marn). In this regard, the lime in the olive groves with the deformed fruits was 3.51% in the first depth (calcareous) and 19.50 % in the second depth (marn).

FIGURE 1
Undeformed fruits

FIGURE 2
Deformed fruits
TABLE 2
Soil properties of the experimental groves

<table>
<thead>
<tr>
<th>Soil Classification</th>
<th>Depths</th>
<th>Texture</th>
<th>pH</th>
<th>Lime %</th>
<th>Ca (mg kg⁻¹)</th>
<th>B (mg/kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groves with</td>
<td>Typical Rendoll</td>
<td>0-30</td>
<td>Loamy</td>
<td>7.8</td>
<td>3.51</td>
<td>3985</td>
</tr>
<tr>
<td>undeformed fruits</td>
<td>Typical Rendoll</td>
<td>30-60</td>
<td>Loamy</td>
<td>8.0</td>
<td>5.85</td>
<td>6641</td>
</tr>
<tr>
<td>Groves with</td>
<td>Typical Rendoll</td>
<td>0-30</td>
<td>Clayey Loamy</td>
<td>7.6</td>
<td>3.51</td>
<td>3698</td>
</tr>
<tr>
<td>deformed fruits</td>
<td>Typical Rendoll</td>
<td>30-60</td>
<td>Clayey Loamy</td>
<td>7.8</td>
<td>19.50</td>
<td>20544</td>
</tr>
</tbody>
</table>

TABLE 3
Average B concentrations of the leaves during 2011-2013

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Boron (mg/kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trees with deformed fruits</td>
<td>22.46</td>
</tr>
<tr>
<td>Trees with undeformed fruits</td>
<td>21.20</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.58</td>
</tr>
<tr>
<td>September, 2011</td>
<td>15.93a</td>
</tr>
<tr>
<td>August, 2012</td>
<td>24.96b</td>
</tr>
<tr>
<td>September, 2013</td>
<td>24.60b</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.71</td>
</tr>
<tr>
<td>September, 2011</td>
<td>16.45</td>
</tr>
<tr>
<td>Trees with undeformed fruits**</td>
<td>15.40</td>
</tr>
<tr>
<td>Trees with deformed fruits**</td>
<td>24.57</td>
</tr>
<tr>
<td>September, 2013</td>
<td>25.36</td>
</tr>
<tr>
<td>Trees with undeformed fruits**</td>
<td>26.38</td>
</tr>
<tr>
<td>Trees with deformed fruits**</td>
<td>22.83</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>1.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
</tr>
<tr>
<td>Sampling Date</td>
</tr>
</tbody>
</table>

*: overall average of the B concentration of the olive leaves

**: average of the leaf B concentrations with respect to sampling date

ab means within column with no common superscripts differ significantly (P < 0.05).

In terms of soil texture, results revealed that the olive groves with undeformed fruits had loamy soils whereas the olive groves with deformed fruits had loamy clayey soils. In this present study, the findings related to the calcium (Ca) content of the soils in the deformed groves showed that the amount of Ca was 3698 mg kg⁻¹ in the first depth and 20544 mg kg⁻¹ in the second depth whereas it the other grove under consideration was 3985 mg kg⁻¹ in the first depth and 6641 mg kg⁻¹ in the second depth.

The B content of the soils in the olive groves where the fruits were not deformed was 3.66 mg kg⁻¹ in the first depth and 4.15 mg kg⁻¹ in the second depth whereas it was 2.99 mg kg⁻¹ in the first depth and 3.72 mg kg⁻¹ in the second depth in the olive groves with deformed fruits.

Boron Concentration of the Leaves. The mean leaf B concentration of the olive trees with healthy undeformed fruits determined at the below given three sampling dates were 15.40 mg kg⁻¹, 25.36 mg kg⁻¹ and 22.83 mg kg⁻¹ (Table 3). The mean leaf B concentrations of the olive trees with deformed fruits were 16.45 mg kg⁻¹, 24.57 mg kg⁻¹ and 26.38 mg kg⁻¹ respectively. Statistical results showed that the leaf B significantly change with respect to sampling date; however, not with the fruit deformation.

Boron Concentration of the Fruit Flesh. The mean B concentration of the fruit flesh samples from the olive trees with undeformed fruits were 11.44 mg kg⁻¹ in the first sampling date, 9.45 mg kg⁻¹ in the next sampling date, and 9.81 mg kg⁻¹ in the last sampling date (Table 4).

The mean B concentration of the fruit fleshes taken from the olive trees with deformed fruits were 11.19 mg kg⁻¹ in the first sampling date, 7.51 mg kg⁻¹ in the next sampling date, and 14.25 mg kg⁻¹ in the last sampling date. According to the results of statistical analysis, fruit flesh B contents did not change with respect to fruit deformation but did change with respect sampling dates. However, B measurements in September 2011 did not statistically differ from September 2013. Similarly, August 2012 and September 2011 resultswas in the same statistical group.
TABLE 4
Boron Concentrations of the Fruit Flesh (mg/kg⁻¹)

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Deformed fruits</th>
<th>Undeformed fruits</th>
<th>Pooled SEM</th>
<th>Deformed fruits</th>
<th>Undeformed fruits</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>September, 2011</td>
<td>10.98</td>
<td>10.23</td>
<td>0.82</td>
<td>11.32abc</td>
<td>10.23</td>
<td>0.84</td>
</tr>
<tr>
<td>August, 2012</td>
<td>8.48b</td>
<td>9.45</td>
<td>1.01</td>
<td>9.45</td>
<td>9.81</td>
<td>1.42</td>
</tr>
<tr>
<td>September, 2013</td>
<td>12.03a</td>
<td>9.81</td>
<td>1.01</td>
<td>12.03a</td>
<td>9.81</td>
<td>1.42</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Probability

<table>
<thead>
<tr>
<th></th>
<th>Fruit</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5205</td>
<td>0.0374</td>
</tr>
</tbody>
</table>

a,b Means within column with no common superscripts differ significantly (P < 0.05).

DISCUSSION

In the study, the deformation of olive fruits, soil properties and tree performances with respect to B was examined in two groves, one grove with deformed fruits and the other one without deformed fruits. Therefore, soil, leaf and fruit flesh and seed samples were analyzed and results of the two groves, with and without fruit deformation, are compared and evaluated with the worldwide cited reference values.

The soil of the olive groves was determined to be between slightly and moderately alkaline [16]. In a study carried out in Ayvacık-Çanakkale where the olive cv Ayvalık is intensively cultivated, the soil reactions were reported to be neutral, slightly alkaline and mild alkaline [17]. Similarly, in several studies conducted by different researchers in different parts of the Mediterranean Basin, soil pH in olive groves was stated to vary between 6.5 and 8.1 [18].

Our experimental site where the sampling was conducted is a great Typical Rendoll soil group formed on the marn parent material rich in lime. The lime contents of the soils were evaluated according to [19]. The first depths of the soils were calcareous, whereas their second depths were marn. Reported that [20] lime content of soils where olives are cultivated should range between 9% and 19%. He also reported that olives can be cultivated in the soils containing high levels of lime too.

The texture of our experimental soils on the undeformed olive groves was loamy whereas on the healthy groves was loamy clay. Reported that [21] olive groves in Ayvalık region have clayey or loamy clayey soil. [22] study conducted in the Research and Production Farm of Olive Research Institute, Kızıldağ, İzmir, the soils were mostly loamy or sandy clayey in texture. [17] study conducted in Çanakkale - Ayvacık, olive groves had sandy loam soils. All the pertinent literature shows that olives can be easily grown in soils with different textures.

When the soils of the olive groves were analyzed in terms of their Ca according to [14], results showed that the experimental soils contained very high Ca. [23] claimed that Ca is important and that Ca content of soils should be more than 2000 mg kg⁻¹ for olive cultivation.

The soils, if evaluated with respect to their B contents, were high in this regard [24]. However, B content of the soils in the olive groves where the fruits were deformed was found to be lower than that of the other grove. However, the difference didn't seem to be very important. In addition, although the amount of B in the olive grove where the fruits were deformed was high, B uptake is thought to be blocked due to very high amount of lime in the second soil depth. [17] stated that B content of soils in most of the olive groves was sufficient, some was found low. According to [25], sufficient B is 0.2-0.3 mg kg⁻¹ in sandy soils, and 0.3-0.6 mg kg⁻¹ in the clayey soils.

When examined according to [26], the leaf samples were low or sufficient in B. [17] reported that the leaf B content of Ayvalık olive trees studied for two years in conventionally and organically cultivated olive groves ranged from 3.11 to 19.43 mg kg⁻¹ and from 11.73 to 13.88 mg kg⁻¹, respectively. In the second year, B ranged from 10.87 to 14.88 mg kg⁻¹ and from 9.70 to 16.16 mg kg⁻¹, respectively. [27] determined the in the leaves of Gemlik cv. between 14 to 26 mg kg⁻¹ [28] between 14.63 to 43.50 mg kg⁻¹ [21] investigated Ayvalık cv olive trees and
stated the leaf B concentration of the leaves as 9.24 to 66.17 mg kg⁻¹.

In a study by [29], Ayvalık variety fruits contained between 12.50 and 46.00 mg kg⁻¹ B. In another study of the same author the fruits of the olive trees in Edremit contained B between 11.50 to 48.00 mg kg⁻¹ in their flesh.

CONCLUSION

Deformation is a serious problem for olive growers in Turkey, especially for the edible table varieties. Deformation in this regard is generally related to B deficiency. However, in our present study, no statistically significant relationship was determined between deformation of Domat olive fruits and their B content against the belief of growers.

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MOLECULAR CHARACTERIZATION AND EMERGENCE OF CHILI LEAF CURL VIRUS (CHLCV) INFEETING CHILI CROPS IN PAKISTAN

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4Forestry and Environment Research Institute, Fujian Agriculture and Forestry University, China

ABSTRACT

Among many other plant diseases Chili Leaf Curl Disease (ChLCD) caused by Begomovirus complex is a crucial threat for chili worldwide. Chili Leaf Curl Virus (CHLCV) is monopartites DNA Begomovirus having DNA-A which comprises of 2754 nucleotides having six ORFs and betasetallite of size 1.3kb. Genomic DNA of infected chili leaf samples was extracted by CTAB method, collected from different field areas of Faisalabad. Circular molecules from genomic DNA were amplified by using Rolling Circle Amplification (RCA). Three samples showed successful RCA which further used in PCR amplification. For amplification of full length Begomovirus from RCA product universal primers were used. Betasatellite component associated with ChLCD was amplified with the help of PCR by using full-length universal primers (β01 and β02). RFLP analysis showed that single type of Begomovirus and diverse type of betasatellite molecules observed in infected chili samples.

KEYWORDS:
Chili Leaf Curl Disease, Begomoviruses, Chili Leaf Curl Virus, Betasatellite, RFLP

INTRODUCTION

Pepper (Capsicum annum L.) is one of the most diverse vegetable species and is considered to be a high value crop. Commercially chili is an important vegetable crop cultivated for industrial (oleoresin and capsaicin extraction) purposes and for spice [1]. It is important cash crop in Pakistan, total cultivated area of chili was 63.6000 hectares in which Sindh contribute up to 52.8000 hectares with the annual production of 171.8000 tons [2]. Chili is susceptible to various pathogens including viruses, causes heavy yield loss [3]. Epidemics of CHLCV can result in 100% yield loss both quantitatively and qualitatively resulting in severe economic consequences [4]. The main symptoms of ChLCD are an upward curling, puckering, and bunching of leaves. Severely affected plants produce fewer, smaller, and deformed fruits [5] discovered that ChLCD caused by CHLCV and transmitted by Bemisia tabaci [6].

Begomovirus is divided into two types either having bipartite or monopartite genome. Betasatellites are ssDNA setallite that induce symptoms in host and associated with monopartite begomoviruses, cause diseases in many crops including chili [7, 8]. CHLCV has been reported in different countries like Pakistan [9], Bangladesh and Indonesia [10], Nigeria [11] and USA [12]. Natural occurrence of several viruses including CHLCV, Pepper vein mottle virus and Pepper vein bending virus has been reported by different researchers [13]. Among all, the CHLCV is the most destructive virus in terms of incidence and yield loss. Worldwide it causes a minimum loss of $73 million yearly [14]. The current study shows emergence of leaf curl disease of chili in a sub temperate region in Pakistan.

MATERIALS AND METHODS

Symptomatic plants of eight different varieties of capsicum (Cayenne, Red Pepper) and chili Capsicum annum were collected from field areas belonging to diverse locations of Faisalabad. Samples were labeled as S1 (Pryia), S2 (Skyline), S3 (Hot flame), S4 (forever chili), S5 (Twin star), S6 (Nighat), S7 (Aristole) and S8 (Green pride). Genomic DNA was extracted from infected leaf samples by using CTAB method [15].

Rolling circle amplification (RCA).
Spectrophotometer was used for quantification of genomic DNA. Quantity of genomic DNA was less than 100ng/µL. 950 µl of Phi-29 mixture was prepared. This mixture contained 200µl of 10mM dNTPs, 50µl of 500µM random hexamer primers,
100µl of 10X reaction buffer and 600µl of water. 
13µl of Phi 29 mixture and 0.5µl of template DNA 
were added. It was boiled at 85°C for 5 minutes. 
Then it was immediately placed on ice for 5 minutes. 
Short spin the eppendorfs for few seconds to collect 
the evaporated water on bottom. Then 0.5µl of Phi- 
29 polymerase and 0.5µl of PPase1 were added. 
Everything was placed on ice. The reaction was kept 
on 30°C for 16-20 hours. After 16-20 hours, 15µl 
dH O was added and short spin for 5 seconds. 
Then the reaction was heated at 65°C for 5 minutes 
to inactivate the Phi-29. Short spin the tubes for few 
seconds. 1µl of the product was loaded on gel. 

**Polymerase chain reaction (PCR).** For 
amplification of DNA, the optimum reagents were 
supplied by Ferments. Amplification was carried out 
in 50 µl reaction volume containing template RCA 
product 2µg (5 µl), dNTPs 2 mM (5 µl), PCR Buffer 
10X (5 µl), MgCl2: 1.5 mM (3 µl), PrimerI 5 µM (1 
µl), Primer II 5 µM (1 µl), Taq polymerase 1.5U (0.5 
µl), d d3HO 29.5 µl. For betasatellite amplification 
DNA template was used. In three major steps of 
PCR, timing of denaturation (94°C), annealing (50-
64°C) and extension (72°C) was optimized 
according to requirements; typically 30 seconds, 1 
minute and 1 minute per 1000bases to amplify, 
respectively. 

Eppendorf thermal cycler (Master Cycler) was 
used for PCR and amplified products were analyzed 
by electrophoresis on 1% agarose gels along with 
DNA marker viewed under ultraviolet trans-
illuminator and photographed using the Eagle Eye 
still video system (Stratagene).

**Restriction fragment length polymorphism 
(RFLP).** By this technique variation among DNA 
sample between different plants was observed. Two 
different restriction enzymes PsrI and EcoR1 were 
used for the restrictions of samples because of their 
availability and mode of action. The procedure of 
restriction analysis was performed by making the 
solution of certain ingredients in certain quantity 
such as: template 5µl, enzyme 0.2µl, buffer 2µl and 
dH2O 14µl. 3X solution were made for each enzyme 
due to three samples. After forming 3X solution 
those were transfer in for different eppendorfs. After 
that short spin those eppendorf tube in centrifuge for 
5 seconds, and those were placed at 37°C for 3 to 4 
hours and in the end those were run at 1% agarose 
gel electrophoresis for 45 minutes.

**FIGURE 1**
Typical Symptoms of ChLCV; Vein thickening, darkening of vein and Leaf curling.

**FIGURE 2**
Gel picture showing Rolling Circle Amplification (RCA) of DNA samples (S1, S2, S3 and S5) using 
random hexamer primers (A).PCR amplification of RCA products results by using universal primers (βo1 
and βo2) of begomoviruses which amplify gene fragment of size 2.8 kb. PCR amplification in Lane S5, S2, 
S1 showing the presence of begomovirus infection in sample plants. Gel lane (- & +) represent negative 
and positive control respectively. While gel lane L represent 1kb DNA ladder (B).
RESULTS AND DISCUSSION

The leaf samples were collected from the chili plants showing typical symptom i.e. curling of leaves, thickening of vein and darkening of vein (Figure 1). There are different Begomovirus species infecting chili plants in Pakistan and India; CLCuMV, ToLCNDV, Tomato leaf curl Joydebpur virus (ToLCJoV) and ChLCV [16, 17, 18, 4, 13, 19, 20]. The present study was designed to check diversity of begomoviruses present in chilli plants of Faisalabad region. Genomic DNA was extracted and Presence of total DNA was confirmed by gel electrophoresis. Detection of circular DNA viruses, their characterization and the production of their infectious clones become so much at ease with the help of Φ29 DNA polymerase [21], which specifically amplify circular DNA by rolling circle replication (RCA) [22]. Rolling circular molecules were successfully amplified by RCA from sample S1, S2 and S5 (Fig. 2A). According to [17], revealed that ChLCV is a monopartite Begomovirus having DNA-A which consists of 2754 nucleotides having six ORF. Betasatellite of 1.3kb was identified by PCR using β01/β02 primers. Amplification of 2.8kb fragment with universal primers confirmed the presence of begomoviruses in infected chili plants in three samples (Fig. 2B). For amplification of the betasatellite universal primers β01 and β02 were used. Out of eight samples betasatellites were successfully amplified from four samples labeled as S1, S2, S3 and S4 (Fig. 3A).

FIGURE 3
PCR amplification of samples (S1 and S2) by using universal primers (β01 and β02) of betasatellite. Lane L represent 1kb Ladder, Gel lane (- & +) represent negative and positive control respectively (Panel A). Restriction analysis of PCR product of Betasatellite. S1 and S2 restricted with EcoR1 (Panel B).

FIGURE 4
RFLP analysis of PCR product of DNA-A. A: S1, S2 and S5 samples, restricted with EcoR1. B: S1, S2 and S5 samples, restricted with Pst1. Restriction pattern exhibiting no diversity within samples.
Common restriction enzymes, PstI and EcoR I were used for PCR-RFLP analysis. These restriction enzymes were selected on the bases of \textit{in silico} studies. PCR products of DNA-A were restricted with PstI and EcoR I enzymes. PstI enzyme restricted all three samples at two different sites producing three bands of size 1300bp, 1050bp and 500bp. In case of EcoR I, this enzyme restricted amplified product at three different sites giving us four band of size 1200bp, 1000bp, 350bp and 250bp (Fig. 3B). As all the samples showed same restriction pattern it is assumed that there is only a single type of Begomovirus that is infecting chili plants in the field in Faisalabad region. RFLP analysis of betasatellite was performed with restriction enzyme EcoR I on two PCR products (S1 and S3) which produced multiple bands. The band size of restricted betasatellite was performed with restriction enzyme (Croton bonplandianum and \textit{Ageratum conyzoides}). Begomoviruses found to be associated with these crops were ChLCV, \textit{Ageratum yellow vein mosaic virus}, \textit{Sponge gourd yellow vein mosaic virus}, \textit{Pumpkin yellow vein mosaic virus}, \textit{Bhindi yellow vein mosaic virus}, \textit{Papaya leaf curl virus}, TLCNDV, \textit{Tomato leaf curl Joydepur virus} and \textit{Mungbean yellow mosaic India virus} [23]. In Philippines, genetic diversity of Begomoviruses on pepper plants and tomato plants were analyzed in 2011. Isolates of four Begomoviruses were detected from 20 samples of tomato and 3 samples of chili. Sequence analysis showed pepper plants were infected with \textit{Tomato leaf curl Mindanao virus} and \textit{Tomato leaf curl Cebu virus}. The results showed that chili plants could also assist as alternate host of Begomovirus infecting tomato [24]. Moreover diversity analysis of betasatellites on chili in Punjab and Khyber Pakhtoonkhwa provinces of Pakistan showed that only one species of betasatellite named ChLCB is widespread all over the area [25]. Current studies revealed that diverse betasatellites and single type of Begomovirus are prevailing in fields of Punjab province.

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DEFICIT IRRIGATION EFFECTS ON CAULIFLOWER (BRASSICA OLERACEA L. VAR. SKYWALKER F1) YIELD UNDER UNHEATED GREENHOUSE CONDITIONS

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ABSTRACT

The aim of this study was to determine the effect of deficit irrigation on yield of cauliflower grown under unheated greenhouse conditions. The research was carried out at the Agricultural Research Station of Yenisehir Vocational School of Bursa Uludag University in Bursa, Turkey, in 2008 and 2009. In the study, water was applied to cauliflower as 1.00, 0.75, 0.50, 0.25 and 0.00 % (as control) of evaporation from a Class A Pan corresponding to 2 day irrigation frequency. Irrigation water applied ranged from 70-530 mm to 70-528 mm, and water consumption ranged from 90-548 mm to 90-540 mm. The effect of irrigation water level on the yield, head height, head diameter, head weight and dry matter were found to be significant. The highest yield were 30.4-28.8 t ha⁻¹. Crop yield response factors for cauliflower (kₑ) were found as 0.90 and 0.93 for both years. The highest values of water use efficiency (WUE) and irrigation water use efficiency (IWUE) for 2008 and 2009 years of treatment were calculated to be 0.069 kg m⁻³ and 0.064 kg m⁻³, respectively. Kₑ application (75%) can be recommended as the most effective irrigation level for the cauliflower to which drip irrigation is applied under scarce and unheated greenhouse conditions.

KEYWORDS:
Evapotranspiration, water use efficiency (WUE), cauliflower, yield and quality parameters, irrigation scheduling

INTRODUCTION

Greenhouse cultivation, also known as protected cultivation, is one of the farming systems widely used to provide and maintain a controlled environment suitable for optimum crop production leading to maximum profits. This includes creating an environment suitable for working efficiency as well as for better crop growth [1]. Greenhouse cultivation is a steadily growing agricultural sector all over the world [2, 3]. The type of structure primarily used in Turkey is the so-called Mediterranean greenhouse; low-cost, unheated plastic-covered structures and with soil-grown crops.

Cauliflower is in the cruciferous vegetables group of the family Brassicaceae (also called Cruciferae). It is grown in autumn and winter and consumed as vegetable in our country. Based on agricultural production statistics, cauliflower is widely produced in Marmara, Aegean, Mediterranean and Black Sea Region of Turkey [4]. Cauliflower is scalded, fried and cooked in various forms or regarded as pickle or frozen vegetable in Turkey. Cauliflower production and consumption is very common in developed countries. In recent years, production and consumption of that vegetable is also increasing in our country [5].

Irrigation scheduling involves preventing the soil water deficit from falling below some threshold level for a particular crop and soil condition. This may involve estimating the earliest date to permit efficient irrigation or the latest date to avoid the detrimental effects of water stress on the crop [6]. Scheduling water application is very critical to make the most efficient use of drip irrigation system, as excessive irrigation reduces yield, while inadequate irrigation causes water stress and reduces production.

The optimum use of irrigation can be characterized as the supply of adequate amount of water to meet the crop needs in the root zone, and at the same time, avoiding the leaching of nutrients into deeper soil layers [7]. High frequency water management by drip irrigation minimizes soil as a storage reservoir for water, provides at least daily requirements of water to a portion of the root zone of each plant and maintains a high soil matric potential in the rhizosphere to reduce plant water stress. On the other hand, the intensity of the operation requires that the water supply is kept at the optimum to maximize returns to the farmer.

Irrigation scheduling with drip irrigation relies on approaches based on evapotranspiration estimations [8, 9, 10, 11] and allowable soil-water depletion [12]. A widely adopted method for estimating crop consumptive water use (CWU) is the pan evaporation method, which relates evaporation from a Class A pan to CWU. These two quantities are re-
lated by what is called the pan coefficient $K$. Irrigation scheduling based on the pan coefficient $K$ is one of the simplest methods where no sophisticated instrument is required. Precise values for $K$ are often difficult to establish, given regional and site-specific soil characteristics, crop physiology and cultural practices. Any recommended value of $K$ for regional irrigation scheduling program must be high enough to prevent water stress arising from emergencies and specialized local situations, while remaining low enough for efficient water management [13]. Based on the US Weather Bureau Class A pan evaporation, many studies have been completed on the irrigation of cauliflower [14, 15], broccoli [16], tomato [17]; green bean [18]; pepper [19], cucumber [20], lettuce [21] and potato [22, 23]. Several studies have been performed to investigate the influence of different irrigation levels on cauliflower growth and yield.

The objectives of this study were to provide a guideline for cauliflower growers and to determine drip irrigated cauliflower response to different irrigation regimes.

MATERIALS AND METHODS

Field trials were conducted under unheated greenhouse conditions in the region of Bursa-Yeşilhisar (40°15'09"N latitude, 29° 38'43"E longitude and altitude of 225 m above mean sea level). For experimental purposes, high tunnel type plastic covered greenhouse with the size of 8 m x 40 m was built. The climate characteristics of the experiment field was hot and dry in summer and cold and rainy in winter. Annual average precipitation and temperature values for 2008 and 2009 years for the regions where greenhouse experiments were carried out was 630.7 – 804.4 mm and 12.9 – 14.6 °C, respectively. The average minimum temperature for 2008 and 2009 years were gauged as -6.6 – (-5.9) °C in January and December while the average maximum temperature were gauged as 32.9 and 34.6 °C in August and December respectively. Irrigation water quality was low sodium risk 46% K2O fertilizer was applied when the crops reached to height of 15 cm. 10 L ha⁻¹ chlorophyllous-ethyl was sprayed against insects. Transplantation date of the cauliflower seedlings to the plots were September 01 in 2008-2009 years. In the experiments, row and plant spacing were 0.60 m and 0.60 m, respectively. Each plot has contained 44 plants. 14 plants of middle row were harvested to prevent the water penetration from its neighboring plots. The head height (cm), diameter (cm) and weight (g) of cauliflowers were measured by callipers and the average of measured values was calculated. Dry matter content was determined by the separation and drying (at 65°C in drying oven) of fruits (two samples for each plot). The amount of dry matter of heads was determined by Kjeldahl method [25].

The order of the trial was set as a randomized block design with 3-replication and single factor, and 5 irrigation applications were randomly distributed to each blocks. The irrigation applications were created using five different crop evaporation coefficients ($K_{1p}$: 1.00, $K_{2p}$: 0.75, $K_{3p}$: 0.50, $K_{4p}$: 0.25, $K_{5p}$: 0.00-for control purposes). The amount of irrigation water was determined using below stated equation [26, 27]:

$$IW = E_p \times K_{ip} \times P$$

Where, $E_p$ is cumulative evaporation (mm) for 2-day irrigation frequency, $K_{ip}$ is pan evaporation coefficient, and $P$ is the percentage of wetted area. Evaporations that occurred in the 2 day irrigation frequency were measured using Class A Evaporation Pan that was held in the middle of greenhouse applications and drip irrigation method was used. The amount of irrigation water was measured with flow-meter devices at the gate of each plot. The needed irrigation water was provided from a deep well (3 L s⁻¹) that was drilled in the field. Quality properties of the irrigation water were presented in Table 2.

Irrigation water quality was low sodium risk and classified in C2S1; with medium level EC value. Crop evapotranspiration (Cumulative evapo transpiration - ETc) was calculated for 2 day irrigation interval using the below stated water balance equation:

$$ETc = (SWCt0 - SWCt1) + IW - D,$$

and insect pests. 170 kg ha⁻¹ 21% N, 50 kg ha⁻¹ 46 % P2O5 as bottom fertilizer was applied two weeks prior to sowing process. An additional 170 kg ha⁻¹ 46% K2O fertilizer was applied when the crops reached to height of 15 cm. 10 L ha⁻¹ chlorophyllus-ethyl was sprayed against insects.

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$$ETc = (SWCt0 - SWCt1) + IW - D,$$
TABLE 2

Chemical composition of irrigation water used in the experiment

<table>
<thead>
<tr>
<th>Water source</th>
<th>EC3 x 10⁶ μhos cm⁻¹</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>pH</th>
<th>Class</th>
<th>SAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep well</td>
<td>715</td>
<td>2.3</td>
<td>2.56</td>
<td>9.25</td>
<td>5.7</td>
<td>7.12</td>
<td>C₂S₁</td>
<td>0.85</td>
</tr>
</tbody>
</table>

TABLE 3

Relationship between the decrease in relative water use and decrease in relative yield and yield response factor for drip-irrigated cauliflower for 2008 year

<table>
<thead>
<tr>
<th>Irrigation treatment</th>
<th>Yield (t ha⁻¹)</th>
<th>Applied Water (mm)</th>
<th>ETa (mm)</th>
<th>ETa/ETm</th>
<th>Yₐ/Yₘ</th>
<th>1-(ETa/ETm)</th>
<th>1-(Yₐ/Yₘ)</th>
<th>ky</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₈p</td>
<td>30.4</td>
<td>530</td>
<td>548</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>K₂p</td>
<td>28.6</td>
<td>398</td>
<td>415</td>
<td>0.757</td>
<td>0.941</td>
<td>0.243</td>
<td>0.059</td>
<td>0.243</td>
</tr>
<tr>
<td>K₃p</td>
<td>16.3</td>
<td>265</td>
<td>300</td>
<td>0.547</td>
<td>0.536</td>
<td>0.453</td>
<td>0.464</td>
<td>1.024</td>
</tr>
<tr>
<td>K₄p</td>
<td>6.0</td>
<td>132</td>
<td>180</td>
<td>0.328</td>
<td>0.197</td>
<td>0.672</td>
<td>0.803</td>
<td>1.195</td>
</tr>
<tr>
<td>K₅p</td>
<td>2.0</td>
<td>70</td>
<td>90</td>
<td>0.164</td>
<td>0.066</td>
<td>0.836</td>
<td>0.934</td>
<td>1.118</td>
</tr>
</tbody>
</table>

Where (SWC₀ – SWCₜ) is the change in volumetric soil water content (mm); IW (mm) and D (mm) are, irrigation water depth (mm) and drainage (mm) for the related period, respectively. Prior to irrigation water applications, water content in 0.60 mm soil depth was determined with gravimetric method [28]. Water content of the soil was monitored till 0.90 depth with increments of 30 cm depth following irrigation applications for each irrigation period. In subplots, the percolations below 0.60 m soil depth were omitted. In our study, the relationships between yield and ET were determined by the Stewart model [29]:

\( (1-Y_a/Y_m) = k_y (1-ET_a/ET_m) \)

Where, \( Y_a \) and ETa are actual crop yield productivity (t ha⁻¹) and cumulative evaporation (mm), respectively, under insufficient irrigation conditions; \( Y_m \) and ETm are maximum crop yield productivity (t ha⁻¹) and cumulative evaporation (mm) under sufficient water conditions. Yield productivity response factor of the deficit irrigated cauliflower was presented with \( k_y \). Water use efficiency (WUE) value was calculated to evaluate the irrigation efficiency in the applications. The two terms that are used to encourage the effective use of irrigation water in crop production phases are water use efficiency (WUE) and irrigation water use efficiency (IWUE). Water use efficiency (WUE) is calculated as the efficiency ratio of yield to ETa and depicted as WUE = YLD / ETa (kg m⁻³). Irrigation water use efficiency (IWUE) was estimated with the below stated equation [30]:

\( IWUE(kg \ m^{-3}) = \frac{YLD}{IRGA} \)

Where, YLD is yield value of each treatment plot (kg ha⁻¹), YLDRAINFED is yield value from control (rainfed) treatment plot (kg ha⁻¹), IRGA is seasonal irrigation water amount (mm). Cauliflower seedlings completely grew and fruit had the yield productivity, head height, diameter and weight, color and taste characteristics to its species, 90 days (DOY=100) after plantation, i.e. in harvest season. Yield productivity and quality parameters, i.e. head height, diameter and weight and dry matter ratio, were evaluated for each harvest season.

Variance analysis was conducted with yield productivity and productivity components by using MSTAT-C (version 2.1-Michigan State University 1991) and MINITAB (Texas University, Austin) software. The significance of irrigation applications was calculated at 0.05 and 0.01 probability levels with F-test [31].

RESULTS

Water applied and water used. All treatments received 70 mm in 2008 and 2009 years irrigation water to refill available soil water content of 0-60 cm soil depth up to field capacity level following planting date. Class A pan measurements of evaporation were just started after first irrigation water application. The maximum and minimum amounts of irrigation water applied were 530-70 mm for the first year and 528-70 for the second year for \( K_{1p} \) treatment and \( K_{5p} \) treatment, respectively. The amount of water applied to other treatments for 2008 and 2009 years varied from 398 to 132 mm and from 396 to 132, respectively. An increase in seasonal evapotranspiration (ETa) was observed with an increase at applied irrigation water. The actual evapotranspiration for 2008 and 2009 years ranged between 548-90 mm and 540-90 mm for \( K_{5p} \) and \( K_{1p} \) treatments, respectively (Table 3, Table 4).

Linear relationships between crop evapotranspiration (ETc) with yield productivity (Ya), and irrigation water (IW) with yield (Ya) were observed for 2008 year. The relationship equation is as follows; \( Y_a = 0.0687ET_a - 4.4047 \) with \( R^2 = 0.95 \) and \( Y_a = 0.0666IW - 1.9317 \) with \( R^2 = 0.96 \) (Figure 1).
TABLE 4
Relationship between the decrease in relative water use and decrease in relative yield and yield response factor for drip-irrigated cauliflower for 2009 year

<table>
<thead>
<tr>
<th>Irrigation treatment</th>
<th>Yield (t ha⁻¹)</th>
<th>Applied Water (mm)</th>
<th>ETa (mm)</th>
<th>ETa/ETm</th>
<th>Ya/Ym</th>
<th>1-(ETa/ETm)</th>
<th>1-(Ya/Ym)</th>
<th>ky</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1ᵢᵣᵦ</td>
<td>28.8</td>
<td>528</td>
<td>540</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>K2ᵢᵣᵦ</td>
<td>27.0</td>
<td>396</td>
<td>420</td>
<td>0.778</td>
<td>0.938</td>
<td>0.222</td>
<td>0.062</td>
<td>0.279</td>
</tr>
<tr>
<td>K3ᵢᵣᵦ</td>
<td>14.2</td>
<td>264</td>
<td>320</td>
<td>0.593</td>
<td>0.493</td>
<td>0.407</td>
<td>0.507</td>
<td>1.246</td>
</tr>
<tr>
<td>K4ᵢᵣᵦ</td>
<td>6.0</td>
<td>132</td>
<td>140</td>
<td>0.259</td>
<td>0.208</td>
<td>0.741</td>
<td>0.792</td>
<td>1.069</td>
</tr>
<tr>
<td>K5ᵢᵣᵦ</td>
<td>1.8</td>
<td>70</td>
<td>90</td>
<td>0.167</td>
<td>0.063</td>
<td>0.833</td>
<td>0.937</td>
<td>1.125</td>
</tr>
</tbody>
</table>

FIGURE 1
The relationship between crop evapotranspiration with yield and water irrigation with yield for 2008 year.
(The errors bars are SE of 14 plants)

FIGURE 2
The relationship between crop evapotranspiration with yield and water irrigation with yield for 2009 year
(The errors bars are SE of 14 plants)

Linear relationships between crop evapotranspiration (ETc) with yield productivity (Ya), and irrigation water (IW) with yield (Ya) were observed for 2009 year. The relationship equation is as follows; 

\[ \text{Ya} = 0.0629 \times \text{ETc} \times 3.4476 \]

\[ R^2 = 0.9512 \] (Figure 2). The highest yields were obtained from Kᵢᵣᵦ application with 30.4 t ha⁻¹ and 28.8 t ha⁻¹ for 2008-2009 years, respectively. It was followed by K₂ᵢᵣᵦ, K₃ᵢᵣᵦ and K₄ᵢᵣᵦ applications for 2008 and 2009 years, in order, with yield productivity values of 28.6 – 16.3 – 6.0 t ha⁻¹ and 27.0 – 14.2 – 6.0 t ha⁻¹, respectively. As expected, the minimum yields (0.5 – 1.4 t ha⁻¹) were found from control K₅ᵢᵣᵦ application in which irrigation was not applied. The yield productivity of unirrigated K₅ᵢᵣᵦ application for 2008 and 2009 years were lower at a rate of 1420-1500% in a comparison with Kᵢᵣᵦ application, respectively. Moreover, in first year lower yield productivity levels at a rate of 6.3%, 86.5%, 406.7% and in second year 6.7%, 102.8%, 380.0% from K₂ᵢᵣᵦ, K₃ᵢᵣᵦ and K₄ᵢᵣᵦ applications were observed in a comparison with Kᵢᵣᵦ application, respectively (see Table 5 and Table 6). Crop yields and quality are reduced due to water deficits applied particularly three or four weeks before harvest.

The related equations for 2008 year were as follows; 

\[ \text{head height} = 0.0235 \times K + 8.651 \] with \( R^2 = 0.93 \) (Fig. 3a.), head \( \text{diameter} = 0.037 \times K + 8.0672 \) with \( R^2 = 0.98 \) (Fig. 3b), head \( \text{weight} = 2.511 \times K - 110.58 \) with \( R^2 = 0.98 \) (Fig. 3c.) and dry \( \text{matter} = -0.0428 \times K + 27.097 \) with \( R^2 = 0.95 \) (Fig. 3d.)

(7371-1784)
TABLE 5
Effects of irrigation treatments on cauliflower marketable parameters for 2008 year.

<table>
<thead>
<tr>
<th>Irrigation Treatment</th>
<th>Head Height (cm)</th>
<th>Head Diameter (cm)</th>
<th>Head Weight (g)</th>
<th>Dry Matter (%)</th>
<th>Yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₁₀₁</td>
<td>19.8 a</td>
<td>26.5 a</td>
<td>1150.0 a</td>
<td>6.5 e</td>
<td>30.4 a</td>
</tr>
<tr>
<td>K₂₀₁</td>
<td>19.0 a</td>
<td>24.5 b</td>
<td>995.0 b</td>
<td>8.0 d</td>
<td>28.6 b</td>
</tr>
<tr>
<td>K₃₀₁</td>
<td>16.4 b</td>
<td>18.0 c</td>
<td>555.0 c</td>
<td>14.0 c</td>
<td>16.3 c</td>
</tr>
<tr>
<td>K₄₀₁</td>
<td>11.2 c</td>
<td>12.5 d</td>
<td>180.0 d</td>
<td>22.5 b</td>
<td>6.0 d</td>
</tr>
<tr>
<td>K₅₀₁</td>
<td>9.6 d</td>
<td>10.5 e</td>
<td>70.0 e</td>
<td>24.8 a</td>
<td>2.0 c</td>
</tr>
<tr>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Blocks</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level, ns non-significant

TABLE 6
Effects of irrigation treatments on cauliflower marketable parameters for 2009 year.

<table>
<thead>
<tr>
<th>Irrigation Treatment</th>
<th>Head Height (cm)</th>
<th>Head Diameter (cm)</th>
<th>Head Weight (g)</th>
<th>Dry Matter (%)</th>
<th>Yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₁₀₁</td>
<td>19.4 a</td>
<td>26.0 a</td>
<td>1100.0 a</td>
<td>6.2 e</td>
<td>28.8 a</td>
</tr>
<tr>
<td>K₂₀₁</td>
<td>18.6 a</td>
<td>25.0 b</td>
<td>990.0 a</td>
<td>7.8 d</td>
<td>27.0 a</td>
</tr>
<tr>
<td>K₃₀₁</td>
<td>16.8 b</td>
<td>18.5 c</td>
<td>540.0 b</td>
<td>13.8 c</td>
<td>14.2 b</td>
</tr>
<tr>
<td>K₄₀₁</td>
<td>11.5 c</td>
<td>13.0 d</td>
<td>185.0 c</td>
<td>23.0 b</td>
<td>6.0 c</td>
</tr>
<tr>
<td>K₅₀₁</td>
<td>10.1 d</td>
<td>11.0 e</td>
<td>72.0 c</td>
<td>25.2 a</td>
<td>1.8 d</td>
</tr>
<tr>
<td>Treatments</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>Blocks</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level, ns non-significant

FIGURE 3
Relationship between applied irrigation water and head height (a), head diameter (b), head weight (c), and dry matter (d) of 2008 year (The errors bars are SE of 14 plants)

The related equations for 2009 year were as follows; head height = 0.0235W + 8.651 with R² = 0.9313, head diameter = 0.0354W + 8.846 with R² = 0.91, head weight = 2.424W + 96.482 with R² = 0.97, and dry matter = -0.00448W + 27.648 with R² = 0.95.

Crop yield response factor (kᵢ). Linear relationship between proportional decrease in water consumption and proportional decrease in yield productivity is depicted with crop yield productivity response factor (kᵢ) that represents yield productivity response to be lowered in water consumption. In other words, it explains the decrease in yield productivity in relation with the decrease in water consump-
tion per unit [29, 32]. For irrigation application, seasonal yield productivity response factor ($k_y$) were calculated as 0.90 and 0.93 for 2008 and 2009 years, respectively (see Fig. 5). $k_y$ values increased with parallel to increase in water amount, except $K_{5p}$ application.

**Water use efficiencies.** Values of WUE and IWUE for two years was lowered when the amount of irrigation water was reduced. The highest WUE and IWUE values for 2008 and 2009 years were calculated from $K_{2p}$ application as 0.069–0.064 kg m$^{-3}$ and 0.067–0.064 kg m$^{-3}$, respectively. IWUE value of $K_{2p}$ application for the 2009 years were found higher than other applications as $K_{1p}$, $K_{3p}$, $K_{4p}$ and $K_{5p}$, in order (See Table 7).

![Figure 4](image1.png)
**FIGURE 4**
Relationship between applied irrigation water and head height (a), head diameter (b), head weight (c), and dry matter (d) of 2009 year (The errors bars are SE of 14 plants)

![Figure 5](image2.png)
**FIGURE 5**
Relationship between relative yield decrease and relative crop evapotranspiration for cauliflower throughout the total growing season
TABLE 7

Total water use efficiency (WUE) and irrigation water use efficiency (IWUE) values for drip irrigated cauliflower at different irrigation treatments for 2008 and 2009 years.

<table>
<thead>
<tr>
<th>Irrigation treatment</th>
<th>Yield (t ha⁻¹)</th>
<th>WUE (kg m⁻³)</th>
<th>IWUE (kg m⁻³)</th>
<th>Irrigation treatment</th>
<th>Yield (t ha⁻¹)</th>
<th>WUE (kg m⁻³)</th>
<th>IWUE (kg m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₁</td>
<td>30.4</td>
<td>0.055</td>
<td>0.054</td>
<td>K₁</td>
<td>28.8</td>
<td>0.053</td>
<td>0.051</td>
</tr>
<tr>
<td>K₂</td>
<td>28.6</td>
<td>0.069</td>
<td>0.067</td>
<td>K₂</td>
<td>27.0</td>
<td>0.064</td>
<td>0.064</td>
</tr>
<tr>
<td>K₃</td>
<td>16.3</td>
<td>0.054</td>
<td>0.054</td>
<td>K₃</td>
<td>14.2</td>
<td>0.044</td>
<td>0.047</td>
</tr>
<tr>
<td>K₄</td>
<td>6.0</td>
<td>0.033</td>
<td>0.030</td>
<td>K₄</td>
<td>6.0</td>
<td>0.043</td>
<td>0.032</td>
</tr>
<tr>
<td>K₅</td>
<td>2.0</td>
<td>0.022</td>
<td>0.000</td>
<td>K₅</td>
<td>1.8</td>
<td>0.020</td>
<td>0.000</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, irrigation treatments significantly affected yield, head height, head diameter, head weight and dry matter. Bansod reported that the total depth of water for cauliflower applied (including effective rainfall of 44 mm) in T₁ (furrow irrigation) and T₂ (sprinkler irrigation) was 221 and 321 mm, respectively [33]. A total of 385 and 467.5 mm irrigation water was applied to the cauliflower and red cabbage plant plots, respectively [34]. Koudela et al. determined that for two years and evaluate the influence of a hydrophilic agent, average of irrigation quantity values for cauliflower varied from 129-157 mm to 306-322 mm in Prague [35]. Bozkurt et al. specified that amounts of total irrigation water during the growing period of cauliflower changed from 85 to 337 mm in 2005, from 85 to 265 mm in 2006 and from 127 to 634 mm in 2007 [15]. On the same study, the highest reference crop evapotranspiration was 814 mm. The ET₀ values changed from 213 mm in K₀ to 638 mm in K₂ treatments with respect to overall mean of the three experimental years. Popale et al. stated that water applied for cauliflower varied from 176-353 mm in different treatments in India [36]. Sahin et al. found that the seasonal ET, was determined as 475 mm for cauliflower and 556 mm for red cabbage [14]. Sarkar et al. reported that present study about cauliflower shows higher curd yield (19.1-20.3 mg ha⁻¹) was recorded when 145-155 mm of total water was irrigated to the crop [37]. In the present study, maximum curd yield (20.3 mg ha⁻¹) was obtained when the seasonal actual evapotranspiration value during the cropping period under irrigation regime was 206.9 mm, beyond which an increase in values of SET till to the highest level, i.e. 239 mm resulted in 0.2-38.8% reduction in curd yield of cauliflower. Panda et al. stated that Pusa Bold was superior to SEJ 2 in terms of growth, yield characters, yields (1527 kg ha⁻¹) and consumptive water use (235.5 mm) [38]. Prabhakar and Srinivas conducted a field experiment on sandy loam soil during the winter seasons (Oct.-Feb.), cauliflower cv. Snowball [39]. An increase in irrigation from 25 to 100% of Epan linearly increased curd yield (from 8.06 to 16.22 t ha⁻¹) and crop evapotranspiration (from 238 to 541 mm). Prabhakar and Srinivas observed that irrigation scheduled at -25 kPa required 16 irrigations with a crop evapotranspiration of 393 mm and resulted in the highest net return per ha and maximum net return per rupee invested [40]. These results are notably in accordance with the irrigation water amounts and crop water consumption values obtained from previous studies [41, 39, 40, 38, 33, 34, 14, 37, 35, 15, 36].

The cauliflower yield for 2008-2009 trial years hanged between 30.4-2.0 t ha⁻¹ and 28.8-1.8 t ha⁻¹. Based on to the results of this study, a significant effect of deficit irrigation was observed on total yield. This result is in agreement with those of [42, 39, 40, 43, 44, 45, 33, 46, 47, 48, 49, and 50]. Previous researches have presented similar results under different irrigation regimes [44, 14, 48, 15, and 50]. Yield was considerably lowered as the amount of irrigation water reduced. Quality parameters such as head height, diameter, weight and dry matter have produced a similar response to deficit irrigation as observed at yield. As expected, all irrigation treatments had higher values than the non-watered (K₅) treatment. These values are similar to those of previous studies [38, 51, 52, 33, 54, 56, 57, 58]. Since K₃ treatments have higher head weight than the other treatments, the lowest dry matters were found at K₃ treatments when the highest values were observed at K₄ treatments in every two years of the study. We may conclude that significant increases in dry matter may be experienced by the increasing level of irrigation water deficit. These results are in agreement with those of [59, 60, 53, 61, 35, 57].

The maximum WUE and IWUE values for 2008 and 2009 years found as 0.069-0.064 and 0.067-0.064, respectively. K₂ treatment in 2008 and 2009 years has had the highest as WUE and IWUE values. When the results regarding water use efficiency are compared with the findings of different researchers, they were found to be similar [38, 33, 37, 14, 48, 55, 15, 36, and 62]. Cauliflower variety choice, climate, soil structure and effective use of water also affect these values. As explained by Davis et al., it may be attributed to the variety and applied cultural practices handling under different climate and geographical conditions [63]. Crop yield response factor (kₚ) for 2008 and 2009 year were cal-
culated as 0.90 and 0.93 for cauliflower, respectively. The specified values of $k_y$ (0.90 and 0.93) which is equal and lesser than 1.00 shows that cauliflower is susceptible to the water. Crop yield response factor ($k_y$) also coincides with the values found by researchers who studied on similar issues [14, 55, 15, 62].

**CONCLUSIONS**

The ultimate goals of optimum irrigation management strategies in deficit areas are to enhance yield and quality as much as possible, increase WUE and reduce water consumption. $K_{2p}$ treatment allowed high yield and quality (in terms of head height, diameter and weight), increased WUE and reduced water use. The variety choice of cauliflower, climate and soil structure also influenced to change WUE and IWUE values. Crop yield response factors of cauliflowers were found as 0.90 and 0.93 in 2008 and 2009 years which is equal or lesser than 1.00. The specified values of $k_y$ (0.90 and 0.93) calculated as 0.90 and 0.93 for cauliflower, respectively. The variation of $k_y$ (0.90 and 0.93) can be recommended as the most effective irrigation level for the cauliflowers to which drip irrigation is applied under scarce water resource and unheated greenhouse conditions.

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DESIGN AND PERFORMANCE OF A HYBRID (PHOTOVOLTAIC/WIND) ENERGY-ASSISTED INDUSTRIAL DRYER

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ABSTRACT

An experimental hybrid energy-assisted dryer with a photovoltaic and wind auxiliary power system was designed, fabricated and tested. The developed dryer has been used in various weather and operating conditions. The effects of three drying air temperatures (50, 60, and 70 °C) and two air velocities (3 and 4 m/s) were investigated for maize kernel drying. Drying performance was evaluated in terms of drying kinetics, specific and total energy consumption, color and rehydration ratio. The results showed that total drying time decreased as the air velocity and drying air temperature increased. Depending on the drying times, the power-generation performances of the photovoltaic panels and the wind turbine were between 5.298 and 3.922 W, and 518 and 311 W, respectively. However, the energy consumption of the dryer was between 39.977 and 22.356 W. The best specific energy consumption was 7.561 kWh/kg at 50 °C at 3 m/s for 565 minutes. The color parameters indicated maize darkening in all drying conditions. The rehydration assays showed that rehydrated maize kernels reached a higher capacity with increased air temperature and air velocity.

KEYWORDS:
Drying, Energy consumption, Photovoltaic panels, Wind turbine

INTRODUCTION

Recently, to cover the global electricity needs, oil-fired power generation solutions are in operated [1]. However, numerous research and development activities have been conducted to find reliable and financially viable alternative energy sources in the light of increasing global energy demand. These alternative sources include solar, wind, wave, geothermal and other types of energies [2]. Their development will used as a sustainable energy production tool [3].

Drying is one of the most important process for food industry [4] and energy intensive operation [5]. Total drying (output) of industrial sectors of industrialized countries combined constitutes 7-15% of energy consumption [6, 7]. Drying mostly requires fossil fuels, which keep rising in price. In addition, because fossil fuels emit CO2, mitigation of the effects on the environment is becoming more difficult [8]. Driven by the rise in fossil fuel prices and concerns over the environment, various dryers have been designed and evaluated in the literature. In industrial drying, the goal is maximum drying efficiency under hygienic conditions and minimum energy consumption [9]. Therefore, using relatively renewable energy sources instead of traditional fuels for drying has considerable advantages, such as saving energy [6] and a decrease in the use of fossil fuels [8]. Furthermore, renewable energy systems can have a beneficial impact on the environmental issues of the world [10].

Hybrid energy systems have proven, in many cases, to be able to considerably decrease the total life-cycle cost of independent energy sources. Furthermore, a combination of energy sources has proven to provide a more reliable utility source [11]. It is clear that neither independent solar energy systems nor independent wind energy systems can provide energy consistently, considering seasonal and periodic changes. Therefore, it is common practice to combine solar and wind energy resources, sometimes with energy stored batteries, into a hybrid energy system [12, 13].

Numerous studies of drying have been conducted with hybrid drying, such as solar-biomass [14], solar-electrical [15], photovoltaic (PV) - thermal [16], solar-water heater [9], solar-gas [17], and PV-halogen lamps [18]. No study has yet been reported on PV/Wind energy-assisted drying. The main objectives of this study were to investigate the effects of various air temperatures and air velocities on the drying time, color, rehydration ratio, and specific and total energy consumption of shelf drying thin layer maize kernels.
MATERIALS AND METHODS

Ripe maize used in the drying experiments was grown in the region of Yenisehir, Bursa, Turkey. The material was harvested on 1 September 2015 and then stored at 4.0±0.1 °C to prevent moisture loss. The samples of average radius 6.8±0.2 mm were used. The initial moisture content of the maize kernels was determined by oven drying (ED115 Binder, Tuttlingen, Germany) at 105 °C for 24 h [19]. Based on the initial moisture content analysis, the samples were found to consist of 44% moisture on a wet basis (w.b.).

Experimental Setup. The dryer experiments were performed under the outdoor meteorological conditions of the Field Laboratory of the Department of Biosystems Engineering, Faculty of Agriculture, Bursa, Turkey (40°13’N, 28°51’E). A photograph and schematic view of the dryer are shown in Figures 1a and 1b, respectively.

The apparatus consists of (1) a 4 unit polycrystalline solar panel module of total capacity 1 kW (SFP250, Solarfield, Turkey) with dimensions 400 - 169 cm, inclined at an angle of 30° with the horizontal, always facing south, (2) a 1.1 kW radial fan (1.5 1500S, MTA, Turkey) with a digital potentiometer, (3) a zone with an 8 kW electric resistance heater, (4) a proportional integral derivative (PID) control processor with a PT100 thermocouple (Esm 7730, Emko, Turkey), (5) twelve batteries (DC 12 V 100 A) connected in series to obtain a 48 V power source (SPG100/12, SB, China), (6) an AC/DC inverter and controller, (7) digital electric meters, (8) a drying chamber with shelves (198 - 58 cm) and (9) a vertical axis wind turbine with a 48 V magnetic generator (500 W VAWT, DPL Energy, China) with dimensions 178 - 62 cm.

The system operates as follows. Air is drawn through the 1.1 kW blowers and directed to the electric resistance zone; the desired air velocity is set using the digital potentiometer. The PID control system is used to adjust the air temperature to obtain the desired temperature at different air speeds. Once the air flow becomes laminar, the sieve-type trays allow air circulation through the material to be dried. The air absorbs the humidity of the product and is accelerated through the collapsed structure in the outlet cross-sectional area; it then is directed to the horizontally mounted vertical axis wind turbine to generate electricity. Concurrently, electricity is generated from solar energy as well. These energy outputs transfer to batteries or the electrical grid. The drying cabinet designed with 200 cm in long, 60 cm in wide and 125 cm in high with containing four trays, 8 cm apart. However, in order to accelerate the discharged air from the dryer, the air outlet section was narrowed [20] with 9 cm in high towards to the wind turbine. The bottom tray was used in the experiments.

Test procedure. The study investigated the performance of a PV/wind-energy-assisted dryer designed specifically for local conditions. The experiments were conducted from 02-19 September 2015, between 10:00 am and 7:25 pm, replicated with a second run. The dryer was located far from any shade during the entire duration of the experiment. This is because there are some parameters such as shadow affecting the energy production efficiency [21]. The sample of maize kernels was placed on wire mesh trays for thin-layer drying.

The entire power generation is dependent on the climatic conditions and every different location will have different weather conditions. So, weather data is a major tool for analyzing for the feasibility study of the hybrid system [22]. Such data were recorded for 12 days in September by a datalogger (Cr1000, Campell Scientific, USA). The dataset consists of 1-min averaged measurements of global horizontal irradiance (CM11 pyranometer, Kipp & Zonen, Netherlands), direct normal irradiance (CHP1 pyrheliometer, Kipp & Zonen, Netherlands, mounted on a solar tracker), sunshine duration (CSD 3 Sensor, Kipp & Zonen, Netherlands), ambient temperature (41342, Young, USA), ambient humidity...
from no reflection for black (L= 0) to perfect diffuse reflection for white (L= 100). The value $a^*$ is the redness, ranging from negative values for green to positive values for red. The value $b^*$ is the yellowness, ranging from negative values for blue to positive values for yellow [30]. Prior to the color measurements, the instrument was calibrated using a standard white plate and a standard black plate. During the measurements, maize kernels were superposed to completely cover the surface of the black cylindrical box [31].

**Rehydration.** Rehydration experiments were performed with 10 g of dried samples that were placed in a cloth and put in a beaker containing 250 ml distilled water. The beakers were kept at room temperature, nearly 20 °C, for 14 hours. The samples were then removed and drained over a mesh to eliminate surplus water and weighed on a digital balance (PS4500, Radwag, Poland). Rehydration capacity was defined as the ratio of the total weight after rehydration to the dry weight of the sample [32].

**Statistical analysis.** The results were processed using MS-Excel software. A one-way analysis of variance was used to evaluate any significant differences between the color and the rehydration capacity after each drying method. All calculations were executed using JMP software (version 7.0, NC, USA). Differences were considered significant at P<0.05 unless otherwise specified.

**RESULTS AND DISCUSSION**

The effects of various drying air temperatures and air velocities on the reduction of moisture content are indicated in Figure 2. The results show that the maize kernels were dried in air temperatures of 50, 60 and 70 °C at a constant drying air velocity of 3 m/s for 565, 460 and 370 min, respectively. At a

![FIGURE 2](image)

**FIGURE 2**

Effects of air temperature and air velocity on maize kernel drying
constant drying air velocity of 4 m/s the drying of maize kernels took 530, 420 and 340 min at 50, 60 and 70 °C, respectively. The drying time was shortened by a factor of 1.52 compared with the drying process of 50 to 70 °C at 3 m/s air velocity. Similarly, drying time was increased approximately 1.55 times when the drying temperature decreased from 70 to 50 °C at 4 m/s air velocity. Consequently, the drying time required to reduce the moisture was longest at 50 °C at 3 m/s air velocity and shortest at 70°C at 4 m/s air velocity; the average total drying time decreased approximately 40%. As can be observed, higher drying temperature and air velocity enhances the kinetic energy of water molecules, and stimulates the rate of water evaporation. Thus, drying time decreased with increasing air temperature and air velocity. These results are in agreement with previous studies. The results of increasing drying air temperatures are similar to those reported by Simal et al. [33] for green peas, Rossello et al. [34] for
green beans, Mohapatra and Rao [35] for wheat, and Rordprapat et al. [36] for paddy. Moreover, increasing air velocity has been shown by other researchers to significantly reduce the drying time: e.g., Iguz et al. [37] for rough rice, Fumagalli and Freire [38] for grass seed, and Khatchatourian [39] for soya beans.

The environmental conditions are shown in Figure 3a–f. Experiments were performed in clear sunshine sky conditions and began at 10:00 am until the moisture content was decreased to 14% (w.b.). Within this framework, it was determined that the drying time and environmental conditions affected the amount of electricity generated from the solar panel and wind turbine. It was observed that the dominant wind direction was from north to south in September. The energy generated from wind turbine calculated with rated wind speed.

The variation in energy consumption (electrical resistance and blower) and energy production (PV and Wind) for different drying treatments are shown in Figure 4. When the drying processes were compared with respect to energy consumption, it was noted that increasing the temperature of air with relative humidity is important for electrical resistance energy consumption. In addition, the dryer required less energy when heating low temperature air. The best results among all drying methods with regard to energy consumption were obtained at 50 °C at 3 m/s. The highest energy consumption of all drying methods was at 70 °C at 4 m/s. As observed in Figure 4, energy consumption increased slightly with air temperature and air velocity rates. During drying, the average total energy consumption decreased from 11.14% to 17.68% when the PV panels produced energy. In addition, the wind turbine at rated wind speed decreased energy consumption by 311 to 518 W.

From Figure 5, the specific energy increased with increasing temperature (at constant air velocity), and it increased with air velocity (at constant temperature). Data show that the highest specific energy for drying of maize kernels was 10.912 kWh/kg at 70 °C at 4 m/s and the lowest value was

FIGURE 4
Energy consumption and energy production
FIGURE 5
Specific energy required for drying maize kernels

TABLE 1
Hunter $L^*$, $a^*$, $b^*$ values and Rehydration capacity of maize kernel under drying conditions

<table>
<thead>
<tr>
<th>Temperature</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>Rehydration Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>66.56±1.39 a</td>
<td>12.81±0.83 c</td>
<td>44.53±1.72 a</td>
<td>-</td>
</tr>
<tr>
<td>3m/s 50°C</td>
<td>56.37±1.08 e</td>
<td>17.54±2.54 b</td>
<td>35.14±1.16 f</td>
<td>1.23±0.01 a</td>
</tr>
<tr>
<td>60°C</td>
<td>59.49±0.28 c</td>
<td>18.69±0.75 b</td>
<td>39.27±0.56 d</td>
<td>1.25±0.03 ab</td>
</tr>
<tr>
<td>70°C</td>
<td>60.95±0.87 b</td>
<td>19.08±0.74 ab</td>
<td>42.23±0.82 bc</td>
<td>1.28±0.05 ab</td>
</tr>
</tbody>
</table>
| 7.561 kWh/kg obtained at 50 °C at 3 m/s. This means that by choosing the proper temperature and air velocity, specific energy consumption can be reduced.

Markowski et al. [40] reported that drying barley at 40 °C in a spouted-bed dryer was characterized by a higher total energy consumption than at 30 °C and 35 °C. Taweerattanapanish et al. [41] studied drying of high moisture paddy using the fluidization technique; their results showed that energy consumption decreased when the air flow rate decreased.

Table 1 summarizes the average color values ($L^*$, $a^*$ and $b^*$) of fresh and various dried maize kernel samples. Increasing the drying temperature and/or air velocity led to an evident darkening of the samples. The $L^*$ value decreased with longer duration of drying. Compared with fresh maize kernels, it was observed that the $a^*$ and $b^*$ values increased significantly during drying. From the above discussions, the color, i.e., the $L^*$, $a^*$, or $b^*$ values, of maize kernels processed at 70 °C at 4 m/s was the best, whereas the products dried at 50 °C at 3 m/s were the worst. From the statistical analysis, it was found that the increases both of air temperature and velocity affected the color values significantly (P<0.05). Similar browning changes have been reported by some authors. Gowen et al. [42] reported that the $L^*$ values of chickpeas and soybeans showed significant reductions during drying, after which there was little change in lightness. Jaiboon et al. [43] dried waxy rice at elevated temperatures; the sample dried at 90 °C became relatively darker. Kahyaoglu et al. [44] found that browning reactions depend largely on moisture content, drying temperature and drying time for wheat. Thus, it can be said that drying at higher temperatures and increased air velocity promotes color changes.

The rehydration ratio characteristics of maize kernels dried at different temperatures and air velocities are shown in Table 1. Considering the individual effects, the average rehydration capacity of samples increased with increasing drying temperature and air velocity. Additionally, the statistical analysis showed that there were significant differences of rehydration capacity at the 5% probability level only between 50 °C at 3 m/s and 70 °C at 4 m/s. Rehydration of dried grains has been observed by other researchers as well. Tunaboyu [32] dried wheat with a solar spouted bed in the range of 40 and 68 °C, and in the open sun with air temperature in the range of 25 to 32 °C. The rehydration ratio for the solar spouted bed dried wheat was higher than that for open sun dried wheat. In another study, Doymaz and
Kocayigit [45] showed that green peas dried at 70 °C have the lowest rehydration capacity compared to 55, 60 and 65 °C drying temperatures.

CONCLUSIONS

Based on drying maize kernels, the following conclusions were deduced. The average total drying time decreased approximately 40%, the maximum time being at temperature of 50 °C and velocity of 3 m/s and the minimum at temperature of 70 °C and velocity of 4 m/s. All drying processes induced color deviations, with the highest at 50 °C at 3 m/s and the lowest at 70 °C at 4 m/s. The highest and lowest rehydration capacity of 1.38 was obtained at 70 °C at 4 m/s and 1.23 at 50 °C at 3 m/s, respectively. The minimum specific energy consumed for drying at 50 °C at 3 m/s was 7.561 kWh/kg water, while the maximum energy consumption was 10.912 kWh/kg water at 70 °C at 4 m/s. As overall conclusion, between 11.97% and 19.50% of the total energy consumption of the dryer can be prevented with hybrid system.

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STUDY ON THE BIODEGRADATION CHARACTERISTICS OF ISOFENPHOS-METHYL AND ISOLATION OF AN ISOFENPHOS-METHYL DEGRADING BACTERIUM, BACILLUS ATROPHEAEUS IM-5

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ABSTRACT

A strain IM-5 capable of highly degrading isofenphos-methyl was isolated from soil. The strain IM-5 was identified as Bacillus atrophaeus based on 16S rRNA and analysis of morphology, physiological and biochemical characters. The optimal pH value and temperature of strain IM-5 were 7.0 and 35-40°C. The strain IM-5 also had high tolerance to isofenphos-methyl. The degradation rate of isofenphos-methyl (50mg/L) by the strain IM-5 could reach about 93.2% in 5d. Isopropyl salicylate and salicylic acid were two important intermediate metabolites of isofenphos-methyl biodegradation. The strain IM-5 could degrade isopropyl salicylate quickly, but could not degrade salicylic acid.

KEYWORDS:
Isofenphos-methyl, biodegradation, Bacillus atrophaeus

INTRODUCTION

Isofenphos-methyl(isopropyl(RS)-O-[(isopropylamino) methoxyphosphiniothioyl] salicylate) was widely used to control a variety of soil insects in various grain and cotton crops, as well as in tree farming practices [1]. Isofenphos-methyl was a high-toxic pesticide, and it was also considered to be moderately persistent in soil. The isofenphos-methyl residue might bring some trouble to soil organism, aquatic systems and public health [2-4]. Because of its toxicity and persistence, the remediation of isofenphos-methyl pollutants has caused growing concern [5, 6]. The microbial degradation was usually considered the most important way of isofenphos-methyl degradation, and the use of microorganisms for bioremediation of isofenphos-methyl contaminated sites has received increasing attention as an efficient and cost-effective biotechnological approach [7, 8]. In the biodegradation process of isofenphos-methyl, intermediate compounds such as isopropyl salicylate and salicylic acid are usually produced [9, 10]. In particular, isopropyl salicylate has a strong antibacterial activity and has a higher stability in soil, and can have negative effects on soil microbes and soil enzyme activity [11]. In addition, salicylic acid as an important phytohormone, may also have an unpredictable effect on the growth of plants. Therefore, attention should be paid to such intermediate metabolites [12, 13].

At present, there were only a few studies on the isolation of pure culture capable of degrading isofenphos-methyl without providing other carbon sources. In this study, Bacillus atrophaeus IM-5, a strain that could degrade isofenphos-methyl was isolated. Its degradation potential and the application for environment protection were studied.

MATERIALS AND METHODS

Chemicals and Media. Chemicals: isofenphos-methyl (98.5%) was purchased from Sigma-Aldrich Company. All other chemicals used were of analytical grade and commercially available.

Culture media: (1). Mineral salt medium: (NH4)2SO4 0.1g, K2HPO4 0.1g, MgSO4·7H2O 0.2g, CaSO4 0.05g, FeSO4·7H2O 0.01g, water 1.0 L, pH 7.0. (2). Isolation medium: mineral salt medium containing isofenphos-methyl (50 mg/L) was used to isolate isofenphos-methyl degrading bacteria. (3). Enrichment medium: peptone 7.0 g, beef extract powder 5.0 g, yeast extract powder 5.0 g, sodium chloride 5.0 g, water 1.0 L, pH7.0.

Isolation and identification of isofenphos-methyl degrading bacteria. The soil sample was collected from woodland in Zhenjiang City, China, in which isofenphos-methyl has been used for more than 8 years. The soil sample was placed in 100ml isolation medium for shaking culture at 30°C (100 rpm). The concentration of the residual isofenphos-methyl was determined every 24h [14]. 5ml culture
solution with 3d degradation rate >70% was transferred to enrichment medium containing same concentration of isofenphos-methy, and continuously transferred for more than 5 times. After the degradation effect was verified again, the above enrichment medium was coated on mineral salt culture plate containing isofenphos-methy for inverted culture at 30°C. The eugonic bacterial colonies were selected and repeatedly streaked on the culture plate to obtain the pure culture. The new isolates were identified by using 16SrRNA sequence analysis.

Effect of temperature on the growth of strain IM-5. Inoculums of isofenphos-methy degrading bacteria were prepared in advance [15]. 5% strain IM-5 inoculum was incubated in mineral salt culture medium containing 50mg/L isofenphos-methy under the different temperature. OD<sub>600</sub> was determined every 12h.

Effects of pH on the growth of strain IM-5. pH of mineral salt culture medium (containing 50mg/L isofenphos-methy) was adjusted to 6.0, 6.5, 7.0, 7.5 and 8.0. Then they were inoculated with 5% inoculum respectively. The shaking culture was performed at 35°C. OD<sub>600</sub> was taken regularly.

Tolerance of the strain IM-5 on isofenphos-methy. The concentration of isofenphos-methy in mineral salt medium was adjusted respectively to 50mg/L, 100mg/L, 250mg/L, 500 mg/L and 1000 mg/L. Then the strain IM-5 was inoculated respectively for shaking cultivation at 35°C. OD<sub>600</sub> was determined regularly, so as to evaluate the tolerance of strain IM-5 on isofenphos-methy.

Degradation of isofenphos-methy by strain IM-5. 5% strain IM-5 inoculum was inoculated in mineral salt culture medium containing 50mg/L isofenphos-methy, and then place it in incubator (100rpm, 35°C). The concentration of isofenphos-methy, isopropyl salicylate and salicylic acid were determined every 24h [16,17].

Degradation of isofenphos-methy by combining strain IM-5 and strain SALL-7. The strain SALL-7 was an isocarbofosph degrading bacterium deposited in our laboratory. It was identified as *Bacillus pumilus* according to 16S analytical method (GenBank accession number MH113275). The strain SALL-7 could effectively degrade isocarbofosph, isopropyl salicylate and salicylic acid. Because the strain IM-5 could not degrade salicylic acid, the combined applications of the strain IM-5 and the strain SALL-7 was considered an effective way to rapidly degrade isofenphos-methy.

4% strain IM-5 inoculum and 4% strain SALL-7 inoculum were inoculated simultaneously in mineral salt culture medium containing 50mg/L isofenphos-methy, and then incubate in incubator. The concentration of isofenphos-methy, isopropyl salicylate and salicylic acid were determined every 24h.

**RESULTS AND DISCUSSION**

Identification and characterization of isofenphos-methy degrading bacteria. A strain capable of using isofenphos-methy as the sole carbon sources and energy source was isolated from soil, named IM-5. It was straight bacillus, 0.7-0.8×2.0-3.0µm in size, G+, subterminal or middle spore, motile, aerobic, and form opaque and gray colonies on nutrient broth plates. It was positive in tests for catalase, glucose fermentation, starch hydrolysis, but negative for iodole test and methyl red test [18]. The strain IM-5 was identified as *Bacillus atrophaeus* according to 16S sequence analysis (GenBank accession number MH236803).

Effects of temperature and pH on the strain IM-5. The strain IM-5 has the highest growth level at 35-40°C, too high or too low temperatures would inhibit the growth of strains (Fig. 1). The experimental results show that strain IM-5 had a strong isofenphos-methy degrading ability. The degradation rate of isofenphos-methy (initial concentration was 50mg/L) was about 74.4% after 3 days of incubation, and could reach 97.2% after 6d at 35°C (Fig. 4). In general, the temperature on the one hand could affect the growth of microorganisms and enzyme activity, on the other hand the temperature could also directly affect the degradation rate of organic compounds. The increase of the temperature would cause the acceleration of enzymatic degradation reaction, but the excessively high temperature would lead to the inactivation of degrading enzymes and other enzymes [19-21]. Based on the above factors, 35-40°C was considered as the optimum temperature of the strain IM-5 for growth and degrading isofenphos-methy.

Environmental pH was also an important factor affecting the growth of microorganisms. The experimental results showed that the growth state of strain IM-5 at pH 7.0 was better than that under other pH conditions (Fig.2), and the optimum pH of the strain IM-5 was pH7.0. Generally, pH could not only cause the change of the biodegrading ability through influencing microbial growth, but also could directly influence the activities of relevant degrading enzymes [22, 23].
Tolerance of the strain IM-5 on isofenphos-methy. When the concentration of isofenphos-methy increased from 100mg/L to 500mg/L, OD$_{600}$ did not change significantly (P>0.05). OD$_{600}$ would slightly decrease if the concentration of isofenphos-methy was 50mg/L (Fig.3), it may be due to 50 mg/L of isofenphos-methy could not meet the carbon source or energy requirements for the strain IM-5 growth. Although 1000 mg/L isofenphos-methy could cause a significant decrease in OD$_{600}$, the strain IM-5 could still grow. Therefore, it was preliminarily considered that the strain IM-5 could tolerate at least 1000mg/L isofenphos-methy under the experimental conditions.

According to the existing results, it was inferred that the strain IM-5 had a strong degradation ability to isofenphos-methy (because isofenphos-methy was used as the sole carbon source in this study), which could be used in a wider range of isofenphos-methy concentration. In addition, it was possible that the strain IM-5 was used in the bioremediation of isofenphos-methy pollution. The degradation ability of the strain IM-5 might be closely related with the long-term use of isofenphos-methy in woodland soil. Generally, soil microorganism that continuously encounter the artificially synthesized compound might be induced the ability to degrade the compound [24, 25].
Degradation of isofenphos-methy by the strain IM-5. The results in Fig. 4 showed that the isofenphos-methy could be rapidly degraded by the strain IM-5. The degradation rate of isofenphos-methy (50 mg/L) by the strain IM-5 could reach about 93.2% in 5 d. Isopropyl salicylate and salicylic acid have been detected during the degradation of isofenphos-methy. The concentration of isopropyl salicylate increased first and then rapidly decreased, indicating that isopropyl salicylate was rapidly degraded. At the same time, it was found that the concentration of salicylic acid continued to increase, showing that salicylic acid could not be effectively degraded during this process. It was presumed that strain IM-5 could not degrade salicylic acid, but could degrade isopropyl salicylate quickly.

Degradation of isofenphos-methy by the strain IM-5 and the strain SALL-7. It was considered that the biodegradation pathway of isofenphos-methy was as follows: Isofenphos-methy $\rightarrow$ Isopropyl salicylate $\rightarrow$ Salicylic acid $\rightarrow$ $\ldots$ $\rightarrow$ CO$_2$+H$_2$O. During the degradation of isofenphos-methy, the degradation rate of intermediate metabolites and the accumulation of intermediates will affect the entire degradation process. The results in Fig. 5 showed that isofenphos-methy could be rapidly degraded by the strain IM-5 and
the strain SALL-7, the degradation rate of isofenphos-methy (50mg/L) could reach about 97.0% in 5d. In addition, the continuous decrease of salicylic acid concentration in Fig. 5 indicated that strain SALL-7 could rapidly degrade salicylic acid. By comparing Fig. 4 with Fig. 5, it could be seen that the combination of two strains could increase the degradation rate of isofenphos-methy. This should be attributed to the fact that strain SALL-7 could degrade salicylic acid and release the accumulation of intermediate products.

CONCLUSION

In this study, *Bacillus atrophaeus* IM-5 was isolated from soil, which could degrade isofenphos-methy. The optimal pH value and temperature of strain IM-5 were 7.0 and 35-40°C. The strain IM-5 also had high tolerance to isofenphos-methy. It could tolerate at least 1000mg/L isofenphos-methy under the experimental conditions. The degradation rate of isofenphos-methy (50mg/L) by the strain IM-5 could reach about 93.2% in 5d. Isopropyl salicylate and salicylic acid were two important intermediate metabolites of isofenphos-methy biodegradation. The strain IM-5 could degrade isopropyl salicylate quickly, but could not degrade salicylic acid. The combined applications of the strain IM-5 and the strain SALL-7 was considered an effective means for rapidly degrading isofenphos-methy. The degradation rate of isofenphos-methy (50mg/L) by the strain IM-5 and the strain SALL-7 could reach about 97.0% in 5d.

ACKNOWLEDGEMENTS

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REFERENCES


ASSESSING CLIMATE CHANGE IMPACTS ON WATER RESOURCES OF THE HOTAN OASIS USING SWAT MODEL, NORTHWEST CHINA

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ABSTRACT

Assessing the water resources of Oasis areas was very important for the ecological environment protection and sustainable development in arid area, especially under the threat of global climate change. This study predicted the runoff of Hotan Oasis over the period of 2020-2050, using the SWAT model driven by China Meteorological Assimilation Driving Datasets (CMADS) on the possible Scenarios, based on the analysis of the trends of temperature, precipitation, and runoff over the period of 1980-2015. The results indicated that the trend of temperature, precipitation, and runoff were increased especially from the 21 Century. Both the significant increasing of annual mean temperature from 2002 and slightly increasing of annual mean precipitation from 2004 due to the increasing tendency of runoff of Hotan river. SWAT model demonstrated well satisfactory performance for the upstream Hotan river basin when driven by CMADS. The runoff into Hotan Oasis is generally predicted to increase of 10.34%, 22.45%, 34.61% and 47.46% in 2020, 2030, 2040 and 2050 by assuming climate scenarios analysis using the SWAT model, which provides useful information for water allocation and management of Hotan Oasis to meet the country’s future needs.

KEYWORDS:
Climate change, SWAT model, CMADS, Hotan Oasis

INTRODUCTION

Climate change has emerged as one of the most important global environmental challenges of the 21st century, and further climate change will increase human vulnerability to depleted water resources. Nearly all regions of the world are expected to experience a net negative impact of climate change on water resources systems [1-3]. The responses of hydrology and water resources to climate change has been investigated all over the world [4-7]. There have been many studies which showed that climate change is a major driven factor of the variation of runoff, especially in areas with less human activities [8, 9]. The influence of climate change on runoff are complex and multifarious through the variation of precipitation and temperature, which mainly affecting the supply of runoff, evaporation and so on.

The global climate change influence was obvious in Xinjiang science 1980s, where the temperature trend has rising, and therefore, the runoff volume of many rivers has increased and some lakes water level has been increasing continuously [10]. Hotan River is located in Xinjian, the arid area of northwest China, as one of the four headwaters of Tarim River. The sources of runoff of Hotan River are precipitation and melting glacial [11]. In recent years, many researchers investigated the trend of runoff [12-16], climate [17, 18] and other issues in Hotan River Basin. Several previous studies pointed out that the runoff of Hotan River was decreased continuously while temperature and precipitation were increased, which presented a contrary situation compared with the other three headwaters of Tarim River [19, 20]. However, the dates studied in almost all existing papers were more than ten years ago. The impact of climate change on runoff in Hotan river basin changed is still not clear, especially for the last ten years.

Hotan Oasis is located in the Takalaman Desert. As a result of the harsh climatic conditions and water scarcity in the region, the river basin is very vulnerable to climate change [18]. The water resources of Hotan Oasis mainly come from the runoff of Hotan River, that is the key factor of social and economic development for Hotan city, and it plays a very important role in the ecological environment protection of Hotan basin. As one of headwaters of the Tarim River, runoff of Hotan River also directly effects on supplying to Tarim river. On the other hand, irrigated area of the basin...
continues to expand, and agricultural water usage continues to increase. The amount of water flowing in the main stream can’t be guaranteed, and the watershed-management departments lack powerful monitoring approaches for unified scheduling and control, causing unsustainable allocation of water resources [21]. Therefore, assessing the runoff into Hotan Oasis was very practical.

Many researches in different study areas assessed the climate change impacts on runoff using Soil and Water Assessment Tool (SWAT), which is one of the most widely used watershed models throughout the world [2, 6, 22]. The water resources of Hotan Oasis areas come from the head waters of Hotan River (i.e. Yulongkasi River and the Kalakasi River). The runoff of Yulongkasi River was simulated by SWAT in previous studies [23, 24]. However, the runoff of Kalakasi River has not been simulated, and the runoff into Hotan Oasis areas has not also been assessed by SWAT, especially based on the trends analysis of climate change. Therefore, it is of great practical significance to improved knowledge of the impact of climate change on runoff of Hotan River and to assess climate change impacts on water resources of the Hotan Oasis.

The objectives of this study were: (1) to analyse the trends of climate and runoff during 1980-2015, and to analyse the impact of climate change on runoff; (2) to test the adaptability of SWAT in Kalakasi River, and to predict the amount of future runoff under climate change with SWAT to provide scientific support for allocation of water resources and sustainable development of Hotan Oasis.

METHODS

Study area. The study area is the upstream area of the Hotan River basin (39°38′-41°45′N, 85°42′-89°17′E) (Fig 1). The Hotan River flows for 1127 km [25], with a mean annual water discharge of 4.478×10⁹ m³, which is the second biggest river on the northern slope of the Kunlun Mountains of southern Xinjiang in China. The Hotan River water comes from the Yulongkasi River and the Kalakasi River, that are converged at Kuoshilasi, then passes through the Taklimakan Desert from south to north before flowing into the Tarim river. The upper reaches of the study area are high mountains with elevations varying from 1192 m to 6858 m asl and ridges covered with snow all the year round. The area is located in a semi-arid climate influenced by continental climate variation with an annual precipitation range between 5.4 and 89.6 mm and mean annual temperature of 12.2°C.
Model and input data. The watershed model used in this study is the SWAT 2012. SWAT is one of the most widely used hydrologic models under different climate changes, which are important tools used for the development of water management strategies. Many studies of runoff simulation were carried out by SWAT model, and the areas at these studies were contained plains, mountains, and even high elevation area [22]. In order to evaluate the performance of the calibrated and validated results provided by the model, statistical performance indicators, such as Nash-Sutcliffe efficiency coefficient (NSE), the ratio of root mean squared error to standard deviation (RSR) and the percentage of bias (PBIAS) were used. The statistical performance indicators are expressed as follows [26]:

\[
\text{NSE} = 1 - \frac{\sum_{i=1}^{n}(Y_{i,\text{obs}} - Y_{i,\text{sim}})^2}{\sum_{i=1}^{n}(Y_{i,\text{obs}} - Y_{\text{mean}})^2}
\]

\[
\text{RSR} = \frac{\sum_{i=1}^{n}Y_{i,\text{obs}} - Y_{i,\text{sim}}}{\sqrt{\sum_{i=1}^{n}(Y_{i,\text{obs}} - Y_{\text{mean}})^2}}
\]

\[
\text{PBIAS} = \frac{\sum_{i=1}^{n}Y_{i,\text{obs}} - Y_{i,\text{sim}}}{\sum_{i=1}^{n}Y_{i,\text{obs}}} * 100
\]

Where \(Y_{i,\text{obs}}\) is the observed stream flow at time step \(i\), \(Y_{i,\text{sim}}\) is the simulated stream flow at time step \(i\), \(Y_{\text{mean}}\) is the mean of observed stream flows, and \(n\) is the total number of observations. Normally, when RSR≤0.7, NSE>0.5 and PBIAS≤±25%, the modeling results of the runoff are considered to be satisfactory. Furthermore, the modelling results can be evaluated as good if 0.6≤RSR<0.7, 0.75≤NSE<0.65 and ±15%≥PBIAS≥±10%, and very good if 0.5≤RSR<1.0, 0.75≤NSE<0.75 and ±10%≥PBIAS [3, 27].

Geographical information used in this study includes digital elevation data (DEM), land use data and soil type. The DEM data with a resolution of 30 m were obtained from National Aeronautics and Space Administration (NASA) database. We extracted DEM of the study area, mentioned the river network and divided basin. Land use data in the 2010s with a resolution of 30 m were obtained from Data Center for Resources and Environmental Sciences, Chinese Academy of Sciences (RESDC) (http://www.resdc.cn). The soil data were provided by Cold and Arid Regions Sciences Data Center at Lanzhou (http://westdc.westgis.ac.cn). Parts of the attribute of soil database of SWAT model, such as bulk density, field capacity and saturated hydraulic conductivity were calculated with Soil-Plant-Atmosphere-Water Model (SPAW) [28]. The other attributes were obtained from Soil Topography of China and SWAT soil database.

Measured annual runoff data (from 1980 to 2015) and meteorological data (i.e., annual precipitation and temperature; from 1980 to 2015) were collected from two hydrologic stations (i.e. Wuluvati Hydrometric Station and Tongguziuluoke Hydrometric Station). The weather data (from 2008 to 2014), which includes the daily maximum temperature, daily minimum temperature, 20–20 h precipitation, average relative humidity, average solar radiation, and average wind speed, used for SWAT in this study were from the China Meteorological Assimilation Driving Datasets (CMADS) which were used in many watersheds in China [29]. It allows the SWAT models to utilize the datasets directly, thus eliminating the need for any format conversion or calculations using weather generators. Consequently, significant improvements to the modeling speed and output accuracy of SWAT models were achieved [29, 30].

The SWAT-CUP and its SUFI-2 algorithm [31], for SWAT model sensitivity, calibration, and validation, as well as for uncertainty analysis, were used in the study. While SWAT offers a wide range of parameters for sensitive analysis, 18 parameters related to variation in snow processes, groundwater, infiltration, evaporation, soil and basin parameters were selected that are known to influence the stream flow in similar catchments [32, 33]. In this study, the periods 2008, from 2009 to 2012 and 2013 to 2014 were taken as warm-up period, calibration period and validation period respectively.

Meteorological and hydrological trends analysis. Inspect historical trends of hydro-meteorology factors can help to understand the effects of climate change on water resources systems. Moving average, linear trend analysis and Mann-Kendall test were widely applied to analyze trends in meteorological element time series and hydrological time series [34-36]. In the study, 5-year moving average, linear trend analysis by R value from the significance test of the correlation coefficient [34], and Mann-Kendall test used by MATLAB2010 were applied.

RESULTS AND DISCUSSION

Trends analysis of climates and runoff. Figure 2-3 show a significant increasing of annual mean temperature and annual mean precipitation from the linear trend analysis during 1980–2015 (\(R_{\text{p}}=0.42\); \(R_{\text{tp}}=0.33\); \(R_{\text{m}}=0.34\); \(R_{\text{tm}}=0.33\); 0.05 significance level \(P=0.05\), respectively. The linear trend analysis shows that a rate of 0.31 °C/decade and 16.5 mm/decade in the upstream of Hotan River basin, respectively. Furthermore, the increasing rate of temperature is more significant compared to the Hotan plain, where the rate is 0.2°C/decade, whereas the increasing rate of precipitation is 7.1 mm/decade [36, 37]. Affected by the topography, the temperature rises from the mountain to the plain in the Hotan River basin, and the precipitation displayed the opposite result [18]. The mean annual temperature was roughly stable from 1980 to 1997, then increased, by 5-year moving average analysis. While from the Mann-
FIGURE 2
The change trend of annual mean temperature in Hotan upstream basin from 1980 to 2015, (a) 5-year moving average and liner trend, (b) M-K test.

FIGURE 3
The change trend of annual mean precipitation in Hotan upstream basin from 1980 to 2015, (a) 5-year moving average and liner trend, (b) M-K test.
Kendall test, the trend was increased from 1997, and kept increasing until 2015. The increased trend of mean annual precipitation was begun at about 2002 by 5-year moving average analysis, and that happed at 2004 from the Mann-Kendall test. The change-points of temperature were mainly happed at 1990-1995, while change-points of precipitation were mainly happed 2000-2005.

The trend of runoff is the least obvious and the most complex compared to the temperature and precipitation [17]. Figure 4 indicates that there was a non-significant increasing trend of annual mean runoff during 1980–2015 from the linear tendency analysis ($R_{\text{runoff}}=0.154 < R_{0.05}=0.256$; 0.05 significance level $P=0.05$), and the runoff increased about $3.4 \times 10^8$ m$^3$/decade over past thirty-six years in the study catchment. The results are different to the study by Shen et al. [17, 19, 20, 38], Fu et al. [13], Chu et al. [39], and Xu et al. [40], who found that the trend of runoff was non-significant decreased during about 1957 to 2005. However, the results are approximately consistent with the previous results [41, 42], which were found the trend of runoff was increased during 1953 to 2014. Therefore, time series studied in the research may be the mainly reason accounted for the difference (the increasing trend of runoff from the change point which happed at 2004 may due to the increasing of the whole time series). In addition, the runoff of the last ten years was increased more than the earlier years was the other reason.

From the Mann-Kendall test of runoff, there was also indicated that a decreasing trend happened in 1985-2005 and an increasing tendency happened from about 2005, while the change-points are mainly happed at about 2005. These results can be described as a fact that the runoff is increasing evidently in the last ten years. That happened due to the superimposed effect of temperature significant decreasing trend from 2002 and the change-points of precipitation happened at about 2003.

Generally, the above results suggested that trend of runoff of the studied river basin was increasing especially from the 21 Century. Additionally, both the significant increasing of annual mean temperature (accelerating melting ice) from 2002 and slightly increasing of annual mean precipitation from 2004 due to the increasing tendency of runoff.

**Model Calibration and Validation.** The range and final parameter values of SWAT after calibration for the Kalakasi River catchment (Wuluwati hydrological station) and the Yulongkasi River catchment (Tongguziuke hydrological station) were shown in Table 1.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value Range</th>
<th>Fitted Value</th>
<th>Wuluwati</th>
<th>Tongguziluoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>v_SFTMP.bsn</td>
<td>Snow fall temperature (°C)</td>
<td>[-5, 5]</td>
<td>-1.65</td>
<td>-0.78</td>
<td></td>
</tr>
<tr>
<td>v_SMTMP.bsn</td>
<td>Snowfall melt base temperature (°C)</td>
<td>[-5, 5]</td>
<td>1.75</td>
<td>2.02</td>
<td></td>
</tr>
<tr>
<td>v_SMFMX.bsn</td>
<td>Maximum melt rate for snow during the year (mm/°C·day)</td>
<td>[0, 10]</td>
<td>1.65</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>v_SMFMN.bsn</td>
<td>Minimum melt rate for snow during the year (mm/°C·day)</td>
<td>[0, 10]</td>
<td>1.45</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>v_TIMP.bsn</td>
<td>Snowpack temperature lag factor</td>
<td>[0, 1]</td>
<td>0.64</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>v_SNO50COV.bsn</td>
<td>Snow water equivalent at 50% snow cover</td>
<td>[0, 0.9]</td>
<td>0.32</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>v_SNOCOVMAX.bsn</td>
<td>Minimum snow water content at 100% snow cover (mm)</td>
<td>[0, 500]</td>
<td>257.50</td>
<td>118.75</td>
<td></td>
</tr>
<tr>
<td>v_PLAPS.sub</td>
<td>Precipitation lapse rate (mm/km)</td>
<td>[-100, 500]</td>
<td>143</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>v_TLAPS.sub</td>
<td>Temperature lapse rate (°C/km)</td>
<td>[-10, 10]</td>
<td>-3.50</td>
<td>-4.65</td>
<td></td>
</tr>
<tr>
<td>v_GW_DELAY.gw</td>
<td>Groundwater delay time (days)</td>
<td>[30, 450]</td>
<td>376</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td>v_GWQMN.gw</td>
<td>Groundwater ‘revap’ coefficient</td>
<td>[0.02, 0.2]</td>
<td>0.17</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>v_REVAPMN.gw</td>
<td>Threshold depth of water in the shallow aquifer required for return flow to (mm)</td>
<td>[0, 2]</td>
<td>1.31</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>v_REVAP.gw</td>
<td>Threshold depth of water in the shallow aquifer for ‘revap’ to occur (mm)</td>
<td>[0, 500]</td>
<td>87.5</td>
<td>150.01</td>
<td></td>
</tr>
<tr>
<td>v_ESCO.hru</td>
<td>Soil evaporation compensation factor</td>
<td>[0, 1]</td>
<td>0.51</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>r_CN2.mgt</td>
<td>SCS runoff curve number for moisture condition II</td>
<td>[-0.2, 0.2]</td>
<td>-0.062</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>r_SOL_K.sol</td>
<td>Available water capacity of the soil layer</td>
<td>[-0.8, 0.8]</td>
<td>0.71</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>v_SURLAG.bsn</td>
<td>Surface runoff lag time</td>
<td>[0.05, 24]</td>
<td>13.82</td>
<td>10.41</td>
<td></td>
</tr>
</tbody>
</table>

Note: a, “v_” means that the existing parameter value is to be replaced by a given value, and “r_” means that an existing parameter value is multiplied by (1 + a given value)

### Table 2

<table>
<thead>
<tr>
<th>Performance indices at different stations in Hotan catchment</th>
<th>Period</th>
<th>Index</th>
<th>Wuluwati</th>
<th>Tongguziluoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration (2009–2011)</td>
<td>NSE</td>
<td>0.80</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSR</td>
<td>0.44</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bias</td>
<td>-9.8%</td>
<td>-2.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSE</td>
<td>0.80</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Validation (2012–2014)</td>
<td>RSR</td>
<td>0.44</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bias</td>
<td>-6.1%</td>
<td>9.6%</td>
<td></td>
</tr>
</tbody>
</table>

The model performance results of Kalakasi River catchment (Wuluwati hydrological station) and the Yulongkasi River catchment (Tongguziluoke hydrological station) were shown in Table 2 and Figure 5. NSE values are both 0.80 for the Wuluwati hydrological station during calibration and validation periods at the monthly time step, which demonstrated that the model simulation met “very good” according to the studies [3, 27]. Additionally, the values of RSR with both 0.44 and Bias with -9.8% and -6.1% during calibration and validation periods at the monthly time step respectively, which also indicated that the model simulation performed very well. The model performance of Tongguziluoke hydrological station was “very good” and “good” during calibration and validation periods at the monthly time step from the values of three indicators. The SWAT model simulation results of Tongguziluoke hydrological station were almost the same to the previous researches [23, 24]. Over all, SWAT model performance was well satisfactory performed during calibration periods and validation periods at the monthly time step for both the Kalakasi River catchment and the Yulongkasi River catchment. It was also demonstrated that CMADS could be well used in the Tongguziluoke [23] and Wuluwati hydrological stations.

Effect of Climate Change Scenarios with SWAT. There are two ways to quantify the impact of climate change on the hydrology of a watershed by SWAT. The first method of climate change impact quantification involves the calculation of change in the basin behavior towards change in the concentration of greenhouse gases in the atmosphere, while the second method facilitates to input specific changes of the precipitation and temperature [2]. In this study, the second method (i.e., specific incremental scenarios for the precipitation and temperature), which has been used in many past studies [2, 22, 43], was selected to
estimate the impacts of climate change on runoff. The precipitation, maximum temperature and minimum temperature on a monthly time step in calibration periods during 2009 to 2011 were used as a baseline condition. Due to the precipitation and temperature increased 12.5% and 0.3% during 1980-2015 above in the study respectively, also considered the conclusion drawn from the IPCC AR5 in the earlier studies in the study area [24]. Six Combined Scenarios (1-6) (Table 3) were adopted to estimate the impacts of climatic variables on runoff. Furthermore, based on the increasing trends of the precipitation and temperature during 1980-2015, also assuming the precipitation and temperature will increase 12.5% and 0.3% respectively. In the future, four Combined Scenarios (7-10) (Table 3) were also considered in this study to analyze the combined effects of future changes in both precipitation and temperature on the runoff of the study area.

![Observed vs Simulated Runoff](image)

**Simulation and observation of the model performance during the calibration and validation periods from 2009 to 2014, (a) Wuluwati hydrological station, (b) Tongguziluoke hydrological station.**

**TABLE 3**

Climate change scenarios based on independent changes in temperature and rainfall

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Precipitation increase (%)</th>
<th>Temperature increase (°C)</th>
<th>Runoff increase (%)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.5</td>
<td>-</td>
<td>8.19</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>-</td>
<td>6.45</td>
<td>-</td>
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<tr>
<td>3</td>
<td>-10</td>
<td>-</td>
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<td>-</td>
</tr>
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<td>4</td>
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<td>0.3</td>
<td>1.96</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>1</td>
<td>7.74</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>2</td>
<td>12.99</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>12.5</td>
<td>0.3</td>
<td>10.34</td>
<td>2020</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>0.6</td>
<td>22.45</td>
<td>2030</td>
</tr>
<tr>
<td>9</td>
<td>37.5</td>
<td>0.9</td>
<td>34.61</td>
<td>2040</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>1.2</td>
<td>47.46</td>
<td>2050</td>
</tr>
</tbody>
</table>
When the precipitation of the Hotan upstream catchment was assumed to change (i.e., P+12.5%, +10% and P-10%), and the other parameters were kept constant, the runoff of Hotan (i.e., total runoff of Tongguziuloke and Wuluwati) will be increase 8.19%, 6.45% and -6.23%. Meanwhile, when the temperature of the Hotan upstream catchment was assumed to change (i.e., T+0.3°C, T+1°C and T+2°C), and the other parameters were kept constant, the runoff of Hotan (i.e., total runoff of Tongguziuloke and Wuluwati) will be increase 1.96%, 7.74% and 12.99% (Table 3). The result indicated that both the rise in the precipitation and the snowmelt with the temperature increased due to the increase of runoff [42]. Additionally, the runoff is more sensitive to temperature than precipitation, for that the runoff with precipitation +10% added was less than that with +1°C added.

While the precipitation and temperature increase 12.5% and 0.3% for one decade respectively in the future, the runoff of Hotan River will be predicted to increase 10.34%, 22.45%, 34.61% and 47.46% in 2020, 2030, 2040 and 2050, compared to the average value during 2009 to 2011(Table 3). Thus, runoff is generally predicted to increase [24] and the water-resource deficiency of Hotan Oasis in the current situation would be reduced. In fact, that result was based on the Scenarios 7-10. Currently, the period of drought is becoming longer in Tarim River [21]. Meanwhile, the lines of water resource demands of Tarim River, which defined amount of quota from the upstream of Hotan River should be given to meet the ecological environmental protection, was made by the government of Xinjiang Uygur Autonomous region in 2013. Thus, water-saving irrigation, recycling of water resources and the efficiency of the exploitation of water resources should be improved to maintain the oasis development of socioeconomic sustainable [21, 44, 45]. In addition, this study provides a probable guideline of water allocation and management of Hotan Oasis in future for local decision makers.

CONCLUSIONS

This study concludes that the trends of annual mean temperature, annual mean precipitation, and annual runoff from the upstream regions of the Hotan River basin were increased especially from the 21 Century. Both the significant increasing of annual mean temperature from 2002 and slightly increasing of annual mean precipitation from 2004 due to the increasing tendency of runoff. SWAT model demonstrated well satisfactory performance for both Tongguziuloke station and Wuluwati station in the studied ungauged Hotan River Basin, which was driven by CMADS. The runoff is more sensitive to the changes in temperature than in precipitation.

The runoff into Hotan Oasis from the upstream of the Hotan River using SWAT model generally is predicted to increase 10.34%, 22.45%, 34.61% and 47.46% in 2020, 2030, 2040 and 2050, respectively, based on the increasing tendency of climate change (temperature and precipitation) in 1980-2015. The research provides useful information for water allocation and management of Hotan Oasis to meet the country’s future needs.

ACKNOWLEDGEMENTS

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REFERENCES


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INVESTIGATION OF ACCESSIBILITY IN RECREATIONAL AND LEISURE AREAS IN BURSA-TURKEY

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ABSTRACT

Recreational areas, where people spend their leisure time, have become increasingly important due to the unfavourable living conditions caused by population growth and rapid urbanization. Recreational areas need to be accessible in order to be available and to increase their potential of use, in addition to the fact that they offer various activity possibilities and functionalities. The purpose of the study is to reveal the importance of transportation in recreation and to examine the relationship of transportation with recreational areas and recreational activities. The study was carried out in Bursa and conducted with "Weighted Criteria Method". The study proposes improvements for accessibility levels and transportation for recreational areas. This study intends to contribute to the effective use of recreational areas.

KEYWORDS:
Accessibility, Leisure, Recreation, Recreation areas, Green spaces, Weighted criteria method

INTRODUCTION

The concept of leisure in the life process has become increasingly prevalent. Today, the notion of leisure is perceived in many cultures as it is in the past, an integral part of everyday life rather than the antithesis of business life. As a result of the great convenience provided by the rapid progress in industrial and technical fields, which started with the industrial revolution, increased leisure time and income have initiated a new process of valuing spare time in line with social values. Leisure depends not only on business and other responsibilities but also on all-time activities together with play and recreation [1-3]. Leisure is an important element in people's lives and should be encouraged in any community [4].

Because of the artificial environments brought by the urbanization and industrialization, which presented negativities in everyday life, people have been searching for different places to rest and have fun [3]. The need for recreation areas to help unhealthier urbanization and human-environment rela-

tions has come into prominence [5]. Along with providing recreation opportunities for physical activities, adequate physical activity contributes to the health status of the person throughout their lifetime [6]. At the same time, recreation areas should be planned according to the growing population, taking into consideration the recreational needs of people while making urban planning important in economically developed societies, as participation in recreational activities is at the top of the developmental level. Recreation areas should be easily accessible to all who live in the city [7].

Urban green areas like parks, forests, green roofs and hobby gardens provide opportunities for ecosystems for cities [8]. The green spaces that play an important role in recreation areas in urban areas should be at the distance of equipment and transportation that the city-dwellers can use them at all times of day and meet daily recreational needs. In this context, urban parks and regional parks within the green spaces offer recreation opportunities to the city dwellers. Other than these, picnic areas, botanical gardens, zoos plantation areas and coppice forest with recreational activities constitute other green spaces [9, 10].

For green spaces to be evaluated as recreational areas, the following conditions should exist [11];

- The position should be appropriate for the purpose; on the urban and regional scale, they should be accessible by public transportation on the neighbourhood and pedestrian on the subscale,
- The size of the area should overlap for the intended use; as the distance between users and the area increases, the size of the area and the increase in the variety of activities should be developed in direct proportion,
- Ergonomics, security, aesthetics, etc. should be designed for purposes appropriate to the principles.

Urban green spaces play an important role in increasing the quality, liveability, and sustainability of our cities and districts when designed in accordance with the functions and purposes required [12]. At the same time, green areas represent physical activity, psychological welfare and general public health of the city-dwellers [8]. Apart from the personal preferences and characteristics of people in urban areas, the facilities provided by these areas
and their social spaces and activities help determine the travel requirements and preferences in urban areas. At the same time, personal transport and accessibility opportunities also affect local mobility [13]. Accessibility is an important criterion for recreation. The number of people who benefit from a recreational area and the problem-free transportation increase the recreation potential of the area [14]. Access to the activity areas designed for comfort and convenience in urban open spaces and transportation within the area is also very important for the individuals with disabilities. One of the most important elements for the socialization of pregnant women, mothers with children, and the senior citizens, as well as individual with physical disabilities, is the availability of accessibility [15]. While transportation systems are being planned, supporting the plant material, which can contribute to urban ecology, adds aesthetic and functional values to urban areas, urban roads and recreational areas. Active transportation can be a convenient way to exercise in the daily routine, and this can be an effective policy in urban planning [16].

The negativities caused by transportation systems can be reduced by plants and more identifiable, healthy, readable, positive and meaningful environments can be created [17].

In this context, the aim of the study is to demonstrate the importance of accessibility, an important criterion in outdoor recreation decision-making process, and to examine its relation to recreational activities in recreational areas. For this purpose, the areas that serve recreation within the scope of green spaces serving to all city and districts have been evaluated. In this context, urban parks, regional parks, urban forests, picnic areas and zoos that allow recreation within the boundaries of Bursa city have been determined. Along with other 34, primarily Atatürk Urban Forest, Hüdavendigar Urban Park, Bursa Zoo, Teoman Özalp Regional Park and Ziraat Picnic Area were identified. These were evaluated by selecting one area representing each green space type.

MATERIALS AND METHODOLOGY

Material. Bursa city is located to the south of the Marmara Region, between 39°30’-40°37’ north latitudes and 28°06’-29°58’ east longitudes. It is surrounded by Bilecik, Sakarya in the east, Kocaeli, Yalova, Istanbul and the Marmara Sea in the north, Kütahya in the south and Balıkesir in the west. There are important lowlands in Bursa Provincial borders (Figure 1). The Bursa lowland is the most important of these and covers a large area in the northwestern Uludağ [18]. The average height of the city from the sea is 100 meters and it varies between 100-150 m in the urban development area.

The current settlement area of the city is 62.100 ha, and the population settled in this area is 1.949.034 according to Turkish Statistical Institute. The research area consists of urban parks, regional parks, urban forests, picnic areas and a zoo located within the borders of Osmangazi, Nilüfer, Yıldırım, Gürsu and Kestel districts forming the Bursa urban fabric. A total of 34 areas were identified from the recreational areas indicated in the green spaces. From these, 5 recreational areas with the greatest spatial size and user capacity were evaluated from each recreational area type (Figure 2). These areas are; Atatürk Urban Forest, Hüdavendigar Urban Park, Bursa Zoo, Teoman Özalp Regional Park and Ziraat Picnic Area.

The study utilised the existing maps and application development plan with 1/1000 scale, satellite images, on-site views and plans and reports prepared about the area. AutoCAD and Photoshop programs were used to create the map and edit the photos.

FIGURE 1
Location of Study area

FIGURE 2
Boarder of Study Areas

Methods. The working method aims:
- Determining the presence of recreational area (urban park, regional park, picnic area, zoo and urban forest) at citywide and 5 district levels,
- Determining recreational area accessibility criteria to be used in the study,
- Evaluation of the criteria according to the research areas, determination of the area accessibility scores,
- Evaluation of the criteria by the expert group and evaluation of the work by the weighted criteria method,
- Establishing recreational areas and accessibility improvement proposals within the area which were applied in 5 stages.

In the first stage of the study – during the determination of the recreational area existence – the list at the urban and district level was derived from the data which were taken from the metropolitan municipality and district municipalities. The satellite image of the study area with its borders was transferred as a base to the AutoCAD program.

In the second stage of the study, the criteria for evaluating the accessibility of recreational areas and the quantities to be used for evaluation within the scope of these criteria have been determined. For this purpose, accessibility to recreational areas and access to recreation areas have been investigated. Research on accessibility and transportation have been examined. The recreational areas were provided in Table 1 [19-22]. In the third stage of the study, these criteria were evaluated in each recreation area within the scope of the specified criteria. An evaluation was made on a scale between 1 and 5. One is the lowest criterion score, five is the highest benchmark. The accessibility score of the recreation areas was reached by collecting all the criteria scores that were taken into evaluation.

In the fourth stage of the study, in assigning and calculating the values determined in the study of the recreational areas of the research area the “Weighted Criteria Method”, which was first described by Gold (1980) [23] in the case of Santa Barbara city (USA) and which was used by the researchers [24, 25, 26, 27, 28, 21] in the evaluation of urban facilities such as bicycle path, square, pedestrian road and active green spaces, and the method yielded successful results, were taken as basis. While each criterion used in the study is important in terms of its own characteristics, differences in significance levels may naturally occur when all the criteria are compared with each other. Based on this prediction and the suggestions [23-28] efforts were made to determine the importance levels of the criteria. For this purpose, interviews were carried out with the expert group (landscaping architects, architects, and urban area planners) related to the accessibility criteria in the recreation areas and a questionnaire was carried out in the standardized forms through the interviews with the organized network.

The importance level scores of the criteria were investigated with a scale ranging from 1 to 5. One is the lowest importance score, 5 is the highest grade point. By taking the arithmetic averages of the efficiency scores investigated through the questionnaire study, the significance level coefficient for each criterion was calculated separately.

In the designated recreational areas, the conditions of the criteria and the features were determined and the scores in the fields were calculated. Weighted scores were obtained for each criterion by multiplying the score given for each criterion by the coefficient of the measure, depending on the recreational area. This process can be summarized by the following formula [21].

\[
\text{Accessibility weighted score} = \text{WSa (Weighted score a)} = \text{Coa (Coefficient a)} \times \text{Ca (Criterion a)}
\]

The total score of each recreational area has been reached with the addition of weighted points with the following formula [21].

\[
\text{Total accessibility weighted score} = \sum_{n=1}^{n} \text{K} \times \text{O}_{1..n}
\]

In the fifth stage of the study, the highest score to be obtained from the criteria for each recreational area accessibility was calculated in order to determine the suitability levels of recreational areas. The highest score was obtained by multiplying each measure by the highest value that it could take with significance. As a result of this process, the calculated criterion scores were collected and the highest value that the recreational areas could take was determined. After this process, the proportional value (%) between the highest score of the recreational area and the score of each field was calculated and the compliance scores of the fields were determined. If the range of the values for the appropriateness level is defined in 5 equal parts and the points for which the recreational areas have been taken are within the range of these values, this range determines the accessibility suitability level of the related recreational area.

RESULTS

Scoring was made in the research area in accordance with the determined areas and accessibility criteria (Table 1).

Atatürk Urban Forest located in Oduńluk District of Osmangazi District of Bursa Province has 150-hectare area [29]. For picnics, rest, sightseeing, and sports, the area is used mostly at weekends. Transportation to the area is usually provided by private vehicles. Public transport (bus) is only about 3 kilometres from the entrance of the area. This area has an entrance fee and is open from 08:00 to 20:00. In the scope of the study, access to the Atatürk Urban Forest is 36 points with the criteria determined for accessibility, while the accessibility score of the area in itself is 37. In total, the Area Accessibility Score is 73.
## TABLE 1
Accessibility Score in Recreational Area

<table>
<thead>
<tr>
<th>Accessibility Criteria (Acc. Cr.) (Score)</th>
<th>Atatürk Urban Forest</th>
<th>Hüdavendigar Urban Park</th>
<th>Bursa Zoo</th>
<th>T.O. Regional Park</th>
<th>Ziraat Picnic Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recreation Area Name</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transportation to Recreation Areas</td>
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<td></td>
</tr>
<tr>
<td>A1. System Loyalty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main road+Secondary road+Collecting road+Local road+ pedestrian roads + bicycle path</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate 6 -property (5) - Appropriate 4-3 property (4) - Appropriate 2 property (3) - Appropriate 1 property (2) - There is no appropriate property (1)</td>
<td>4 5 4 3 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2. Existence of motor vehicle roads to access the area: Present (5) Absent(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3. Existence of pedestrian road:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A4. existence of crosswalk to access the area: Present (5) Absent(1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A5. Existence of Bicycle paths:</td>
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<td>A6. Existence of car park:</td>
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<tr>
<td>A7. Easy access with public transport</td>
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<tr>
<td>Metro(Rail system)+Train+ Bus +Minibus +Shared Taxi</td>
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</tr>
<tr>
<td>Appropriate 5-4 property (5) - Appropriate 3 property (4) - Appropriate 2 property (3) - Appropriate 1 property (2) - There is no appropriate property (1)</td>
<td>2 3 2 2 3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A8. Sidewalk width on connected boulevards and streets to the area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>According to Haris and Dines (1998);</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25m&lt; Sidewalk width (5)- 1.5m&lt; Sidewalk width&lt;2.25m (4)- 1&lt; Sidewalk width&lt;1.5m (3)- 0.5&lt; Sidewalk width &lt;1 (2)- There is no Sidewalk width</td>
<td>2 4 5 4 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9. Accessibility for disabled person on the roads reaching the area: Present (5) Absent(1)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A10. Guidance to access the area(Signboards): Present (5) Absent(1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>36</td>
<td>39</td>
<td>38</td>
<td>28</td>
<td>29</td>
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<tr>
<td>Part B</td>
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<tr>
<td>Transportation in Recreation Areas</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B1. Usability of selected material in transportation within recreation area- Material properties suitability (Ender, 2011); Surface feature do not affect pedestrian usage (excessive roughness, pt, bump, etc.)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Appropriate seam feature Reflection on the surface(albedo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Having Appropriate features in rainy weather (not slippery) Having Appropriate infrastructure features (compressed floor, stabilizing fill, blocking, etc.)</td>
<td>5 5 4 2 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate 5-4 property (5) - Appropriate 3 property (4) - Appropriate 2 property (3) - Appropriate 1 property (2) - There is no appropriate property (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2. In-area pedestrian access:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3. Road width in in-area transportation According to Haris and Dines (1998); 2.25m&lt; Road width (5)- 1.5m&lt; Road width&lt;2.25m (4)- 1&lt; Road width&lt;1.5m (3)- 0.5&lt; Road width &lt;1 (2)- Road absence (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4. Presence of routing in transportation: In the context of road and usage relationship; There is a relationship (5) No relationship (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5. The presence of routing and signalling by planting; It means the fact that the plants make their entries prominent and designed as a router along the road</td>
<td></td>
<td></td>
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<tr>
<td>B6. Presence of guiding sign in-area:</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B7. Presence of entrance emphasis to recreation area: Present (5) Absent(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8. Appropriate path widths for disabled person access</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedestrian width 1.5m (5)</td>
<td>Pedestrian width&lt; 1.5m (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B9. Appropriate ramp for disabled person access Width of ramps should be 90cm and slopes should be 8% according to TSE 12576 (Ender, 2011).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10. Accessibility of disabled person to usage</td>
<td>Present (5) Absent(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>42</td>
<td>45</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>OVERALL TOTAL</td>
<td>73</td>
<td>81</td>
<td>83</td>
<td>53</td>
<td>64</td>
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</table>
TABLE 2
Averages of the Weighted Levels of Criteria Used in the Evaluation of Recreation Areas

<table>
<thead>
<tr>
<th>Acc.</th>
<th>Cr.</th>
<th>A</th>
<th>Av</th>
</tr>
</thead>
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<tr>
<td>A1</td>
<td></td>
<td>4 4 5 4 5 3 5 5 4 5 4 5 5 5 5 5 4 5 3 5 5 4 4</td>
<td>4.4</td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td>3 3 4 4 5 3 4 5 4 1 3 5 5 5 4 4 3 4 5 3 5 5 4</td>
<td>3.9</td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td>5 2 5 4 5 5 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 3</td>
<td>4.7</td>
</tr>
<tr>
<td>A4</td>
<td></td>
<td>5 3 5 4 5 3 2 5 5 5 5 5 5 5 4 4 4 5 5 5 5 5 3</td>
<td>4.5</td>
</tr>
<tr>
<td>A5</td>
<td></td>
<td>3 5 4 3 4 3 1 3 4 5 5 5 5 5 3 5 5 5 3 4 5 5 5 2</td>
<td>4.0</td>
</tr>
<tr>
<td>A6</td>
<td></td>
<td>5 3 4 5 5 4 2 5 5 4 5 5 5 5 4 4 5 4 5 5 5 5 5 2</td>
<td>4.2</td>
</tr>
<tr>
<td>A7</td>
<td></td>
<td>5 5 5 5 5 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 4</td>
<td>4.8</td>
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<td>A8</td>
<td></td>
<td>5 4 5 5 5 2 4 4 3 3 3 3 4 5 5 5 3 5 5 3 2 1 5 1 3</td>
<td>3.9</td>
</tr>
<tr>
<td>A9</td>
<td></td>
<td>5 5 4 5 5 2 3 5 4 5 5 5 5 5 4 5 5 5 5 5 5 5 5 2</td>
<td>4.6</td>
</tr>
<tr>
<td>A10</td>
<td></td>
<td>3 4 5 3 4 3 3 5 5 5 4 5 5 5 4 5 5 5 5 5 5 5 5 3</td>
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<table>
<thead>
<tr>
<th>Acc.</th>
<th>Cr.</th>
<th>B</th>
<th>Av</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td></td>
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</tr>
<tr>
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</tr>
<tr>
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<td>4.4</td>
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<td>4.2</td>
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<td>4.3</td>
</tr>
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<td>4.7</td>
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<td>4.8</td>
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<td>B10</td>
<td></td>
<td>5 5 4 5 5 2 5 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 4 7</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Note: Acc.Cr. = Accessibility Criteria, Av. = Average

Hüdavendigar Urban Park, through which Nilüfer Stream passes, covers an area of 510,000 m2. It houses concerts, showgrounds, hiking, cycling routes, seating, recreation, picnic areas, various sports activities, buffets, cafes, restaurants, children's playgrounds, administrative units and peripherals [29-30]. Hüdavendigar Urban Park is an area surrounded by Odunluk neighbourhood, Dikkaldrım neighbourhood, and Doburca neighbourhood. There are also important business centres, shopping centres, hotels and residences around the park. Within the scope of the study, the transportation to the park is 39 points in terms of the criteria determined for accessibility, while the accessibility of urban park in itself is 42 points. In total, the Area Accessibility Score is 81.

Bursa Zoo is a zoo [30] that is in compliance with European standards and is located within the borders of Osmangazi District, in the 2nd km of the nearby road in Soğanlı neighbourhood [31]. Within the scope of the study, access to Bursa Zoo is 38 points in terms of the criteria determined for accessibility, while the accessibility score of Zoo in itself is 45. In total, the Area Accessibility Score is 83.

Teoman Özalp Regional Park located in the district of Osmangazi in Bursa, Hamitler neighbourhood, contains areas such as walking paths, children's playgrounds and an amphitheatre [32]. Within the scope of the study, Teoman Özalp Regional Park transportation point is 28 points with the criteria determined for accessibility, while the accessibility point of the park is 25 within itself. In total, the Area Accessibility Score is 53.

Ziraat Picnic Area covers an area of 2.600 m2 at the Karaman neighbourhood of the Osmangazi district of Bursa [33]. Within the scope of the study,
in terms of the criteria determined for accessibility, the transportation of Ziraat Picnic Area is 29 points while the accessibility score of the picnic area in itself is 35. In total, the Area Accessibility Score is 64.

Within the scope of "Weighted Criteria Method", a questionnaire was conducted with 30 expert groups (Landscape Architects, Architects and Urban and Regional Planners) about the accessibility criteria in the recreational areas in order to determine the importance levels of the criteria. The significance level coefficient for each criterion was calculated by taking the arithmetic averages of the efficiency scores between 1 and 5 questioned by the questionnaire study (Table 2).

The highest score obtained from the criteria for each recreational area transportation was calculated in the course of determining the transportation suitability levels of the research areas. The highest score was substituted in the form, and each measure was multiplied by the highest value that it could attain the importance level, and the "Accessibility Weight Score" was obtained. As a result of this process, the calculated criterion points were collected and the highest value that the recreational areas could obtain and the "Weighted Accessibility Points" to the recreational areas were calculated (Table 3).

The total points reveal that Bursa Zoo has the highest weighted accessibility score with 369.19, Hüdavendigar Urban Park’s accessibility score is 360.12, Atatürk Urban Forest’s accessibility score is 303.13, Ziraat Picnic Area’s accessibility score is 286.63, and the lowest weighted accessibility score is Teoman Özlal Regional Park’s. When the results are evaluated ranging from 89.36-446.8, the accessibility suitability level has been obtained. With these results, Bursa Zoo was evaluated as “Good”, Hüdavendigar Urban Park as “Good”, Atatürk Urban Forest, T.Ö. Regional Park and Ziraat Picnic Area as “Moderate” (Table 4).

In the next step, the proportion of the area's appropriateness level was determined by calculating the ratio (%) between the highest score for the recreational area and the score for each area. Accessibility proportional values of the areas were calculated as 82.6% for Bursa Zoo, 80.6% for Hüdavendigar Urban Park, 67.9% for Atatürk Urban Forest, 64.1% for Ziraat Picnic Area and 52.9% for Teoman Özlal Regional Park. When we look at the ratio of transportation to recreational areas (Part A), we obtain the value 77.22% for Hüdavendigar Urban Park. The highest score for recreational area (Part B) accessibility value was 89.49% in Bursa Zoo (Table 5). If the range of values for the appropriateness is defined in 5 equal parts and the points for which the recreational areas have been taken are within the range of values, this range determines the transportation appropriateness level of the related recreational area (Figure 3).

### TABLE 3
**Total weighted accessibility scores of recreation areas**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>17.88</td>
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<td>23.5</td>
<td>5</td>
<td>23.5</td>
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<td>1</td>
<td>4.03</td>
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<tr>
<th>Part A Weighted Total Score</th>
<th>135.46</th>
<th>169.12</th>
<th>165.28</th>
<th>122.11</th>
<th>127.55</th>
<th>219</th>
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</table>

<table>
<thead>
<tr>
<th>Part B Weighted Total Score</th>
<th>168.13</th>
<th>191</th>
<th>203.88</th>
<th>114.5</th>
<th>159.08</th>
<th>227.8</th>
</tr>
</thead>
</table>

Total | 89.36 | 303.59 | 360.12 | 369.16 | 236.61 | 286.63 | 446.8 |

**TABLE 4**

<table>
<thead>
<tr>
<th>Range of value</th>
<th>Suitability level</th>
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</thead>
<tbody>
<tr>
<td>89.36 – 160.86</td>
<td>Very Weak</td>
</tr>
<tr>
<td>160.87 – 232.37</td>
<td>Weak</td>
</tr>
<tr>
<td>232.38 – 303.88</td>
<td>Moderate</td>
</tr>
<tr>
<td>303.89 – 375.39</td>
<td>Good</td>
</tr>
<tr>
<td>375.39 – 446.8</td>
<td>Very Good</td>
</tr>
</tbody>
</table>

**TABLE 5**

Proportional value (%) between the highest score for accessibility of recreational area and the score of each area

<table>
<thead>
<tr>
<th>Atatürk Urban Forest</th>
<th>Hüdavendigar Urban Park</th>
<th>Bursa Zoo</th>
<th>Teoman Özalp Regional Park</th>
<th>Ziraat Picnic Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A</td>
<td>61.85</td>
<td>77.22</td>
<td>75.47</td>
<td>55.75</td>
</tr>
<tr>
<td>Part B</td>
<td>73.80</td>
<td>83.84</td>
<td>89.49</td>
<td>50.26</td>
</tr>
<tr>
<td>Total</td>
<td>67.94</td>
<td>80.59</td>
<td>82.62</td>
<td>52.95</td>
</tr>
</tbody>
</table>

**FIGURE 3**

Coverage Ratio for Criteria of Recreational Area

**DISCUSSION**

Urban equipment, which has great importance in terms of urban culture, urban landscape and recreational activities of urban people, constitutes the components of the quality of urban life from a physical point of view. Social equipment was evaluated in the study with regard to urban living quality; pieces of equipment that are used in areas such as squares, pedestrian zones, bicycle paths, parks, children's playgrounds, picnic areas, botanical and zoo gardens, urban parks, and afforestation areas. The total open green space presence in Bursa city is 5,114,132m². Open and green spaces are urban parks, regional parks, neighbourhood parks, children playgrounds, sports areas as active green spaces, open spaces and other green spaces [34, 35].

The need for active green spaces has been increasing as a consequence of the intensive construction brought by rapid urbanization and the erroneous land use of this construction, which does not comply with scientific data and standards. As people move away from nature, and as the environment they live in becomes increasingly urban, they feel the need to be more rested and renewed. With this situation, recreation and recreational activities have begun to gain importance. However, the limited number and unbalanced active green spaces in the cities do not meet the needs of the society. Since there are no quantitative and qualitative legal criteria or determinants designated to increase the effectiveness of active green spaces, different interpretations, and different decisions can be made in planning and implementation [21, 36, 37].

The quality of the active green spaces in the city and the quality of the green space that quantities can be revealed using evaluation criteria [21]. Transportation, which is an important parameter in increasing the qualifications of these areas, was evaluated with the studies of [9, 19, 20, 21, 22, 23, 38, 39] and the criteria were set. Access to leisure [40], which transforms the urban space of a range of spatial scales, has many options.

In the short and long term, where leisure habits can promote a more environmentally friendly change, new policies need to be developed to
change the existing strong links between economic growth and leisure time consumption. It has been observed that the use of private vehicles has a larger share when looking for transportation for leisure activities. People participating in leisure time activities can change their leisure time activities and provide easy transportation for children, etc. It is more practical to use special vehicles because of these reasons. In addition, if the group is crowded, the members of the group may use private vehicles more economically. However, recent research shows that it is becoming more and more preferred to use public transport for short-distance leisure activities [41]. When the city of Bursa, was taken into consideration, it was determined that it would be possible to use motor vehicles / special vehicles if the transportation to the leisure areas such as city parks, picnic areas and zoos that exist in the city is considered as a group [34]. Apart from this, there are different travel-transportation requests for sports activities in leisure activities. For these people, distances did not appear to be a barrier [42]. Leisure activity areas and the service sector's position in this respect are important for economic growth and local market opportunities [43].

With mixed-use destinations, parking, street design, etc. modes of transportation can be diversified by modifying the characteristics of the neighbourhood with walking activities. In this way, it will be possible to go not only to the parks but also to the workplaces and schools on foot. In this way, planning approaches can be implemented with a model that includes the surrounding areas and route characteristics of destinations (parks, green spaces, workplaces, schools, residences, etc.) where walking behaviour is an important determinant [44].

In the evaluation carried out in the city of Bursa, it is necessary to prioritise a balanced distribution that meets the standard of citywide and neighbourboods by associating the amount of space per person to the distance to the user in the open and green space planning in the city by evaluating the way out of the work [34].

Urban outdoor green spaces, urban recreation areas, urban parks, regional parks, neighbourhood parks, etc., are important points for leisure activities. According to Özdemir (2009), in urban areas, health, social communication, psychological relaxation and increasing environmental qualities play a major role and have added value. Again, it is important to focus on bringing these areas into a position where people from all walks of society can easily access them. This situation is inevitable for increasing urban life, publicity, and environmental quality [45].

In urban areas, transportation, planning and design work is supported not only in motorized transport but also in non-motorized transport (walking, cycling) to be associated with physical activity [46]. Given the problems in transportation to desti-
is also important to promote open-air activities with families, to focus on facilities and resources, and to collect and promote them [53, 54, 55].

An important component of the urban open green space system is road trees. While the road trees make up the city and the path it creates is the identity and continuity, it creates continuity and occupancy effects. They take away the monotony of the roads giving them an interesting look and create effective routes. Road trees will also provide positive contributions to the urban ecosystem when they are used correctly [17].

To summarize, the study provides important contributions to multidisciplinary research on environmental contributions in the name of transport, urban design and planning-related physical activity levels [45, 56]. According to the findings of the study, the most accessible area is Bursa Zoo and the second is Hüdavendigar Urban Park. Apart from all these assessments, in his study, Park (2017) added a different point of view to the accessibility of parks, emphasized the importance of perceptual dimension together with the physical dimension in accessibility, and mentioned the psychological park accessibility [57]. According to the findings of his study, Park classified psychological park accessibility as surrounding settlements that can be measured both quantitatively and qualitatively, park quality and distance perception. He concluded that future research should examine psychological accessibility at the physically or socio-economically disadvantageous points in the sense of park accessibility. This study, on the other hand, emphasizes the criteria of transport, which is one of the most important criteria for the urban public recreational areas in green fields to be utilisable, that have to be paid attention to at the planning and design stage. The study will also provide the ascertainment of the accessibility approaches that are to contribute to the effective usage of recreational areas which contribute to a healthy urban life.

CONCLUSION AND RECOMMENDATION

Recreational areas, which allow people a bit of breath in urban life, and accessibility to urban green spaces are important components of the city's quality of life. In terms of population density, Bursa, which is the 4th biggest city of Turkey and an important industrial centre, has been featured as "Green Bursa" from past to present day. However, this feature, which began to disappear with the pressure of intense urbanization, will continue without the loss of the creation of new recreational spaces. One of the most important components that enhance the usability of these areas is that they are accessible. In the study, the extent of accessibility of the open green spaces, which have an important place in Bursa city's leisure time usage, is examined. The accessibility of Atatürk Urban Forest, Hüdavendigar Urban Park, Bursa Zoo, Teoman Özalp Regional Park and Ziraat Picnic Area were evaluated among the recreation areas in Bursa.

Bursa Zoo has the highest area accessibility point. Teoman Özalp Regional Park has the lowest point. When the accessibility criteria for recreation areas were evaluated, Ziraat Picnic Area got the lowest score while Hüdavendigar Urban Park got the highest score. When the accessibility criteria in the recreation areas are evaluated, Bursa Zoo has the highest score and Teoman Özalp Regional Park has the lowest score. The evaluation of the accessibility of recreation areas reveals that there are both motor vehicles and pedestrian ways in all areas. However, observation exhibits the fact that it is only possible to reach the Hüdavendigar Urban Park by bicycle routes. It has been determined that it is possible to reach all selected areas by bus from public transport vehicles. Hüdavendigar Urban Park has the highest score when we evaluate the roads around the area (system's loyalty). There are secondary roads, collecting roads, local roads, pedestrian roads and the existence of bicycle paths and interrelationships with each other.

The availability of road type diversity and continuity in reaching the area strengthens the access to this area. In addition to these, it has been determined that there is no solution to the disabled for transportation in any area. When reaching the area, it was determined that there were signboards for Atatürk Urban Forest and Bursa Zoo, and it was determined that this was not the case for other areas. This leads to negative results for usage in the other three recreational areas.

Atatürk Urban Forest and Hüdavendigar Urban Park got the highest points in the suitability of the selected ground materials within the scope of the evaluated criteria. Teoman Özalp Regional Park and Ziraat Picnic Area got the lowest score. Hüdavendigar Urban Park, Bursa Zoo, and Ziraat Picnic area have the highest score in the road widths and routing and signalling. Directional signs within the area are available at Atatürk Urban Forest and Bursa Zoo. The accent of the entrance to the area could not be determined in Teoman Özalp Regional Park and Ziraat Picnic Area. Although all recreational areas assessed in the framework of the study have sufficient road widths for the access of individuals with disabilities, it has been determined that access to the usage area of the disabled is not in any case appropriate. In addition to this, Hüdavendigar Urban Park, Bursa Zoo and Ziraat Picnic Area have suitable slopes and ramps for disabled transportation. In some places in Atatürk Urban Forest, the slope up to 30-35% is not suitable for transportation of disabled people. Appropriate slopes and ramps should be arranged to facilitate access to these areas by disabled people and to facilitate in-area use. The presence of in-area signboards was...
observed in Atatürk Urban Forest and Bursa Zoo. There are no in-area redirection signs in other areas. The presence of in-area routing boards may provide ease of use for area users.

After calculating the current area scores, the total weight points were calculated by substituting the importance levels of the criteria obtained by expert questionnaires into the formula. When looking at the total amount, Bursa Zoo has the highest weighted transportation score with 369.19. Teoman Özalp Regional Park received the lowest weighted transportation score. As a result of this study, 82.62% Bursa Zoo, 80.59% Hüdavendigar Urban Park, 67.94% Atatürk Urban Forest, 52.95% Teoman Özalp Regional Park and finally 64.15% Ziraat Picnic Area values were obtained when the coverage ratios for the criteria of recreational areas were compared.

In general, when we look at these recreational areas in Bursa city, it is seen that the urban infrastructure, the widths of the roads are not sufficient and the public transportation diversity is not enough. As a result of observations, it was determined that the vast majority of visitors to the area are living in the recreational area. It is suggested to improve the transportation (width, material, orientation, etc.) for the use of these recreational areas. The diversity of the connections from the neighbouring settlements, directional signs and types of transportation (public transportation, pedestrian transportation, bicycle transportation, etc.) should be increased, and improvements should be made. While working on planning and design, the principle of ‘design for everybody’ should be internalized. This will increase the usability of the areas and ensure that their effective use.

Accessibility is an important element for the use of recreational areas. The lack of adequate infrastructure in existing recreation areas may make it more difficult to correct these criteria. However, if planning in the recreation areas to be newly installed is made taking these transportation criteria into consideration then accessibility, which plays a big role in increasing the utilization of those areas, will be provided. In order to increase area utilization, it is necessary to increase diversity in public transport. It should be acknowledged that diversity of transportation is one of the most important elements of transportation in the city planning process, not just the recreation areas. Planning should be done taking the people (elderly, young, children, disabled, etc.) into consideration.

REFERENCES


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RESEARCH ON THE POSSIBILITIES OF USING SOME ESSENTIAL OILS ON CONTROL OF DAMPING-OFF AND SEEDLING ROOT ROT OF COTTON

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ABSTRACT

Seedling diseases caused by fungi are the most widespread and devastating biotic stresses that subsequently affect cotton yield. Interest to the aromatic and medicinal plants has been increasing steadily both in industrial and scientific researches due to their potential in the fields of medicine and plant protection. The aim of this research was to evaluate the effects of essential oils of some medicinal plants such as Lavandula stoechas, Ocimum basilicum, Origanum onites, Rosmarinus officinalis, Salvia sclarea and Thymus serpillum against number of pathogenic fungi causing damping-off and seedling root rot of cotton. Results showed that all essential oils except Lavandula stoechas inhibited mycelial growth of Fusarium solani, Macrophomina phaseolina and Rhizoctonia solani ranging from 100 % to 10%. The most effective essential oils were Origanum onites and Thymus serpillum with the doses of 2000 and 2500 ppm. Although these essential oils also provided high germination rates ranging from 85-90 % for cotton seeds in vitro, success could not be confirmed in vivo conditions.

MATERIALS AND METHODS

In this study, "Carmen" variety cotton seeds were used. All laboratory analyzes were carried out at Ege University Seed Technology Center.

Isolation of pathogens and pathogenicity test. Seedling root-rot pathogens were isolated from the root and root neck of naturally infected seedlings in the trial fields of Nazilli Cotton Research Institute. PDA was used for Macrophomina phaseolina, Rhizoctonia solani and Fusarium solani, whereas selective medium was used for Thielaviopsis basicola isolation [25].

For identification and selection of the most virulent pathogen isolates, surface-sterilized cotton seeds were placed on pure pathogenic colonies developed on PDA medium in Petri dishes and germination and first development stages were observed.

In vitro antifungal activity of essential oils. For sensitivity of the isolated pathogens to essential oils, essential oils were added on the PDA medium,
which was sterilized in the autoclave in 50 °C and cooled and then poured into petri dishes. Later pathogen dishes were located on solid medium. Experiments were carried out as four replicates.

**Determination of the phytotoxic effects of Essential Oils.** To determine the phytotoxic effects of essential oils at doses of which were effective, phytotoxicity experiments were carried out with cress seeds.

**Treatment of Essential Oils to Cotton Seeds.** Essential oils that do not show phytotoxicity at effective doses to pathogens were applied to cotton seeds by using spraying and polymer coating methods before sowing. However, it was determined that seed coating homogeneity was not provided in the spraying method. Therefore, the experiments were continued with seed coating. For each dose selected, 500 mg/l CMC (Carboxymethyl Cellulose) was added to the coating material to act as a adhesive. After coating, the seeds were subjected to germination test.

**Germination tests.** The germination tests were carried out in accordance with ISTA rules in randomized complete blocks design with four replications. At the end of germination period, normal/abnormal seedlings were determined.

**Preparation of pathogen inoculums.** Although selective medium was used, *T. basica* was not isolated. For this reason, *F. solani*, *M. phaseolina* and *R. solani* isolates that have high pathogenicity were used in pot experiments.

Pathogens to be used in pot experiments were grown in the wheat bran medium. For this purpose, wheat bran was mixed with pure water at a ratio of 5:1 (v/v) and sterilized in glass bottles (1/2 lt) or in Erlenmeyer.

The 10 day-old pathogen cultures which filled the petri dish was divided into 8 triangular pieces by inoculation needle. Two pieces were placed in each bottle containing the wheat bran medium and left to incubate at 24 °C for 3-4 weeks.

**Pathogen contamination of peat.** The pathogen cultures grown in the glass bottles were mixed with peat on a clean plastic cover at a ratio of 1:20 and filled in the pots. Sterile wheat bran and peat (1:20 v/v) were placed in the pots for control. Experiments were carried out in 76 pots.

These were the applications:
- Negative control: sowing in the sterile peat (four pots)
- Positive control: sowing into the peat contaminated with a, b and c separately, four pots each
- *O. onites* (total 24 pots)
  - 2000 ppm + 500 mg/l CMC (R. solani, F. solani, M. phaseolina):
  - 3 x 4 = 12 pots
  - 2500 ppm + 500 mg/l CMC (R. solani, F. solani, M. phaseolina):
  - 3 x 4 = 12 pots
  - *T. serpillum* (total 24 pots)
  - 2000 ppm + 500 mg/l CMC (R. solani, F. solani, M. phaseolina):
  - 3 x 4 = 12 pots
  - 2500 ppm + 500 mg/l CMC (R. solani, F. solani, M. phaseolina):
  - 3 x 4 = 12 pots

**Statistical analyzes.** In the study, although all experiments were carried out in laboratory and homogeneous conditions, but in order to eliminate possible faults, the randomized complete block design were set up in four replications. The experiments were analyzed in the Tarist Statistics Packet Program. Five % significance level was chosen for the LSD test.

To determine the susceptibility of pathogenic isolates to essential oils, essential oil was injected into the PDA medium that was sterilized and cooled to °C 50, then they were poured into the petri dishes and pathogen discs were inoculated after solidification [26]. Experiments were carried out with four replicates.

**RESULTS AND DISCUSSIONS**

**In vitro tests.** Comparing the data obtained using essential oils of six plant species revealed that all essential oils tested except for *L. stoechas* were capable of inhibiting mycelial growth of *F. solani*, *M. phaseolina* and *R. solani* ranging from 100 % to 10% (Table 1).

The highest level of mycelial growth inhibition against *F. solani*, *M. phaseolina* and *R. solani* was obtained using *O. onites* and *T. serpillum* without significant differences between the concentrations of 2000 and 2500 ppm.

*R. officinalis* had the lowest inhibition rates (10%) at the concentrations ranging from 2000 and 2500 ppm for *M. phaseolina* and *R. solani*. Essential oil of *L. stoechas*, which is mentioned to be effective against some pathogens in literature, was found to be ineffective against the all pathogens in our study.

For this reason, the doses of 2000 ppm and 2500 ppm of essential oils of *O. onites* and *T. serpillum* were chosen for *in vivo* study.

Researchers announced that oil of *L. vera* which most strongly inhibited the growth of bacteria, exhibited the lowest antifungal activity against fungal pathogens [27].

Some researchers found that lavender extracts reduced the mycelium growth of *R. solani* and *Fusarium spp* in varying degrees in *in vitro* studies against cotton damping-off agents [20].

Our results obtained against fungal pathogens
with all essential oils except for lavandula were found to be effective and these results were compatible with many results of some researchers who investigated the essential oils and components of some medical plants [10, 11, 19].

**Seed treatments.** The effects on the germination rate of essential oils are shown in Table 2.

The highest germination rate was obtained from the control application (93%). Seeds treated with a rate of 2500 ppm concentration of *O. onites* showed drastic abnormal germination.

However, since all doses of both essential oils gave above 85% germination, pot trials were carried out with essential oils of *O. onites* and *T. serpillum*.

Some researchers working on the effect of essential oils of many medicinal plants including lavender on the germination rates of different weed seeds, found that both plant species and essential oil concentrations had different effects on germination [28].

On the other hand, researchers who investigated the effects of essential oils of *O. onites* (Ori) and *R. officinalis* (Ros) on wheat germination found that both volatile oils had a detrimental effect on the germination rate [29].

Researchers suggested that inhibition of the germination rates were higher for the essential oil of Ori and germination inhibition was between 87.1% and 37.3%.

Also, some researchers [30] who investigated the effect of the essential oils of seven plants including *O. onites* on the germination of cotton seeds reported that all essential oils affected negatively the germination of cotton seeds.

In addition, essential oils reduced the germination of cotton seeds by 40 to 100%. Similarly, in our study, the *O. onites* affected the germination of cotton seeds at different levels and the concentration of 2500 ppm reduced the germination rate significantly.

### Table 1

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Doses</th>
<th>Pathogens</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>F. solani</em></td>
<td><em>M. phaseolina</em></td>
<td><em>R. solani</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lavandula stoechas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>0</td>
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<tr>
<td></td>
<td>1500 ppm</td>
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<tr>
<td></td>
<td>2000 ppm</td>
<td>0</td>
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<td>0</td>
<td></td>
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<td></td>
<td>2500 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Origanum onites</td>
<td>500 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
<td>60</td>
<td>50</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1500 ppm</td>
<td>95</td>
<td>85</td>
<td>90</td>
<td></td>
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<tr>
<td></td>
<td>2000 ppm</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2500 ppm</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>500 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td>45</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2500 ppm</td>
<td>45</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Salvia sclarea</td>
<td>500 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
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<td>0</td>
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<td></td>
<td>1500 ppm</td>
<td>70</td>
<td>70</td>
<td>80</td>
<td></td>
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<tr>
<td></td>
<td>2000 ppm</td>
<td>90</td>
<td>70</td>
<td>90</td>
<td></td>
</tr>
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<td></td>
<td>2500 ppm</td>
<td>90</td>
<td>70</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Ocimum basilicum</td>
<td>500 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>1500 ppm</td>
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<tr>
<td></td>
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<td>45</td>
<td>15</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>2500 ppm</td>
<td>50</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Thymus serpillum</td>
<td>500 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
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<td>70</td>
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<td></td>
<td>2500 ppm</td>
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<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

LSD : 4.875*

* Means within a column followed by the same letter are not significantly different (P = 0.05)
TABLE 2
Effects of essential oils on germination rate (%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination rate (%)</th>
<th>Abnormal germination rate (%)</th>
<th>Non-vital seed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><em>Origanum onites</em></td>
<td>88</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>2000 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Origanum onites</em></td>
<td>85</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2500 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thymus serpillum</em></td>
<td>90</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>2000 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thymus serpillum</em></td>
<td>89</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>2500 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>3.57*</td>
<td>3.99*</td>
<td>1.24 ns**</td>
</tr>
</tbody>
</table>

*: Means within a column followed by the same letter are not significantly different ($P = 0.05$)
**: ns: not significant

TABLE 3
Emergence rates of *Origanum onites*-treated cotton seeds in pot trial (%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination rate (%)</th>
<th>Abnormal germination rate (%)</th>
<th>Non-vital seed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control R.Solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Positive control F. solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Positive control M. Phaseolina</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2000 ppm O.Onites +R.solani</td>
<td>0</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>2500 ppm O.Onites +R.solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2000 ppm O.Onites +F.solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2500 ppm O.Onites +F.solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2000 ppm O.Onites +M. Phaseolina</td>
<td>0</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>2500 ppm O.Onites +M. Phaseolina</td>
<td>0</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>LSD</td>
<td>-</td>
<td>3.99ns</td>
<td>3.24 ns</td>
</tr>
</tbody>
</table>

*: ns: not significant

TABLE 4
Emergence rates of *Thymus serpillum* -treated cotton seeds in pot trial (%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination rate (%)</th>
<th>Abnormal germination rate (%)</th>
<th>Non-vital seed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control R.Solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Positive control F. solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Positive control M. Phaseolina</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2000 ppm O.Onites +R.solani</td>
<td>0</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>2500 ppm O.Onites +R.solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2000 ppm O.Onites +F.solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2500 ppm O.Onites +F.solani</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2000 ppm O.Onites +M. Phaseolina</td>
<td>1</td>
<td>2</td>
<td>97</td>
</tr>
<tr>
<td>2500 ppm O.Onites +M. Phaseolina</td>
<td>3</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>LSD</td>
<td>-</td>
<td>3.27ns</td>
<td>4.74 ns</td>
</tr>
</tbody>
</table>

*: ns: not significant

**In vivo trials.** After the application of essential oils of *O. onites* and *T. serpillum*, emergence test was carried out and the results were shown in Table 3 and Table 4.

As seen in tables, the success of *in vivo* trials was not obtained *in vitro* conditions. Both essential oils caused very little or no germination.

However, it is thought that it is possible to achieve success in *in vivo* studies by using some different techniques such as diffusion of essential oil and seed treatments.

Researchers who studied the effect of essential oil of *O. onites* (Ori) and *R. officinalis* (Ros) on the seedling growth of wheat found that essential oils significantly reduced seedling shoot length of wheat cultivars compared to controls.

They mentioned that the reduction rate was relatively increased with the increasing dose of both essential oil types.

The essential oil of Ori proved more
detrimental effect compared to the essential oil of Ros. The mean shoot length of wheat cultivars was inhibited by 87% and 75%, respectively, by application of essential oil of Ori and Ros [29].

Also, some researchers [31] who studied some plant extracts against cotton seedlings damping-off reported that clove and garlic extracts significantly suppressed the growth of all tested R. solani AGs growth.

It was mentioned that both extracts could be promising as sources of natural ecofriendly phytofungicidal compounds for in vivo applications.

In some studies, the successful results of essential oils have not been achieved in vivo. One of reasons for this result might be the possibility of degrading the compounds of essential oils in soil. However, further studies are needed to confirm in vitro results in field conditions.

CONCLUSION

Data obtained from our study showed that all essential oils except L. stoechas inhibited mycelial growth of F. solani, M. phaseolina and R. solani ranging from 100% to 10%. The most effective essential oils were O. nutis and T. serpillum with the doses of 2000 and 2500 ppm.

These essential oils also provided high germination rates for cotton seeds. The success achieved in vitro conditions could not be confirmed in vivo conditions. Further work is needed to confirm in vitro results in field conditions.

REFERENCES


DETERMINATION OF ENERGY USE EFFICIENCY OF COTTON PRODUCTION IN TURKEY:
A CASE STUDY FROM HATAY PROVINCE

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2 Siirt University, Agricultural Faculty, Department of Biosystems Engineering, Siirt, Turkey
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4Hatay Mustafa Kemal University, Agricultural Faculty, Department of Agricultural Economics, Hatay, Turkey

ABSTRACT

This study aimed at determining the energy efficiency of cotton production in the Hatay region of Turkey. Data were gathered in the season of 2016-2017 from 136 enterprises which were chosen by the Simple Random Sampling Method. In order to determine the energy efficiency of cotton, data provided by farmers were utilized. The energy input and output in cotton production were calculated as 57134.25 MJ ha⁻¹ and 63270.54 MJ ha⁻¹, respectively. Energy inputs consist of; electrical by 20914.02 MJ ha⁻¹ (36.61%), chemical fertilizer by 15466.06 MJ ha⁻¹ (27.07%), diesel fuel by 13828.61 (24.20%), irrigation by 4378.50 MJ ha⁻¹ (7.66%), machinery by 1136.59 MJ ha⁻¹ (1.99%), chemical by 830.85 MJ ha⁻¹ (1.45%), seed energy by 311.99 MJ ha⁻¹ (0.55%), and human labour by 267.62 MJ ha⁻¹ (0.47%). Energy in cotton production could be classified as 68.94% direct, 31.06% indirect, 8.67% renewable, and 91.33% non-renewable. Energy efficiency, specific energy, energy productivity, and net energy in cotton production were calculated as 1.11, 10.66 MJ kg⁻¹, 0.09 kg MJ⁻¹, and 6136.29 MJ ha⁻¹.

KEYWORDS:
Cotton, Energy use efficiency, Specific energy, Turkey

INTRODUCTION

Cotton produced in Turkey is considered to be a fundamental source of material for the textile, oil, and animal feed industries. Cotton production creates a source of income for many families as it is an important source of employment in the country. World cotton fibre production is 19 million tonnes from 33.4 million hectares. Turkey has the sixth largest cotton cultivation area and is sixth in terms of seed cotton production after China, USA, India, Pakistan, and Uzbekistan. Cotton production areas in Turkey are in the Aegean, Antalya, Çukurova, and Southeast Anatolia regions [1]. Cotton production has an important place in terms of providing added value and labor force. Global cotton demand has been increasing along with the increasing population. Due to the ecological demands for the cotton plant, 80% of the global cotton production is being produced by eight countries including Turkey [2]. In terms of fiber productivity, Australia takes first place in the world; and with the increase in cotton productivity in recent years, Turkey took second place in the season of 2015-2016. India and Uzbekistan are two of the main cotton producers in the world that are under the global average in cotton productivity. An increase in these two countries’ productivities would provide a significant increase in global cotton production [3, 4]. Agricultural production is widely mechanised which is powered by fossil fuels. Although this provides more income, it decreases the level of labor force usage. Especially in developed countries, fossil fuel usage levels in agricultural production are quite high, and the side effects of unconscious energy consumption makes planned energy consumption inevitable [5, 6]. In order to assess productivity, it is a more realistic approach to compare the total energy value that is used in agricultural production, to the energy value that is gained from agricultural production [7, 8]. It is important to analyse inputs and outputs carefully to increase productivity and to decrease inputs that are being used in production [6]. Energy input-output analyses are essential to use energy efficiently in terms of providing sustainability in agricultural production, and protecting the environment [8, 9]. Several researches were conducted on the cotton energy efficiency in agricultural production such as; [1, 10, 11, 12, 13, 14, 15, 16, 17]. Other agricultural studies were done on the energy use efficiency of; corn [18]; sugar beet [19], colza [20], wheat [21], sunflower [22], sweet sorghum [23], vetchs [24], apple [25], apricot [26], grape [27], peach [28], pear [8], tomato [29], etc. As there was no related research for the Hatay province, this study aimed at determining the energy efficiency of cotton production in that area.
TABLE 1

<table>
<thead>
<tr>
<th>Inputs and outputs</th>
<th>Unit</th>
<th>Energy equivalent coefficient (MJ unit⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human labour</td>
<td>h</td>
<td>1.96</td>
<td>[36, 37]</td>
</tr>
<tr>
<td>Machinery</td>
<td>h</td>
<td>64.80</td>
<td>[38, 39]</td>
</tr>
<tr>
<td>Chemical fertilizers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>kg</td>
<td>60.60</td>
<td>[38]</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>kg</td>
<td>11.10</td>
<td>[38]</td>
</tr>
<tr>
<td>Potassium</td>
<td>kg</td>
<td>6.70</td>
<td>[38]</td>
</tr>
<tr>
<td>Micro elements</td>
<td>kg</td>
<td>120</td>
<td>[34, 38, 40, 41]</td>
</tr>
<tr>
<td>Chemicals</td>
<td>kg</td>
<td>101.20</td>
<td>[42]</td>
</tr>
<tr>
<td>Diesel fuel</td>
<td>L</td>
<td>56.31</td>
<td>[38, 43]</td>
</tr>
<tr>
<td>Electricity</td>
<td>kWh</td>
<td>3.60</td>
<td>[44]</td>
</tr>
<tr>
<td>Irrigation</td>
<td>ton</td>
<td>0.63</td>
<td>[42]</td>
</tr>
<tr>
<td>Seed</td>
<td>kg</td>
<td>11.80</td>
<td>[1, 38]</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton plant</td>
<td>kg</td>
<td>11.80</td>
<td>[1, 38]</td>
</tr>
</tbody>
</table>

**MATERIALS AND METHODS**

The region of Hatay is located in southern Turkey neighboring the regions of Adana, Osmaniye, and Gaziantep. Syria is located at the east side, and the Mediterranean Sea at the west side of the area. Hatay is split into 15 districts, which consist of 46.1% mountain, 33.5% plain, and 20.4% plateau. The weather is dry and hot in summers, and warm and rainy in winters which is typical for the Mediterranean climate. The annual temperature average is between 15.1 °C and 20 °C, and average precipitation is between 562.2 mm and 1216.3 mm [30].

**Material.** Parent material of this study consisted of primary data that were gathered from cotton enterprises in the Hatay region by means of the face-to-face interview method in the season of 2016/2017. Also, secondary data were gathered from the Food and Agriculture Organisation of the United Nations (FAO), the International Cotton Advisory Committee (ICAC), the Republic of Turkey Ministry of Food and Agriculture and Livestock (MFAL), Republic of Turkey the Ministry of Customs and Trade (MCT), and the Turkish Statistical Institute (TSI). National and international reports were also used which were published by several organisations.

**Method. Sampling Method.** Primary data of the study were gathered from cotton enterprises which were determined by the Simple Random Sampling Method [31]. The formula of the Method that was used to determine the sample size is given below [32]:

\[
n = \frac{\sum(NhSh)^2}{N^2D^2 + \sum Nh(Sh)^2}
\]

(1)

\(n\) = Sample size
\(Nh\) = Number of unit at \(h^{th}\) layer
\(Sh\) = Standard deviation at \(h^{th}\) layer

**N= Total unit number that belongs to the sampling frame**

**D= The margin of error \((d/z)\)**

**d=Deviation ratio from average**

**z=t value in the distribution table at a degree of freedom (N-1) and at a confidence limit [31].**

In this study, 136 cotton enterprises were determined as the sample size with a 5% margin of error and at a 95% confidence interval. In order to determine the sample villages and enterprises, the Simple Random Sampling Method was used by means of data that were obtained from the Farmers’ Registration System’s (FRS) records of the MFAL.

**Survey Distribution.** According to the TSI data of 2015; cotton was grown in eight districts of Hatay, and 93.32% of that cotton was produced in the districts of Kirkhan, Antakya, Reyhanli, and Kumlu. The 136 surveys were carried out in these four districts since they provided the majority of the cotton production in Hatay.

Total energy input in unit area (ha) constitutes each total of input energy. Human labour, machinery, chemicals, chemical fertilizers, diesel fuel, irrigation energy, electricity and seed were calculated as inputs. The cotton plants were the output. In Table 1, the agricultural production inputs, and energy equivalents of input and output were taken as energy values. Energy efficiency calculations were made to determine the productivity levels of cotton plant production. The units shown in Table 1 were used to find the input values in cotton plant production. Input amounts were calculated, and then these input data were multiplied by the energy equivalent coefficient. When determining the energy equivalent coefficients, previous energy analysis sources were used. By adding energy equivalents of all inputs in MJ unit, the total energy equivalent was found. For example, in order to determine the energy efficiency in wheat production, [33] reported that; “The energy ratio (energy use efficiency), energy productivity,
specific energy, and net energy have been calculated by using the following formulas [34, 35].

\[
\text{Energy use efficiency} = \frac{\text{Energy output (MJ ha}^{-1}\text{)}}{\text{Energy input (MJ ha}^{-1}\text{)}}
\]

(1)

\[
\text{Energy productivity} = \frac{\text{Energy output (kg ha}^{-1}\text{)}}{\text{Energy input (MJ ha}^{-1}\text{)}}
\]

(2)

\[
\text{Specific energy} = \frac{\text{Energy output (MJ ha}^{-1}\text{)}}{\text{Yield output (t ha}^{-1}\text{)}}
\]

(3)

\[
\text{Net energy} = \text{Energy output (MJ ha}^{-1}\text{)} - \text{Energy input (MJ ha}^{-1}\text{)}
\]

(4)

Following the analysis of data through the Microsoft Excel program, by referring to the inputs, the results were tabulated. Cotton energy efficiency values were determined and the calculations were given in Table 2. [45] reported that; “The input energy can also be classified into direct and indirect, and renewable and non-renewable forms. The indirect energy consists of pesticide and fertilizer while the direct energy includes human and animal power, diesel, and electrical energy used in the production process. On the other hand, non-renewable energy includes petrol, diesel, electricity, chemicals, fertilizers, and machinery, while renewable energy consists of human and animal labour [34, 46].” The energy input-output and efficiency calculations in cotton production were given in Table 3. Energy input of cotton production in the form of direct, indirect, renewable, and non-renewable energy were given in Table 4.

**RESULTS AND DISCUSSION**

According to studies on cotton farms, the average amount of cotton produced per hectare during the 2016 production season was calculated as 5361.91 kg ha\(^{-1}\). The average farm size was 10.79 ha. For the 2016 cotton production season, the energy efficiency analysis of cotton production related to this study was provided in Table 2. If the average values are investigated by referring to Table 2, it can be seen that the highest energy inputs in cotton production were; electrical by 20914.02 MJ ha\(^{-1}\) (36.61%), chemical fertilizer by 15466.06 MJ ha\(^{-1}\) (27.07%), diesel fuel by 13828.61 (24.20%), irrigation by 4378.50 MJ ha\(^{-1}\) (7.66%), machinery by 1136.59 MJ ha\(^{-1}\) (1.99%), chemical by 830.85 MJ ha\(^{-1}\) (1.45%), seed by 311.99 MJ ha\(^{-1}\) (0.55%), and human labour by 267.62 MJ ha\(^{-1}\) (0.47%), respectively.

**TABLE 2**

**Energy balance in cotton production**

<table>
<thead>
<tr>
<th>Inputs</th>
<th>Unit</th>
<th>Energy equivalent (MJ unit(^{-1}))</th>
<th>Input used per hectare (unit ha(^{-1}))</th>
<th>Energy value (MJ ha(^{-1}))</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human labour</td>
<td>h</td>
<td>1.96</td>
<td>136.55</td>
<td>267.62</td>
<td>0.47</td>
</tr>
<tr>
<td>Soil preparation</td>
<td>h</td>
<td>1.96</td>
<td>7.35</td>
<td>14.41</td>
<td></td>
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<tr>
<td>Sowing</td>
<td>h</td>
<td>1.96</td>
<td>1.44</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>Hoeing</td>
<td>h</td>
<td>1.96</td>
<td>103.46</td>
<td>202.78</td>
<td></td>
</tr>
<tr>
<td>Fertilization</td>
<td>h</td>
<td>1.96</td>
<td>1.38</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>Spraying</td>
<td>h</td>
<td>1.96</td>
<td>6.30</td>
<td>12.35</td>
<td></td>
</tr>
<tr>
<td>Irrigation</td>
<td>h</td>
<td>1.96</td>
<td>13.71</td>
<td>26.87</td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>h</td>
<td>1.96</td>
<td>2.91</td>
<td>5.70</td>
<td></td>
</tr>
<tr>
<td>Machinery</td>
<td>h</td>
<td>64.80</td>
<td>17.55</td>
<td>1136.59</td>
<td>1.99</td>
</tr>
<tr>
<td>Soil preparation</td>
<td>h</td>
<td>64.80</td>
<td>5.10</td>
<td>330.48</td>
<td></td>
</tr>
<tr>
<td>Sowing</td>
<td>h</td>
<td>64.80</td>
<td>1.11</td>
<td>71.92</td>
<td></td>
</tr>
<tr>
<td>Hoeing</td>
<td>h</td>
<td>64.80</td>
<td>3.56</td>
<td>230.68</td>
<td></td>
</tr>
<tr>
<td>Fertilization</td>
<td>h</td>
<td>64.80</td>
<td>1.04</td>
<td>67.38</td>
<td></td>
</tr>
<tr>
<td>Spraying</td>
<td>h</td>
<td>64.80</td>
<td>4.91</td>
<td>318.16</td>
<td></td>
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<tr>
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<td>h</td>
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<td>1.83</td>
<td>118.58</td>
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<td>8.21</td>
<td>830.85</td>
<td>1.45</td>
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<tr>
<td>Chemical fertilizers</td>
<td></td>
<td></td>
<td></td>
<td>15466.06</td>
<td>27.07</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>kg</td>
<td>60.60</td>
<td>233.79</td>
<td>14167.67</td>
<td>24.80</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>kg</td>
<td>11.10</td>
<td>102.38</td>
<td>1136.42</td>
<td>1.99</td>
</tr>
<tr>
<td>Potassium</td>
<td>kg</td>
<td>6.70</td>
<td>7.16</td>
<td>47.97</td>
<td>0.08</td>
</tr>
<tr>
<td>Micro elements</td>
<td>kg</td>
<td>120</td>
<td>0.95</td>
<td>114</td>
<td>0.20</td>
</tr>
<tr>
<td>Diesel fuel</td>
<td>l</td>
<td>56.31</td>
<td>245.58</td>
<td>13828.61</td>
<td>24.20</td>
</tr>
<tr>
<td>Electricity</td>
<td>kWh</td>
<td>3.60</td>
<td>5809.45</td>
<td>20914.02</td>
<td>36.61</td>
</tr>
<tr>
<td>Irrigation</td>
<td>m(^3)</td>
<td>0.63</td>
<td>6950</td>
<td>4378.50</td>
<td>7.66</td>
</tr>
<tr>
<td>Seed</td>
<td>kg</td>
<td>11.80</td>
<td>26.44</td>
<td>311.99</td>
<td>0.55</td>
</tr>
<tr>
<td>Total inputs</td>
<td></td>
<td></td>
<td></td>
<td>57134.25</td>
<td>100.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outputs</th>
<th>Unit</th>
<th>Energy equivalent (MJ unit(^{-1}))</th>
<th>Output per hectare (unit ha(^{-1}))</th>
<th>Energy value (MJ ha(^{-1}))</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton plant</td>
<td>kg</td>
<td>11.80</td>
<td>5361.91</td>
<td>63270.54</td>
<td>100.00</td>
</tr>
<tr>
<td>Total outputs</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100.00</td>
</tr>
</tbody>
</table>
TABLE 3
Energy balance calculations in cotton production

<table>
<thead>
<tr>
<th>Calculations</th>
<th>Unit</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yields</td>
<td>kg ha⁻¹</td>
<td>5361.91</td>
</tr>
<tr>
<td>Energy input</td>
<td>MJ ha⁻¹</td>
<td>57134.25</td>
</tr>
<tr>
<td>Energy output</td>
<td>MJ ha⁻¹</td>
<td>63270.54</td>
</tr>
<tr>
<td>Energy use efficiency</td>
<td></td>
<td>1.11</td>
</tr>
<tr>
<td>Specific energy</td>
<td>MJ kg⁻¹</td>
<td>10.66</td>
</tr>
<tr>
<td>Energy productivity</td>
<td>kg MJ⁻¹</td>
<td>0.09</td>
</tr>
<tr>
<td>Net energy</td>
<td>MJ ha⁻¹</td>
<td>6136.29</td>
</tr>
</tbody>
</table>

It can be seen from these tables that the first, second, and third highest energy inputs in cotton production were electrical energy by 36.61%, chemical fertilizer energy by 27.07%, and diesel fuel energy by 24.20%. Similarly, in previous studies, [1] and [15] concluded in their cotton study that fertilizer application energy had the second share with 14354.10 MJ ha⁻¹ (28.86). [1] determined that diesel fuel energy had the largest share among 15468.40 MJ ha⁻¹ (31.10%). [15] determined that diesel fuel energy had the largest share with 24863 MJ ha⁻¹ (47.40%). As seen in Table 2, the amount of chemical fertilizers used for cotton growth was 344.28 kg ha⁻¹. Nitrogen was the most common chemical fertilizer used in cotton production, with 233.79 kg ha⁻¹, followed by phosphorus 102.38 kg ha⁻¹, potassium 7.16 kg ha⁻¹, and micro elements 0.95 kg ha⁻¹. The energy input-output and efficiency calculations in cotton production were given in Table 3.

According to Table 3, cotton plant, energy input, energy output, energy efficiency, specific energy, energy productivity, and net energy in cotton plant production have been calculated as; 5361.91 kg ha⁻¹, 57134.25 MJ ha⁻¹, 63270.54 MJ ha⁻¹, 1.11, 10.66 MJ kg⁻¹, 0.09 kg MJ⁻¹, and 6136.29 MJ ha⁻¹. In previous studies; [1] determined energy efficiency as 0.74, [12] determined it as 1.51, [14] determined it as 1.63, [13] determined it as 2.36, [15] determined it as 0.70, [11] determined it as 2.52, [16] determined it as 3.79, and [17] determined it as 1.92. The distribution of inputs used in the production of cotton in accordance with the direct, indirect, renewable, and non-renewable energy groups, were given in Table 4.

The total energy input consumed in cotton production could be classified as 68.94% direct and 31.06% indirect. The total energy input consumed in cotton plant production could be classified as 8.67% renewable and 91.33% non-renewable. Similarly, it was determined that the ratio of non-renewable energy was higher than the ratio of renewable energy in cotton [12], cotton [13], cotton [15], cotton [1], cotton [14].

In this study, the energy efficiency of cotton plant production in the Hatay province has been defined. According to the evaluated results, cotton production is a low profit production in terms of energy usage. The research results indicate that the ratio of non-renewable energy is higher than the ratio of renewable energy. The reason for chemical fertilizer energy being so high is due to the fact that chemical fertilizers were used instead of manure. [47] reported that: “Energy resources are limited and are being rapidly consumed; therefore, energy needs to be used efficiently. The importance of energy increases each day, as fossil fuels have a limited period of usage, and renewable energy resources are eco-friendly and sustainable energy systems”. Energy use in agriculture has been increasing in response to the ever increasing population, limited supply of arable land, and a desire for higher standards of living. Continuous demand to increase food production has resulted in intensive use of chemical fertilizers, pesticides, agricultural machinery, and other natural resources. However, intensive usage of energy causes problems, which threaten public health and the environment. Efficient use of energy in agriculture may minimize environmental problems, may prevent the destruction of natural resources, and promote sustainable agriculture as an economical production system and efficient use of energy in agriculture.
[48]. Solar power is also suggested to be utilized in order to decrease the level of electricity usage.

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ANALYSING ENVIRONMENTAL NOISE IN URBAN PARKS IN THE CITY OF GAZIANTEP, TURKEY

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ABSTRACT

This study aims to analyse environmental noise levels in three urban parks in the city of Gaziantep, Turkey, considering measurements performed in two different seasons, and GIS based maps. A-weighted continuous equivalent sound level (Leq) was collected in homogenously distributed 38 points, and the results have been compared with the cut-off value established for outdoor living areas by the World Health Organization. The results revealed environmental noise does not pose a significant threat to park visitors’ well-being from mean noise level point of view, but more than half of the measurement points exceeded the cut-off value, depending on the increase in the number of vehicles. Noise maps have been assessed as potential tools to have information on noisy parts of the parks, to make possible to develop specific measures to decrease the noise levels. But, to produce different approaches on mitigation of noise, the effectiveness of other environmental sources of noise should be analysed in detail.

KEYWORDS:
Noise pollution, environmental noise, urban parks, Gaziantep, Turkey

INTRODUCTION

The world’s population has concentrated in cities [1]. In Europe it is expected that around three quarters of the population will live in urban settings by 2020. Urban living limits access to nature and can increase exposure to certain environmental hazards, such as air and noise pollution [2]. These developments had increased the demand for green spaces in urban areas [3] viewed as the last remnants of nature [4]. Parks and open spaces, serving important social and environmental functions, are valued leisure and amenity resources in cities [5]. But, urban green spaces and their environmental and social benefits are becoming inadequate due to environmental problems [3, 6]. Cities experience increasing signs of environmental stress, particularly in the form of poor air quality and excessive noise. Among them, environmental noise is one of the key determinants of life quality in urban areas [7, 8]. Noise in urban areas results from the combination of various sources, such as transportation, industries, construction sites, recreational activities, schools and commercial areas [9]. Noise pollution is among the most common complaints regarding environmental issues in Europe. It is estimated that more than 40% of European citizens are exposed to road noise levels above 55 dB(A), which disturbs sleep, affects cognitive functions in children, causes stress reactions and can lead to cardiovascular health problems [10, 11, 12].

The aim of this study is to analyse the environmental noise in three urban parks in the city of Gaziantep, Turkey. Noise levels have been measured on pre-defined measurement locations, and Geographical Information Systems (GIS) based maps have been prepared to quantify spatial distribution of noise pollution. Measurement results have been compared with the cut-off value established for outdoor living areas, by the World Health Organization (WHO). Some proposals have also been produced to increase the benefits of urban parks and the visitors’ well-being.

MATERIALS AND METHODS

Study area. The study has been performed in the city of Gaziantep which is one of the most rapidly urbanizing cities in Turkey. The areal coverage of the urban built-up land was 11,393 ha in 2000 [13], reached up to 13,640 ha in 2015 [14]. On the other hand, the urban population was 1,175,000 in 2007, but increased by 30% during the past 10 years, reaching 1,663,000 in 2017 [15]. The number of motor vehicles has also risen almost two-fold during the past decade, from about 240,000 in 2007 to 455,000 in 2017 [16].

Noise measurements have been performed in three urban parks located in the city core. With its 9.5 ha of areal coverage, Çamlık Park is the largest among others. The park is surrounded mostly by low-rise buildings (Figure 1). Vegetation cover is dense and mainly consists of Pinus brutia. Masal Park has 8.4 ha area, and surrounded by 4-5 storey buildings. Vegetation cover is
not dense, and consists of deciduous tree species. Yüzünçü Yıl Park has 8.8 ha area, and surrounded mostly by 4-5 storey buildings. Great part of the vegetation cover is consists of well developed deciduous tree species.

Measurement procedure and evaluation. To determine the seasonal differences in noise levels, measurements have been performed in two months, in March and September. Each month, 38 measurements have been collected to analyze the environmental noise in urban parks. A homogenous distribution was considered while locating the measurement points in the park areas. According to a real width of the parks, the number of measurement points has ranged from 10 to 14 (Figure 1).

The measurements have been carried out during weekdays, between 10:00 a.m. and 5:00 p.m., under ideal meteorological conditions when there was no rain or wind. The measurement schedule represents the most suitable activity period in terms of both the traffic and park visitors’ density. The duration of each measurement was 3 min [3, 6, 17] during which A-weighted continuous equivalent sound level Leq was collected. To determine the effects of traffic density on noise levels, motor vehicles have been counted in 29 measurement points located close to the roads. Relationship between noise levels and number of vehicles has been statistically analysed by using Spearman’s Correlation approach. The measurement results have been evaluated by considering the cut-off value established by WHO. WHO’s limitations regarding environmental noise specify that recommended noise levels for outdoor living areas should be 55 dB(A) [6, 18]. GIS based maps were also produced for each urban park to analyze the spatial distribution of noise pollution in the entire area. This method enables us to interpolate data values for the entire area based on pre-defined measurement locations. Noise maps were generated using Spatial Analyst extension in ArcGIS 10.5 software.

RESULTS

Within this research, it is aimed at measuring environmental noise levels in three urban parks in the city of Gaziantep. During field surveys, a wide variety of noise sources have been determined. Motor vehicle traffic is assessed as the main source of noise due to roads which completely surround the parks. The other environmental noises have been originated from park visitors, building constructions, cafeterias, schools, and peddlers.

The measurements realised in three urban parks revealed mean noise level is 56 dB(A) both in March and September, 1 dB(A) more than the limit value specified by the WHO (Table 1). It means that environmental noise does not pose a significant threat to park visitors’ wellbeing, from mean noise level point of view.

<p>| Number of measurements according to WHO limit, mean noise levels, and number of motor vehicle |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Number of Measurements</th>
<th>&lt;55 dB(A)</th>
<th>&gt;55 dB(A)</th>
<th>Mean dB(A)</th>
<th>Number of Motor Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>Sept</td>
<td>March</td>
<td>Sept</td>
<td>March</td>
</tr>
<tr>
<td>Çamlık Park</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Masal Park</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yüzünçü Yıl P.</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>9</td>
<td>2</td>
<td>1</td>
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</table>
TABLE 2
Spearman’s Correlation Analysis results according to relationship between noise level and number of motor vehicle

<table>
<thead>
<tr>
<th>Noise Level</th>
<th>P March</th>
<th>P September</th>
<th>r March</th>
<th>r September</th>
<th>N March</th>
<th>N September</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>0.001</td>
<td>0.614</td>
<td>0.578</td>
<td>29</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 2
Noise levels in the parks

The highest mean noise level has been determined in Çamlık Park with the values of 57 and 58 dB(A) for March and September, respectively. The second highest mean noise level was 56 dB(A) in Yüzüncü Yıl Park for both months. In Masal Park, mean noise level for both months was 55 dB(A). These results have showed that there is no significant differences between March and September, from the viewpoint of mean noise levels. On the other hand, 58% and 74% of the total 38 measurements exceeded the limit of 55 dB(A) in March and September, respectively (Table 1) (Figure 2). It is thought that this difference between months has been occurred due to traffic density. The total number of motor vehicle was 1628 in March, but reached to 2042 in September.

The relationships between noise levels and the number of motor vehicle have been statistically analysed. Spearman’s Correlation approach has been preferred due to the number of sampling is less than 30, and the number of motor vehicle does not have a normal distribution. According to analysis results revealed for both months, it is determined that p-values were smaller than 0.01, and r-values were between 0.50-0.69 (Table 2). Considering these results, it is possible to say that there is statistically significant and moderately positive linear relationship between the noise level and the number of motor vehicles.
To analyse the spatial distribution of noise pollution in the entire area, three GIS based maps have been produced considering March, September, and mean values of measurements. The results have displayed that noise levels show roughly similar distribution in the maps. Generally noise levels are high in outer parts of the parks, while inner parts show relatively low levels (Figure 3). Especially in Masal and Yüzünçü Yıl Parks, the corners located close to intersections have reflected higher levels. Considering these results, it is possible to say that analysing spatial distribution of noise pollution provide useful information by enabling us to describe the sources of noise, and to develop measures to decrease the effects of noise on park visitors.

**DISCUSSION AND CONCLUSIONS**

Within this study, environmental noise levels have been analysed in three urban parks in the city of Gaziantep, considering measurements performed in different seasons, and GIS based maps. The results of the measurements revealed environmental noise does not pose a significant threat to park visitors’ wellbeing, from mean noise level point of view. On the other hand, more than half of the measurement points exceeded the cut-off value established by the WHO [18], and it was determined that this ratio increased depending on the increase in the number of vehicles [10, 11, 12]. Noise maps have showed that the parts of the parks close to the intersections have reflected relatively high noise levels. Besides, the statistical analysis put forward that there is statistically significant and moderately positive linear relationship between the noise levels and the number of motor vehicles. Considering the findings of this research, it is possible to say that motor vehicle traffic is one of the most important reason of noise pollution [6], but the effectiveness of the other environmental sources of noise should be analysed in detail. Some mitigation measures should be developed to reduce and control noise pollution with the help of noise maps. Especially in noisy parts of the parks, vegetative barriers should be formed between source and receiver, by using deciduous shrubs. Limiting vehicle speed, and covering the roads by silent asphalt material should be assessed as alternative mitigation methods for decreasing noise levels.

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This study has been realised in the context of the MSc thesis entitled “Analysing Environmental Noise in Urban Parks in The City of Gaziantep”. The authors also acknowledge The University of Kahramanmaraş Sütçü Imam for financial support of this study (Project no. 2005/5-10).

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IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF FOUR GYPSOPHILA L. SPECIES PLANT EXTRACTS

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ABSTRACT

This study is carried out for the research of antimicrobial and antioxidant effects of Gypsophila perfoliata L. var. perfoliata, G. perfoliata L. var. araratica Kit Tan, G. pilosa Huds. and G. osman- gaziensis Ataslar & Oacak plant extracts. The species of genus Gypsophila L. which are used in this study are collected from two different localities. The antimicrobial effects of plants extracts are researched against bacteria Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhimurium, Staphylococcus epidermidis, Staphylococcus aureus and against fungi Aspergillus niger, Aspergillus fumigatus, Fusarium solani. Antimicrobial activities were examined using disc diffusion technique. The minimum inhibitory concentrations (MIC) was determined by the micro dilution method. Phytochemical scans are made to examine the types of plant secondary metabolites. Free radical scavenging effect of plant species are made with DPPH. Petroleum ether and ethyl acetate extracts of G. pilosa and methanol extracts of G. perfoliata var. perfoliata against P. vulgaris are formed high inhibition zone. The results show that the three extracts (1A, 1C and 4B) produced favorable results against P. vulgaris microorganism with MIC values between 230.4 and 460.9 μg/mL.

KEYWORDS:
Caryophyllaceae, Gypsophila L., antimicrobial, antioxidant, minimum inhibitory concentration.

INTRODUCTION

The family Caryophyllaceae is one of the richest in Caryophyllales including about 3000 species (100 genera) which are primarily distributed in the Holoartic region and have a center of diversity in the Mediterranean and Irano-Turanian areas [1-3]. Caryophyllaceae is represented by over 470 species and 32 genera, among these genera Gypsophila L. which is represented in Turkey [4-6].

Gypsophila is a polymorphic Eurasian genus (north-temperate part of the Old World, mainly between the latitudes of 30° and 60°) belonging to the subfam. Caryophyllidoideae [7]. In Turkey, Gypsophila is represented by 60 species and 10 sections [5, 8-10].

A comprehensive study was carried out by Böttger and Melzig (2011) about triterpenoid saponins of Caryophyllaceae family [11]. A study was carried out by Yotova et al. in 2012 triterpenoid saponins of Gypsophila trichotoma Wender [12]. Another study was carried out by Arslan et al. (2012) about a cytotoxic triterpenoid saponin was isolated from the under-ground parts of G. pilulifera Boiss. & Heldr. [13].

Gypsophila species contain saponins [14-16]. Saponins were used for washing wool and silk, giving halva its fragility, and as fire extinguisher agents, while in medicine they were believed to have expectorant, laxative, and emetic properties [16, 17-20].

Some of the perennial Gypsophila species are known as soapwort in Turkey and used especially in making halva. The first and most comprehensive study about saponin contents of Gypsophila species was carried out by Ekrem Sezik in 1982 in Turkey. In his study Sezik (1982) detected raw saponin percentages of four Gypsophila species. According to this study, G. bicolor (Freyn. & Sint.) Grossh. includes 20-25%, G. arrostit Guss. var. nebulosa (Boiss. & Heldr.) Barkoudah includes 19-22%, G. perfoliata Ser. var. anatolica (Boiss. & Heldr.) Barkoudah includes 15-19%, G. eriocalyx Boiss. includes 10-14% raw saponins [14].

Another study carried out by Shafaghata and Shafaghatonbar (2011) about G. bicolor is related with antimicrobial activity and chemical constituents of the species. Moreover in this study it was stated that Gypsophila species include saponins, flavonoids and sterols and Gypsophila saponins have anticarcinogenic properties, including direct cytotoxicity, immune-modulating effects, and normalization of carcinogen-induced cell proliferation [15].
Acebes et al. (1998) stated that new saponin was isolated from the methanol extract of the roots of G. bermejoi G. López and identified by a combination of chemical degradation and spectral methods. Moreover in this study it was also stated about Gypsophila species that the saponins from the roots of G. paniculata L. and G. arrostii Guss. have been used as detergent and expectorant. G. struthium Loefl. is known as a source of saponins since the antiquity and is also used in gastronomy in Arabic countries [21].

Also some Gypsophila species aerial parts, forming a large number of branches, ending with tiny flowers, are used for decorative purposes to adorn bouquets [16].

In recent years it has been observed that there are lots of side effects in synthetic drugs while there are scarcely any or even no side effects in natural-origin drugs which drives people to phytotherapy once again [22, 23]. From this point, in this study antimicrobial activity of extracts of Gypsophila perfoliata L. var. perfoliata, G. perfoliata L. var. araratica Kit Tan, G. pilosa Huds. and G. osman- gaziensis Ataşlar & Oçak species against specific Gram (+) and Gram (-) bacteria and fungi and specific phychochemical characteristics were presented. With this study which was carried out for the first time with these four Gypsophila species, it was aimed to ensure people use the plant consciously, and to shed light on future studies about Caryophyllaceae family or Gypsophila species and to provide data for studies to be carried out about medicinal plants.

### MATERIALS AND METHODS

**Plant material.** In the study, four different species of Gypsophila belonging to Caryophyllaceae family was used. In Table 1, taxons and localities used in the study were given. Plant species were collected from two different localities (Table 1) and dried.

**Test microorganisms.** The bacterial strains were recovered from long term storage at −85 °C in the cryobank. The bacteria were refreshed in Nutrient Broth (Merck, Germany) at 35-37 °C and then inoculated on Nutrient Agar (Merck) plates to check the microbial purity. The fungal strains were refreshed in Malt Extract Agar (Merck) at 27 °C. The strain numbers and sources of the acquired microorganisms are listed in Table 2.

**Preparation of the extracts.** Fresh gametophytic samples of G. perfoliata var. perfoliata, G. perfoliata var. araratica, G. pilosa and G. osman-gaziensis were treated with 0.8% Tween 80 aqueous solution to remove the epiphytic hosts normally found on the surface, extensively washed in tap and distilled water, and dried on filter paper at room temperature.

Extraction procedures were applied as described elsewhere [24, 25]. Extraction was carried out through two different processes. First, 10 g of the sample in powder form was extracted with 250 mL of 80% petroleum ether for 8 h using Soxhlet equipment. After filtering with Whatman filter paper (#1), all extracts were concentrated by rotary
evaporation to dryness in vacuum (yield = 2.20 %, 0.86 %, 1.70 %, and 1.40% respectively) and stored in desiccators for future use (extract A).

Plant material was degreased with petroleum ether, spread on filter paper and dried overnight. In the second step, 250 ml 70% methanol (MeOH) solution on the material which was degreased with petroleum ether the night before. The flask was closed with foil and mixed for half an hour in the mixer, the fluid part was sieved and put in another store. This was repeated three times and after each process the mixture was sieved. Finally the total filtrate were sieved with whatman filter paper. Then extract was concentrated by rotary evaporation to dryness in vacuum. In this way “extract B” was obtained (yield = 6.90 %, 5.00 %, 8.96 %, and 7.36 % respectively). It was stored in a closed box for future use. Sediments of plants were thrown at the end of this process.

In the third step of extraction, plant material was three times maseder with 70% of methanol as it was in the second step. After part of methanol was concentrated, liquid-liquid extraction was applied with the liquid phase attained. The phase was evaporated of methanol, measured and put into phase seperating funnel. Ethyl acetate was measured in the same amount with phase and added on the phase into separating funnel. Separating funnel was shaken so that ethyl acetate and phase would be mixed. Some time later, phase was sieved into volumetric flask and this process was repeated three times. Later on ethyl acetate phase was concentrat-ed by rotary evaporation to dryness in vacuum. In this way “extract C” was obtained (yield = 0.53 %, 1.00 %, 0.43 %, and 0.56 % respectively). It was stored in a closed box in refrigerator for future use.

Liquid phase was frozen at -80ºC in lyophilizator (Christ Alpha 1,4). Later on this part was concentrated with vacuum and “extract D” was obtained.

Extracts were stored at 4ºC and diluted with dimethyl sulphoxide (DMSO) as 200 mg/mL as its final concentration when to be used in activity studies.

**Antimicrobial activity.** This experiment was implemented pursuant to the method defined by the National Committee for Clinical Laboratory Standards [26], with some alterations. The test-cultures of bacteria were incubated in MHB (Mueller-Hinton Broth) at 35 to 37ºC until they became apparently sedimented. The density of these cultures was set to the same turbidity of the 0.5 McFarland standard (at 625 nm, 0.08 to 0.1 absorbance) with sterile saline. Alternatively, in order to stimulate spore formation, the molds were bred on Potato Dextrose Agar (PDA) slants at 27ºC for 5 to 7 days. After being counted with the Thoma slide, the spore concentration for each mold was set right to 10^6 CFU/mL with sterile 0.1% Tween 80. Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar plates, each of which was cooled down to 45 to 50ºC and sterilized, were used for bacteria and fungi, respectively and then the plates were placed into the sterilized Petri dishes (9 cm). The whole surface of the MHA plates and the Sabouraud 4% Glucose Medium plates were inoculated with the bacteria and fungi by being spread with a sterile swab dipped into the adapted suspensions. Six wells, each of which were 6 mm in diameter, were cut out of the agar, and 20 μL of the extract solutions was placed into each well. The plates were incubated with bacteria at 37ºC for 24 hours or with fungal strains at 30ºC for 48 hours. After these incubations, the dishes were kept at 4ºC for 2 hours. The diameters of the inhibition zones were measured in terms of millimeters. As a positive control for bacteria vancomycin and oxacillin and for fungi, amphotericin B (Sigma) was used. DMSO was used as the negative control. All assays were done in duplicate [27].

**Minimum inhibitory concentration (MIC).** Micro dilution method was utilized through a 96 well plate pursuant to NCCLS for the determination of MIC [26]. Firstly, 100 μL of MHB or Sabouraud Dextrose Broth was placed in each well. The stock solutions of the extracts were diluted and transferred into the first well, and serial dilutions were performed so that concentrations in the range of 58,59 to 30000 μg/mL could be acquired. The inoculums were set right to contain approximately 10^3 CFU/mL bacteria and 10^4 CFU/mL fungi as defined above. 100 μL of the inoculums was added to all the wells and the plates were incubated at 37ºC for 24 hours for bacteria or at 30ºC for 48 hours for fungi. MIC values were determined by adding 20 μL of 0.5% trefenil tetrazolium chloride (TTC) aqueous solution. The MIC value was considered as the lowest concentration of the extract that inhibited any visible bacterial or fungal growth, as indicated by TTC staining following the incuba-tion [26]. As the reference antibiotic control, vancomycin and oxacillin were used again.

**Phytochemical Tests.** Phytochemical tests were done with all plant extracts and the existence of anthraquinone, alkolo id, flavanoid and terpene were observed. To test the existence of anthraquino-ne in extracts 0,5 mg plant extract was mixed with 10 ml sulphuric acid (H_2SO_4) and then filtered. The filtrate was mixed with 5ml chloroform and chloroform layer was taken to another tube. Then 1 ml diluted ammonia was included into filtrate and the change of color was observed.

To observe terpenes in plant extracts, 2 ml chloroform was included in 0,5 mg plant extract. 3 ml concentrated sulphuric acid (H_2SO_4) was includ-ed on it carefully. Reddish brown color proves the existence of terpenes. To observe the flavanoids in
plant extracts, 5 ml diluted ammonia was added to some part of plant extracts. 1 ml concentrated sulphuric acid (H₂SO₄) was added on this mixture. Yellow color proves the existence of flavanoid. To show the alkaloids in plant extracts, 0.5 mg plant extract was diluted with 10 ml acid alcohol and then filtered. 2 ml diluted ammonia was added to 5 ml filtered amount of the mixture. 5 ml chloroform was added to the mixture and stirred. Reddish Brown color proves the existence of alkaloids [28, 29].

RESULTS

Antimicrobial activity. The results of the antimicrobial activity of extracts A, B, C, and D, from *G. pilosa*, *G. osmangaziensis*, *G. perfoliata* var. *araratica* and *G. perfoliata* var. *perfoliata* are presented in Tables 3. The applied concentration of all extracts did not demonstrate any activity against fungal strains.

Table 4 illustrates the MIC ranges of extracts against bacterial strains. Our results show that the three extracts (1A, 1C and 4B) produced favorable results against *P. vulgaris* microorganism with MIC values between 230.4 and 460.9 μg/mL.

### TABLE 3

<table>
<thead>
<tr>
<th>Code of extract</th>
<th>Bacterial Strains</th>
<th><em>P. vulgaris</em> (mm)</th>
<th><em>S. aureus</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1B</td>
<td>18</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1C</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1D</td>
<td>21.5</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>2A</td>
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<td>NS</td>
<td></td>
</tr>
<tr>
<td>3C</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3D</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>4A</td>
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<td>22.5</td>
<td></td>
</tr>
<tr>
<td>4B</td>
<td>14.5</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>4C</td>
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<td>25</td>
<td></td>
</tr>
<tr>
<td>VA*</td>
<td>17</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>OX**</td>
<td>14.5</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS: not sensitive; * Vancomycin (10 μg/disc); ** Oxyacillin (30 μg/disc)

### TABLE 4

<table>
<thead>
<tr>
<th>Code of extract</th>
<th><em>P. vulgaris</em> (μg/ml)</th>
<th><em>S. aureus</em> (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>460.9</td>
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<tr>
<td>1B</td>
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<td>1C</td>
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<td>30000</td>
</tr>
<tr>
<td>2A</td>
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<td>-</td>
</tr>
<tr>
<td>2B</td>
<td>30000</td>
<td>-</td>
</tr>
<tr>
<td>2C</td>
<td>3750</td>
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</tr>
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<td>-</td>
</tr>
<tr>
<td>4D</td>
<td>30000</td>
<td>30000</td>
</tr>
</tbody>
</table>

### TABLE 5

<table>
<thead>
<tr>
<th>Code of extract</th>
<th>200 (μl) Inhibition% (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C</td>
<td>0.245 48.95</td>
</tr>
<tr>
<td>2C</td>
<td>0.180 62.50</td>
</tr>
<tr>
<td>3C</td>
<td>0.141 70.62</td>
</tr>
<tr>
<td>4C</td>
<td>0.136 71.66</td>
</tr>
<tr>
<td>1B</td>
<td>0.129 73.12</td>
</tr>
<tr>
<td>2B</td>
<td>0.130 72.91</td>
</tr>
<tr>
<td>3B</td>
<td>0.470 2.08</td>
</tr>
<tr>
<td>4B</td>
<td>0.186 61.25</td>
</tr>
<tr>
<td>BHT (Control)</td>
<td>0.480 -</td>
</tr>
</tbody>
</table>

Plant extracts were tested in the sense of specific phytochemicals and the findings were presented in Table 6. There was no flavanoid in any of petroleum ether (A) extracts; alkoloid was observed in three of them (1A, 2A, 3A); terpene was observed in two of them (1A, 2A) and anthraquinone was observed in one of them (3A). There was no alkoloid in any of methanol (B) extracts; terpene was observed in one of them (2B), flavanoid was observed in one of them (2B) and anthraquinone was observed in one of them (3B). While there was no flavanoid and anthraquinone in any of ethyl acetate extracts (C); alkoloid was observed in three of them (1C, 2C, 3C), terpene was observed in one of them (4C). Alkoloid and terpene were not observed in any of lyophilisation extracts (D), flavanoid was observed in two of them (1D, 2D) and anthraquinone was observed in two of them (2C, 4D).
<table>
<thead>
<tr>
<th>Code of extract</th>
<th>Alkaloid</th>
<th>Terpene</th>
<th>Flavonoid</th>
<th>Anthraquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>+</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
<td>4D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

A. Petroleum ether extract; B. Methanol extract, C. Ethyl acetate extract, D. Lyo-philisation extract

**TABLE 6**

Phytochemical test results of plant extracts

DISCUSSION AND CONCLUSIONS

Turkish soapwort plant is obtained from five different types of Gypsophila (G. bicolor, G. arrostii var. nebulosa, G. eriocalyx, G. perfoliata var. anatolica, and G. venusta). Saponins being derived from the roots of these plants are generally components originating from plants that are used in treatments for centuries due to their different pharmaceutical effects besides their antifungal and antibacterial influences [30]. G. paniculata and G. arrostii species are expectorants and G. struthium species is being used in the meals in Arabic countries since very ancient times [21, 31].

In this study, antimicrobial activity studies have been conducted with four species belonging to Gypsophila. Regarding plant extracts of G. perfoliata var. perfoliata, G. perfoliata var. araratica, G. pilosa, G. osmangaziensis species that are obtained as a result of their interaction with petroleum ether, methanol, and ethyl acetate, and against microorganisms of Escherichia coli NRRL-B.3008, Pseudomonas aeruginosa ATCC 27853, Proteus vulgaris (clinical isolate), Salmonella typhimurium ATCC-14028, Staphylococcus epidermidis, Staphylococcus aureus ATCC 25923, Aspergillus niger ATTC 10549, Aspergillus fumigatus NRRL-163, Fusarium solani (wild type) antimicrobial activity, minimum inhibition concentration (MIC), antioxidant activity, and phytochemical detections have been realized.

In this study, it has attracted attention that extracts being obtained from plants had greater impact on fungi with respect to the bacteria being tried. While various extracts belonging to the plant species being investigated had lower impact with respect to the bacteria being tried, inhibition impact of certain extracts has a significant ratio. Inhibition zones being formed by methanol extracts belonging to the plants being investigated, have more significant ratios with respect to extracts being obtained with other solvents. Furthermore, efficiency percentage of methanol extracts of plants has been determined to have visibly high when compared with other extracts (petroleum ether, ethyl acetate). While it is known that in the composition of certain Gypsophila species generally saponins are present, [28, 32-40], if solubility of saponins in methanol is also considered, it could be stated that the main component in methanol extracts is saponin.

Park et al. (2005) have investigated biological activities and chemistry of saponins from Panax ginseng C.A. Meyer. In this study, more than 30 ginsenosides have been isolated, and known compounds are identified. Pharmacological effects of ginseng have been demonstrated in diabetes mellitus, cardiovascular system, immune system and central nervous system including anti-stress and anti-oxidant activity [31].

As being based on the findings of agar diffusion method, MICs belonging to plant extracts forming inhibition zone against test microorganisms have been specified. MIC values of extracts with 1A, 1C, and 4B codes were found to be 460.9 μg/ml, 230.4 μg/ml and 460.9 μg/ml respectively against P. vulgaris. Meaning that antimicrobial effects of petroleum ether extract and ethyl acetate extract of G. pilosa plant and methanol extract of G. perfoliata var. perfoliata plant have been revealed. Furthermore, it was found out that extracts with codes 1D, 2D, 4A, and 4D had inhibition impact on S. aureus which was gram positive. Shafaghat and Shafaghatlonbar (2011) have determined antimicrobial activity and chemical components of volatile oils being obtained from the flowers, leaves, and roots of G. bicolor by means of hydro-distillation [15]. They have stated that extracts had moderate level of impact on gram positive and gram negative bacteria. Due to the fact that water
solubility of saponins is high, it can be thought that saponin content of (D) extracts, meaning watery extracts is high and that this antibacterial impact originates from the saponins. By inhibiting the growth of gram positive and gram negative bacteria, saponins reveal antimicrobial activities. Some of the saponins are not effective against gram negative bacteria due to the reason that bacteria can not penetrate into the cell membranes [41].

Many of the saponins that are present in the plants have antifungal features and they are present in plants having high endurance against external factors, in high concentrations. These molecules can act as chemical barrier against fungal impact. Many of them inhibit the development of microorganisms in in vitro media. These components are either naturally synthesized during the growth and development of plant or they are not present in healthy plants and they are produced against pathogen attacks and stress [42, 43]. In the antifungal activity study being conducted, it was found out that 16 different extracts belonging to four Gypsophila taxa did not have inhibition impact on the fungi being used. Saponins have two different structures being neutral (steroid derivatives) and acid (triterpenoid structure) types. While Monocotyle Angiosperms have steroid derivative type of saponins, Dicotyl Angiosperms have saponins having triterpenoid structure type. According to the literature, antifungal activity of saponins originates from steroidal type of saponins [41]. Since the plants constituting the subject of our study are part of Dicotyl Angiosperms, they bear more triterpenoid type of saponins with respect to steroidal type of saponins. Experimented extracts’ not revealing antifungal activity, is consistent with these information. Antifungal activity of steroidal saponins is related with the number and structure of monosaccharides that are in their sugar chains. Saponins reveal antimicrobial activity by inhibiting the development of certain gram negative and gram positive bacteria. However since certain saponins can not penetrate in the cell membranes of microorganisms, they are not effective on gram negative bacteria. According to literature information it has been reported that saponins also avoided the development of soil originated fungi at the same time [43-45]. Dianthus versicolor Fisch. ex Link. (Caryophyllaceae), having triterpenoid saponins, is a natural plant, diuretic and anti-inflammatory features of which are known. Many bioactive saponins have been isolated from this plant. [46].

Ertürk et al. (2006) have investigated antimicrobial features of chloroform extract fractions belonging to Silene multijida (Adams) Rohrb. Plant which is part of Caryophyllaceae family. As a conclusion they have found out that fractions of plant with numbers of 1, 2, 3, 4, 5, and 6 revealed antimicrobial activity against the tested bacteria. It has been determined that in fractions with numbers 2 and 4, fractions with numbers 1 and 5 revealed more antifungal impact. Even though they were part of the same family, it has been determined that there wasn’t antifungal effect in any of the studied Gypsophila taxa. It was found that antibacterial activity was present in petroleum ether and ethyl acetate extracts of G. pilosa and in methanol extract of G. perfoliata var perfoliata. In this respect, the study is consistent with the relevant literature [47].

In recent times, biological activity studies being conducted with many seedy plant species are reflected in the literature. Dissolving systems and methods which are used in the extraction of plant material cause for different phytochemicals to be gathered in each extract being obtained in different ratios. For this reason, in the studies being conducted in this direction, different findings are being obtained. Depending on solvent polarity being used, extracts having different type and quantities of dissolved materials, have been obtained and as it can be seen from Table 6, differences have been observed in the composition of each extract. In the extracts being obtained with petroleum ether (A), mainly the presence of alkaloids and terpenes has been detected. It is known that apolar solvents such as ether, hexane, and ethyl acetate, extracted chemical groups such as alkold, terpenoid, coumarin, and flavonoid. In a similar way, presence of flavonoids and anthraquinones was determined in ethanol and water extracts of Gypsophila taxa in the study. Information specifying that polar solvents such as ethanol and methanol extracted chemical groups such as lectin, alkoldoid, flavone, polyphenol, tannin, and saponin, is supporting our findings. It could be stated that extracts being obtained with methanol (B) and watery extracts being obtained following this (D) contained components having antimicrobial effect. Saponins are among the main composites that are present in Gypsophila species [14, 33]. Many saponins having natural antimicrobial activity in the plants provide protection against the effect caused by pathogens. The molecules commonly have strong antifungal activity and their natural function in the plants is to protect the plant against pathogenic microorganisms. These are being used in the production of medicines. Due to the reason that it is easily soluble in water, it is expected for saponin to be highly present in (D) extract. This situation increases the possibility that activity in water extract (D) originates from saponin. Tunç (2000) has determined that solubility of saponins in water was high. The reason for this is correlated with colloidal dissolution of saponin in water [48].

While there aren’t any certain information relating with their physiological effects in the plants, it is known that many saponins have antimicrobial effect and that they protect the plant against certain types of insects in the soil. In addition to this, it is anticipated that in certain plant organs they have a
role for improving the plant resistance [49]. At the same time saponins are compounds which have spread on a wide area in nature as existing in the form of steroidal or triterpenoid glycosides with heavy molecules and having biological activity with wide impact on plants, insects, fungi, and microorganisms. While low dosages of them regulate plant rooting, high dosages reduces root development [31, 49, 50].

Sezik et al. (1982) have investigated the influence of Gypsophila species having antifungal effect on various fungi (Alternari solani, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. versicolor, Fusarium oxysporum and Candida albicans) that reproduce by forming mycelium. As a result of the researches they have stated that raw saponozit being derived from Gypsophila arrostii was effective on fungi that reproduced by forming mycelium in high concentrations following 100-200 mg/ml concentrations of G. albicans and that G. eriocalyx raw saponozit showed antifungal impact on 3/8th of fungi and on 1/8th of G. perfoliata being among fungi that are used in the research. The fact that antifungal impact of G. perfoliata is much less than the impact of other species supports the outcome we have reached in our study [30, 51].

In the antimicrobial activity studies being conducted with various seedy plants, similar findings are observed. Kivrak et al. (2001) have stated that S. aureus, being a gram positive bacteria, showed sensitivity to methanol extract of Ceratonia siliqua L. [52]. Dülger et al. (1998), have determined that besides some other gram negative and gram positive bacteria, Artemisia absinthium L. plant had significant antimicrobial activity with respect to equivalent antibiotic on P. vulgaris bacteria culture with various extracts, and that extracts especially revealed a strong impact on Salmonella and Bacillus species, and that there was no antimicrobial activity against yeast culture being used [53]. Ilçim et al. (1995) have determined that Myrtus communis L. subsp. communis plant extracts inhibited the development of S. aureus bacteria (38 mm inhibition zone) [54]. Dığırak et al. (1998) have experimented antimicrobial activity of certain plant extracts against 11 bacteria and five fungi and as a conclusion they have stated that inhibition zones in P. vulgaris bacteria were 22-31 mm, and that inhibition zones of S. aureus were 12-26 mm [55]. Özcan et al. (2008) have experimented with the extracts they obtained from Thymus sypyleus subsp. rosulans on microorganisms and they have observed that dichloromethane and acetone extracts, dissolving substances having oily character from the plant, were especially effective on Gram positive S. epidermidis and S. aureus test organisms. Regarding MIC values being obtained with micro-dilution method, it was stated that among bacteria they were more effective on P. aeruginosa [56]. Kirbaş et al. (2005) have investigated antimicrobial activities of certain medicinal plants being raised in Elazığ region and they have inhibited development of Bumian paucifolium var. paucifolium microorganisms at varying ratios and they were found to be most effective against S. aureus bacteria with 17 mm of inhibition diameter (being same as the standard) [57]. Şengül et al. (2005) have investigated antimicrobial effects of Verbascum georgicum Bentham extract and they have stated that four fungi species being tested did not reveal antifungal activity on their isolates and that methanol extract of plant contained components having antimicrobial features and they recommended that this could be used as antimicrobial agent in the development of new medicines [58]. İlhan et al. (2006), have investigated antimicrobial activity of Punica granatum L. fruit barks were effective against the bacteria being used and that methanol extract was high against B. mycoides, B. cereus, B. subtilis and Micrococcus luteus were sensitive to acetone extract, gram negative bacteria (Klebsiella pneumoniae, Versinia enterocolitica, P. aeruginosa, E. coli and Enterobacter aerogenes) were sensitive to all of them [27]. Adıgüzel et al. (2005), have investigated antimicrobial activity of Ocimum basilicum (Labiatae) ethanol, methanol, and hexane extracts and they have revealed that none of the three extracts showed antifungal activity but that antifungal activity of Micrococcus flavus and E. coli organisms [60]. Khan et al. (2007), have investigated antibacterial, antifungal, and cytotoxic activities of Amorphophallus campanulatus roots and with disc diffusion method they have found out that the ethanol extracts being used were effective against all test organisms and that antifungal activity was weak [61]. Khan et al. (2001), have investigated antimicrobial activity of Cassia alata plant and they have found out that dichloromethane extract were more effective against test organisms being used when compared with petroleum and ethyl acetate extracts [62]. Ertürk et al. (2003), have tested N-hexane extract of Viscum album subsp. abietis plant against bacteria and fungi by using agar diffusion method and they have determined that plant extract showed antimicrobial activity against microorganisms being used [63]. Al-Zoreky (2009) have stated that methanol extracts of Punica granatum L. fruit barks were effective against the bacteria being used and that methanol extract activity was related with high amount of total phenols in the plant and that MIC value of plant methanol extract was high against S. enteriditis bacteria [64]. Yığit et al. (1993), have investigated antimicrobial activity of walnut (Juglans regia L.) plant with
water and methanol extracts by using disc diffusion method and they have found out that these extracts showed antimicrobial activity against S. aureus, S. epidermidis, P. aeruginosa, C. albicans, C. galabratea, C. tropicalis and C. kefyr strains [65]. Vaghasiya et al. (2007), have investigated antimicrobial activities of methanol and acetone extracts of 14 Indian medicinal plants on five gram positive and seven gram negative bacteria and three fungi and they have found out that most influenced bacteria was K. pneumoniae, and that most resistant bacteria were P. vulgaris, S. typhimurium, P. aeruginosa and E. coli [66]. Parekh et al (2008), have investigated antibacterial activity of liquid and alcoholic extracts of 34 pieces of Indian medicinal plants against some Staphylococcus species and they have found out that the most sensitive bacteria was S. aureus bacteria [67].

As certain extracts (petroleum ether, ethyl acetate, methanol) of G. pilosa and G. perfolutia var. perfolutia being among Gypsophila taxa that are investigated in this study had antimicrobial effect against P. vulgaris bacteria, we have seen that these taxa could be used for treatment purposes and that they could be alternatives against synthetic antibiotics. However, it should not be forgotten that usage for this purpose should also be supported with other chemical examinations. Plants’ having different phytochemical features is a reason why extracts being obtained from them are revealing or not revealing antimicrobial activities. In the future studies to be conducted, as active substances in various extracts of Gypsophila taxa are determined one by one, antimicrobial impact could be more. It is thought that Gypsophila taxa being studied had potential for being used with the purpose of preserving food as antimicrobial agents.

Antioxidant substances are strong as per their capacity to eliminate free radicals in the environment. In free radical sweeping trial being conducted as per DPPH method, absorbance values being measured at 517 nm were compared with BHT.

It was observed that G. osmangaziensis methanol extract with 2B code containing flavonoid, has ranked as the second one with respect to the free radical sweeping impact. The fact that in a previous literature being made it was reported that S. scardica Griseb. Specie had free radical sweeping impact that could be compared with BHT [68] supports the results being given in our study.

Low absorbance shows that high free radical removal impact is present. This study is like the beginning of discovery of new antioxidant substances from natural sources and it is considered to have significant importance. Saponins which are stated to be effective on lipid metabolism, mineral metabolism, blood pressure, reproduction performance, and cancer in mammals, also reveal antioxidant activity [69].

Phytochemical features of plants show variations from one specie to another. In this study, flavonoid was found in the plants with codes of 2B (G. osmangaziensis methanol extract), 1D (G. pilosa watery extract), and 3D (G. perfolutia var. araratica methanol extract), and alkoldow was found in plants with codes of 1A (G. pilosa petroleum ether extract), 2A (G. osmangaziensis petroleum ether extract), 3A (G. perfolutia var. araratica petroleum ether extract), 1C (G. pilosa ethyl acetate extract), 3C (G. perfolutia var. ararica ethyl acetate extract), and 4C (G. perfolutia var. perfolutia ethyl acetate extract), terpene was found in plants with codes of 1A (G. pilosa petroleum ether extract), 2A (G. osmangaziensis petroleum ether extract), 2B (G. osmangaziensis methanol extract), and 4C (G. perfolutia var. perfolutia ethyl acetate extract), and anthraquinone was found in plants with codes of 3A (G. perfolutia var. araratica petroleum ether extract), 3B (G. perfolutia var. ararica methanol extract), 2D (G. osmangaziensis watery extract), and 4D (G. perfolutia var. perfolutia watery extract).

As a conclusion, in our antimicrobial activity findings it was determined that petroleum ether and ethyl acetate extracts of G. pilosa and methanol extract of G. perfolutia var. perfolutia were more effective against P. vulgaris bacteria when compared with other extracts. The remarkable aspect of study is that due to P. vulgaris’ being the main reason of urinary tract infections which are seen in people, it is seen that G. pilosa and G. perfolutia var. perfolutia extracts had antibacterial molecules.

REFERENCES


PRELIMINARY STUDY ON THE ECOLOGICAL QUALITY OF OREN AREA IN GÖKova BAY, THE SOUTHEASTERN AEGEAN SEA

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Department of Hydrobiology, Faculty of Fisheries, Ege University, Izmir, Turkey

ABSTRACT

This study is on the zoobenthic community structures and benthic quality status of a marine protection area in Gökova Bay (the southeastern Aegean Sea). Samples were taken from sand and sandy mud bottoms by means of a van Veen grab at the depths between 5 and 45 m of 7 different stations on 8.11.2014 and 09.11.2014. A total of 67 species belonging to 8 systematic groups were identified in the area, among these, 3 species are new records for the Turkish coasts and 3 species for the Aegean Sea coast of Turkey. 3 of 67 species reported were exotic. Group, Polychaeta was the most dominant in the area contributed 70.1% of the total fauna. The most dominant and frequent species was *Cirrophorus branchiatus* in the study area. Community variables varied significantly among sampling sites; number of species ranged from 4 to 27 per 0.1 m², density from 90 to 780 ind. m⁻², Shannon–Wiener diversity index varied from 1.66 to 4.27. The biotic index values for 7 stations were between 3.00 and 4.52 for Bentix, 1.83 and 2.63 for Ambi, 2.44 and 3.5 for Medocc, and 2.20 and 4.08 for Tubi.

KEYWORDS: Macro–zoobenthos, Community Structure, Biotic Index, New Records, Gökova Bay, The Southeastern Aegean Sea

INTRODUCTION

The Aegean Sea is bounded by the Ionian Sea in the southwestern and the Levantine Sea in the southeastern and by the Dardanelles and the Sea of Marmara and the Bosphorus and the Black Sea in the north [1]. Turkish Aegean Sea has a total of 11 Bays such as Saros, Edremit, Dikili, Çanlarlı, İzmir, Gerence, Sığacık, Kuşadası, Güllük, Gökova, and Datça each having long coastlines and many small islands. Gökova Bay is located at the intersection point of the Aegean and the Mediterranean and one of the largest and richest bays in Turkey. Yet, Gökova Bay is one of the sixteen of the Special Environmental Protection Area (SEPA) in total. Gökova Bay was regarded as “Special Environmental Protection Zone” on 05.07.1988. This area covers a total area of 1092.79 km² [2]. A total of 723 macroscopic species belonging to 19 systematic groups were reported in previous studies conducted on biodiversity of the Gökova SEPA region [3]. 79 of 723 species are flora and other 644 species are macrozoobenthos.

Studies on macrozoobenthos of Gökova Bay is also important in terms of fisheries which is scarce. The presence of species belonging to invertebrate groups in previous studies performed on faunistic and floristic assemblages of several zoogeographic areas in the region were reported by different researchers [4–10].

Benthic macro-invertebrates in transition and coastal ecosystems are generally used as biologic determinants when the ecological quality status of the environment is monitored. Different studies have shown that macrozoobenthic species are relatively sensitive to anthropogenic and natural stress [11–13]. Many biotic indices are used to determine the effects of the increasing organic enrichment on benthic community structures in a special marine ecosystem.

Ecological quality analysis of the environment is carried out by biotic indices such as Shannon–Weaver diversity index (H), Ambi, Bentix, Medoce, and Tubi in the Mediterranean marine environments [14–19].

In this study, the structural characteristics of the benthic communities found in Gökova Bay were tried to be determined. The ecological quality status of the region was also determined by means of various biotic indices.

Thus, negative effects to be appeared in the Gökova Bay can be determined ahead. Based on the results of this study, the environmental problems to be occured in the region in future can be prevented.

MATERIALS AND METHODS

In order to determine the structural characteristics of the benthic assemblages in Gökova Bay located on the Aegean coast of Turkey, benthic
samples were taken from 7 different sampling sites by means of van Veen Grab with an area of 0.1 m\(^2\) on 08–09.11.2014 (Figure 1). Features of sampling sites (biotope type, date, depth) were given in Table 1.

Benthos material was softened with sea water in the basins and then sieved by a sieve with mesh size of 0.5 mm. The materials on the sieve were preserved in plastic bootles containing buffered formalin of 4%. Material fixed in situ was passed through a triple sieve system with the eyes of 0.5, 1 and 2 mm under laboratory conditions with the help of pressurized water and washed. All the faunal specimens remaining on the sieves were preserved in alcohol of 70% in glass tubes on a group basis after removal at macro and micro level. Triocular stereo microscope was used to determine all the specimens found in the study area. Benthos specimens were defined based on the studies of various authors for each systematic groups.

The total number of species and individuals belonging to macrozoobenthos were determined, and the frequency [20], dominance [21] diversity [14], and similarity indexes [22] were calculated for the interpretation of the data obtained. Soyer’s Frequency Index \(F\) was used for classifying species according to their occurrences in samples. According to this index, species with \(F\geq 50\%\) are considered ‘constant’, those with \(F\) between 25 and 49% are ‘common’, while \(F\) values <25% are considered as ‘rare’. Bray–Curtis [22] index was calculated for the similarity matrix at the sampling sites and was used to distinguish species assemblages on soft substratum. In order to determine the relationships between assemblages, along with the contribution levels of the different species at sites SIMPER Analysis [23] was used. The logarithmic transformation \([\log (x+1)]\) was applied to the raw data in the cluster analysis. In this analysis, species contributions to similarity or difference are expressed as \%. These analyzes were performed by PRIMER 6 package programs. According to the results of biotic indices used, the ecological quality status of the study area is evaluated in 5 categories (high, good, moderate, poor, and bad).

**FIGURE 1**
Map showing the sampling sites in the study area

**TABLE 1**
List of sampling sites

<table>
<thead>
<tr>
<th>Sampling sites</th>
<th>Biotopes</th>
<th>Date</th>
<th>Depths (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Muddy sand</td>
<td>8.11.2014</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Sand</td>
<td>8.11.2014</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Sand</td>
<td>8.11.2014</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Mud</td>
<td>8.11.2014</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Mud</td>
<td>9.11.2014</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Sand</td>
<td>9.11.2014</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Mud</td>
<td>9.11.2014</td>
<td>45</td>
</tr>
</tbody>
</table>
RESULTS

As a result of faunistic analysis of macrozoobenthic samples collected from seven different sites in the area to define the ecological quality situation of a marine protection area in Gökova Bay, a total of 8 systematic grubs (Nemertini, Nematoda, Oligochaeta, Polychaeta, Arthropoda, Mollusca, Echinodermata and Cephalochordata) 67 species and 255 individuals were identified (Table 2).

When the systematic groups recorded at 7 sites selected in the study area were compared regarding species and individuals, Polychaetes had

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>List of species found in the study area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>1</td>
</tr>
<tr>
<td>---------</td>
<td>---</td>
</tr>
<tr>
<td><strong>NEMERTINI</strong></td>
<td></td>
</tr>
<tr>
<td>Cereblatulus sp.</td>
<td>-</td>
</tr>
<tr>
<td>Lineus cf. ruber (Müller, 1774)</td>
<td>-</td>
</tr>
<tr>
<td>Tubulanus linearis (McIntosh, 1874)</td>
<td>-</td>
</tr>
<tr>
<td><strong>NEMATODA</strong></td>
<td></td>
</tr>
<tr>
<td>Nematoda (s.p.)</td>
<td>-</td>
</tr>
<tr>
<td><strong>OLIGOCHAETA</strong></td>
<td></td>
</tr>
<tr>
<td><strong>POLYCHAETA</strong></td>
<td></td>
</tr>
<tr>
<td>Arichlidon reyssi (Katzmann, Laubier &amp; Ramos, 1974)</td>
<td>-</td>
</tr>
<tr>
<td>Paralacydonia paradoxa (Fauvel, 1913)</td>
<td>-</td>
</tr>
<tr>
<td>Pilargis verrucosa de Saint Jose, 1899</td>
<td>-</td>
</tr>
<tr>
<td>Prosphaerosyllis marmarae Çinar, Dagli &amp; Açik, 2011</td>
<td>-</td>
</tr>
<tr>
<td>Sphaerosyllis hystrix Claparède, 1863</td>
<td>-</td>
</tr>
<tr>
<td>Sphaerosyllis pirifera Claparède, 1868</td>
<td>-</td>
</tr>
<tr>
<td>Syllis garciai (Campoy, 1982)</td>
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</tr>
<tr>
<td>Leonnates persicus Wesenberg-Lund, 1949</td>
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</tr>
<tr>
<td>Glycera alba (O. F. Müller, 1776)</td>
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</tr>
<tr>
<td>Glycera fallax Quatrefages, 1850</td>
<td>-</td>
</tr>
<tr>
<td>Micronephthys stammeri (Augener, 1932)</td>
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<tr>
<td>Nephtys kersivalensis McIntosh, 1908</td>
<td>-</td>
</tr>
<tr>
<td>Lumbrineris gedilayii Carrera-</td>
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<tr>
<td>Protodorvillea kefersteini (McIntosh, 1869)</td>
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</tr>
<tr>
<td>Cirrophorus branchiatus Ehlers, 1908</td>
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<tr>
<td>Drilonereis filum (Claparède, 1868)</td>
<td>-</td>
</tr>
<tr>
<td>Armandia polyophthalma Kükenthal, 1887</td>
<td>-</td>
</tr>
<tr>
<td>Owenia fusiformis Delle Chiaje, 1844</td>
<td>-</td>
</tr>
<tr>
<td>Lagis koreni Malmgren, 1866</td>
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### Sampling Sites

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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Di%</th>
<th>F%</th>
</tr>
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<tbody>
<tr>
<td><strong>Euchone limnicola</strong> Reish, 1959</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.39</td>
<td>14.3</td>
</tr>
<tr>
<td><strong>Jasminiera caudata</strong> Langerhans, 1880</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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### ARTHROPODA

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<th>7</th>
<th>Di%</th>
<th>F%</th>
</tr>
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<tbody>
<tr>
<td>Cyprideis torosa (Jones, 1850) Jones, 1857</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.78</td>
<td>14.3</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.39</td>
<td>14.3</td>
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<tr>
<td>Caprella sp.</td>
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<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>14.3</td>
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<td>Chondrochelia savignyi (Kroyer, 1842)</td>
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<td>-</td>
<td>1</td>
<td>-</td>
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<td>-</td>
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<td>28.6</td>
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### MOLLUSCA

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<th>6</th>
<th>7</th>
<th>Di%</th>
<th>F%</th>
</tr>
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<tbody>
<tr>
<td>Nerita sanguinolenta Menke, 1829</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>14.3</td>
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<td>Smaragdia viridis (Linnaeus, 1758)</td>
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<td>-</td>
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<td>-</td>
<td>1.18</td>
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<tr>
<td>Tectonatica sagraiana (d’Orbigny, 1842)</td>
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<td>-</td>
<td>-</td>
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<td>Mangelia costata (Pennant, 1777)</td>
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<tr>
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### ECHINODERMATA

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<th>7</th>
<th>Di%</th>
<th>F%</th>
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</thead>
<tbody>
<tr>
<td>Psammechinus microtuberculatus (Blainville, 1825)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>1.96</td>
<td>14.3</td>
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<tr>
<td>Ophiothrix fragilis (Abildgaard, in O.F. Müller, 1789)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.18</td>
<td>28.6</td>
</tr>
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</table>

### CEPHALOCHORDATA

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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Di%</th>
<th>F%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branchiostoma lanceolatum (Pallas, 1774)</td>
<td>11</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.84</td>
<td>28.6</td>
</tr>
</tbody>
</table>

Di%: dominance, F%: frequency.

**FIGURE 2**

The number of species (A) and individuals (B) belonging to systematic groups.
the highest number of species (47 species) and individuals (171 individuals). This group was followed by Arthropoda by 6 species and 14 individuals, Mollusca by 6 species and 10 individuals, and Nemertini by 3 species and 3 individuals, respectively. Other groups (Nematoda, Oligochaeta, Echinodermata, and Cephalochordata) having low number of species and individuals were represented by 5 species and 57 individuals in total (Figure 2A, B).

Sampling site 1, had the highest number of species by 27 species when the distribution of species found at sites with muddy, sandy and muddy-sandy bottom structures were analyzed. Site 1 was followed by site 5 with 21 species and site 4 with 16 species. Other sites had 4 to 12 number of species (Figure 3A). The maximum number of individuals (78) was found at site 1 and this site was followed by site 2 by 54 specimens. The least number of individuals (9) were found at site 6 and the number of individuals at other sites varied between 10 and 44 (Figure 3B).
**FIGURE 5**
Distribution of the species in 3 frequency index groups

**TABLE 3**
Diversity, AMBI, BENTIX, MEDOCC and TUBI index values with the number of species and individuals found at sites.

<table>
<thead>
<tr>
<th>Station</th>
<th>S</th>
<th>N</th>
<th>H'</th>
<th>EQR</th>
<th>EQS</th>
<th>AMBI</th>
<th>EQR</th>
<th>EQS</th>
<th>BENTIX</th>
<th>EQR</th>
<th>EQS</th>
<th>BENTIX</th>
<th>EQR</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>78</td>
<td>4.27</td>
<td>0.85</td>
<td>High</td>
<td>1.83</td>
<td>0.31</td>
<td>Good</td>
<td>4.52</td>
<td>0.75</td>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>54</td>
<td>3.00</td>
<td>0.60</td>
<td>Good</td>
<td>2.00</td>
<td>0.33</td>
<td>Good</td>
<td>4.00</td>
<td>0.67</td>
<td>Good</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>28</td>
<td>3.17</td>
<td>0.63</td>
<td>Good</td>
<td>2.38</td>
<td>0.40</td>
<td>Good</td>
<td>3.67</td>
<td>0.61</td>
<td>Good</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>43</td>
<td>3.57</td>
<td>0.71</td>
<td>Good</td>
<td>2.00</td>
<td>0.33</td>
<td>Good</td>
<td>4.13</td>
<td>0.69</td>
<td>Good</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>32</td>
<td>4.20</td>
<td>0.84</td>
<td>High</td>
<td>2.21</td>
<td>0.37</td>
<td>Good</td>
<td>3.71</td>
<td>0.62</td>
<td>Good</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>9</td>
<td>1.66</td>
<td>0.33</td>
<td>Poor</td>
<td>2.63</td>
<td>0.44</td>
<td>Good</td>
<td>3.00</td>
<td>0.50</td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>10</td>
<td>2.45</td>
<td>0.49</td>
<td>Moderate</td>
<td>2.00</td>
<td>0.33</td>
<td>Good</td>
<td>4.00</td>
<td>0.67</td>
<td>Good</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Station</th>
<th>S</th>
<th>N</th>
<th>MDDOCC</th>
<th>EQR</th>
<th>EQS</th>
<th>TUBI</th>
<th>EQR</th>
<th>EQS</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>78</td>
<td>2.44</td>
<td>0.41</td>
<td>Good</td>
<td>4.08</td>
<td>0.82</td>
<td>High</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>54</td>
<td>2.67</td>
<td>0.44</td>
<td>Good</td>
<td>3.08</td>
<td>0.62</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>28</td>
<td>3.17</td>
<td>0.53</td>
<td>Good</td>
<td>3.04</td>
<td>0.61</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>43</td>
<td>2.67</td>
<td>0.44</td>
<td>Good</td>
<td>3.52</td>
<td>0.70</td>
<td>Good</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>32</td>
<td>2.86</td>
<td>0.48</td>
<td>Good</td>
<td>3.65</td>
<td>0.73</td>
<td>Good</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>9</td>
<td>3.50</td>
<td>0.58</td>
<td>Moderate</td>
<td>2.20</td>
<td>0.44</td>
<td>Moderate</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>10</td>
<td>2.67</td>
<td>0.44</td>
<td>Good</td>
<td>2.81</td>
<td>0.56</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

(S, the number of species; N, the number of specimen; H', Diversity Index; EQS, Ecological Quality Situation; EQR, Ecological Quality Ratio)

**Cirrophorus branchiatus** was the dominant species by a value of 9.41% when the dominance values of the species were considered. **C. branchiatus** was followed by **Branchyostoma lanceolatum** with a dominance value of 7.84%, Oligochaeta (sp.) with 7.06%, Micronephthys stammeri with 6.27%, Prionospio paucipinnulata with 5.88%, and Protodorvillea kefersteini with 4.71%. Other 61 species had a dominance value of 58.82% (Figure 4).

According to Soyer [20], 70.1% of recorded species are rare, 25.4% common, and 4.5% constant (Figure 5). The species in the constant category are **Cirrophorus branchiatus**, Micronephthys stammeri and Nematoda (sp.). Common species are Aricidea (Aricidea) pseudoarticulata, Aricidea (Aricidea) wassi, Protodorvillea kefersteini, Branchyostoma lanceolatum, and Oligochaeta (sp.).

**BIOTIC INDEX**

The values of diversity, Bentix, Ambi, Medocc, and Tubi index of sampling sites were given in Table 3.

According to the ecological analysis results, the diversity index was very good in 2 of 7 sampling sites, good in 3, moderate in 1, and poor in 1. While AMBI values were good at all sites, the values of MEDOCC were good at 6 sites, and moderate at 1 site. BENTIX values were high at 1 of 7 sites, good at 5 sites, and moderate level at 1 sampling point. TUBI values were very good at 1 sampling site, good at 4 sites, and moderate at 2 sites (Table 3).

As the highest diversity index value (H'=4.27) was at site 1 and the lowest value (H'= 1.66) was observed at sampling point 6 (Figure 6A). When the benthic Ecological Quality Situation (EQS) at other sites was examined in terms of diversity index
value, 29% of all sampling sites (2 sites) were at "high" (clean) level in terms of benthic quality status (Figure 6A). The other 5 sites had benthic qualities in the "good" (less dirty) level. BENTIX values at stations ranged from 3.00 (site 6) to 4.52 (site 1) (Figure 6D). According to these results, 14% of the sampling sites, about benthic ecological quality, were "high", 72% "good", and 14% "moderate". AMBI values at all sites were lower than 3 (Figure 6E). The highest AMBI value was recorded at site 6 as 2.63, while the lowest AMBI value (1.83) belonged to site 1. According to the results cited above, all of AMBI values presented for all study area showed a "good" benthic quality status. MEDOCC values were below 3.2 for all sites excluding site 6 (Figure 6C). The highest MEDOCC value (3.50) was presented for site 6, while the lowest value (2.44) was for site 1. These results showed that MEDOCC values were in good "benthic quality" status at all sites except site 6 (at level "moderate benthic quality") represented by a few species. TUBI values in the samples collected from the study area varied between 2.20 (site 6) and 4.08 (site 1) (Figure 6B). 71.4% of the samples had the highest TUBI values (TUBI≥3).

A cluster analysis based on the Bray-Curtis similarity index was used to define the species associations between the sampling sites (Figure 7). The similarity between site, 3 and site 5 which formed the first group (group A) was 20%, approximately. The other two sites (1 and 2) in the study area had a similarity of 40% as group B. Similarity between sampling site 6 and sites 7 was about 25% and these three sites constituted group C. Besides, the lowest similarity value was at site 4.

FIGURE 6
Diversity (A), Tubi (B), Medocc (C), Bentix (D) and Ambi (E) values found at the sampling sites. The Ecological Quality Situation (EQS) of the sites were shown in different colors.
According to the results of SIMPER Analysis, the most significant contributors to the similarity in group A were Nematoda (sp.) (Contribution: 4.5%), Heteromastus filiformis (contribution: 4.0%) and Stenothe sp.; In group B, the species with the highest contribution were Ophiotrix fragilis (10.0%), Aricidea wassi (9.0%), Prionospio maciolacae (7.5%), and Lumbrineris latreilli (5.5%) (Table 4).

A total of 3 exotic species and 18 specimens of these 3 species were recorded in this study. One (Leonnates persicus) of the exotics was transported via the Suez Canal and two of them (Prionospio depauperata, Prionospio paucipinnulata) via ballast water of the ships to the Mediterranean.

**CONCLUSIONS**

A total of 67 species belonging to 8 systematic groups (Nemertini, Nematoda, Oligochaeta, Polychaeta, Arthropoda, Mollusca, Echinodermata and Cephalochordata) were recorded as a result of the benthos samples collected from 7 different sites in Ören Area, Gökova Bay (the southeastern Aegean Sea). 3 of 67 species (Neptys kersivalensis, Chaetazone elakata, and Euchone limnicola) are new for Turkish Fauna and 3 (Paradoneis ilvana, Terebellides mediterranea, and Jasmineira caudata) for the coast of Turkey’s Aegean Sea.

Sampling site which had the highest number of species (28) and the specimens (78) during the study was site 1. This can be an indication that the biological diversity in the region is quite high. Biotopes with muddy sand known as the transition zone (ecoton) between sandy and muddy biotopes, are rich in terms of number of species since they contain species from both adjacent biotopes [24]. This aspect can clarify the reason why there were more species and individuals at site 1 than the other sites.

Group, polychaeta, represented by the highest number of species and specimens in the study area is known to show dense populations on muddy sandy bottoms [25–26]. If there is large amounts of organic matter in any region, abundance of polychaete increases (approximately, 90-100% of the
total zoobenthos), and this group of zoobenthos is known as a significant indicator of ecological status [26]. Table 2 shows that Neanthes caudata, Malaco- 
coceras fuliginosus, Capitella capitata, Polydora cornuta, Hydrodoides elegans, Hydrodoides dianthus, 
Oxydromos pallidus, Schistomerings rudolphi, and Corbula gibba known as pollution indicators 
[26–33]; were not recorded in the study area. The observation of Heteromastus filiformis which is a 
pollution indicator at a single site (site 5) showed that there was no significant pollution suppression 
in the environment. Additionally, Syllids’s records which are quite sensitive to pollution showed that 
the region had a clean environment.

Diversity of arthropods and molluscs were low numerically in the study area where was unusual. Low diversity can be due to the existing biotope structure of the study area. Also, van Veen grab may not have effectively used at the sites with sandy-gravel bottoms and thereby, a standard amount of bottom sample may not have been taken.

In this study, diversity values recorded for several sites were under 3 (H = 1.66 for site 6; H = 2.45 for site 7). If the diversity index values at the sites with deeper bottoms are under 3 and 1.5 in the at shallow waters, a stress factor can be mentioned on the benthic communities in the studied area. Nevertheless, no environmental pressure was observed at the sites 6 and 7 and this difference can be caused by the bottom structure.

Five different indices such as diversity index, bentix, ambi, medocc, and tubi were used to determine the benthic quality status at the sampling sites of the study area and according to the results of the indices, the sites were classified as “high”, “good”, “moderate”, “poor”, “bad”. The index results showed that the benthic quality of several sites were at level of moderate.

Since there was no ecological predominance on the region selected in Gökova Bay and none of the sites were condition in bad ecological condition, we can conclude that the area studied is ecologically in good or very good.

REFERENCES


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EFFECT OF VARIOUS MICROORGANISMS ON NITROGEN CONSERVATION IN ORGANIC WASTES COMPOSTING

Xiaoli Xiong, Ning Li*, Xiaohan Zhang, Sheguang Ding, Shengming Chen
College of Environment and Resource, Chongqing Technology and Business University, Chongqing 400067, China

ABSTRACT

Thermophilic compost is an effective strategy to recycling use of ever-increasing organic wastes, however, a large proportion of nitrogen species (N) can be lost via ammonia (NH₃) volatilization during composting. The study aimed to examine the effects of the acetic acid bacteria (AAB), compound microbes (CM) and the synergetic effect of the two microbes (CM+AAB) on the total nitrogen loss (TN) during composting of organic wastes. Results showed that the CM, AAB, and CM+AAB synergetic treatments reduced the loss of nitrogen by approximately 39.29%, 11.57%, 13.24%, respectively. The germination indexes of CM were maximum in the three treatment groups. Effects of different C/N ratios on the nitrogen conservation during composting were evaluated. Generally, the most effective strategy for reduction of nitrogen losses in composting of organic wastes was to add CM into the compost materials and to adjust the C/N ratio to 20.

KEYWORDS:
Organic wastes, composting, nitrogen fixation, microorganism

INTRODUCTION

Organic wastes, such as food scraps, yard wastes, agricultural wastes, and process residues, account for the largest proportion (46%) of the overall generated solid wastes [1]. With increasing global population, urbanization and a shift in consumption behavior, large quantities and various types of organic wastes were generated from all walks of life [2]. Especially to meet the growing global demand for protein, poultry industry has developed rapidly [3]. It was estimated that the Australian poultry industry produced 1.9 million tonnes of litter in 2010 [4]. In 2015, the production of poultry manure was estimated at 5 million tons in Poland [5]. According to a report, 5 million tonnes of wastes were produced annually across the Russian Federation [6]. These organic wastes which stored in uncontrolled conditions were currently generating the environmental pollution risks, such as soil pollution, water eutrophication, and the emission of odor, nitrogen-based compound and methane [7].

Composting manure has been recognized as one of effective ways to partially solve organic wastes. Composting is a biological stabilization process that can transform the organic fraction of the wastes into a stable and sanitized final product [8, 9], and it is also an effective and reliable treatment technology of organic wastes due to the low operating costs and high social and environmental benefits [8, 10, 11]. However, gaseous emission during composting poses a serious problem, which not only causes serious atmospheric pollution, but also reduces the nutrition of compost. During composting, nitrogen loss is mainly caused by microbial activity, which involved in decomposition of nitrogenous material, nitrification, and denitrification [12]. In the early stage of composting, the loss of N occurs mainly via ammonia (NH₃) volatilization [13]. The high temperature and the high pH values affected NH₃ emissions. Ogunwande et al. [14] found that up to 50-88% of total nitrogen (TN) in poultry manure could be lost as NH₃ during composting. Studies have shown that 9.6-50% of TN in the raw material was released in the form of gases [15].

There had been several strategies to reduce nitrogen loss during the composting of organic wastes, e.g. physical method, chemical method and microbiological method. Biochar addition to composting hen manure and barley straw at low flow rates proved effectively in reducing cumulative NH₃ loss [16]. Madrini et al. [17] reported that natural zeolite reduced the nitrogen loss of 88-89% in comparison with the control group in the food waste compost. Dias et al. [7] found that the addition of biochar made by Eucalyptus grandis significantly decreased nitrogen loss during poultry manure composting. Meng et al. [18] demonstrated that sucrose was better than glucose and starch to be extra carbon sources to reduce nitrogen loss during sewage sludge composting. In the manure composting, the addition of 1.2 % copper complex compound had a positive effect on nitrogen fixation [19]. By adding phosphate to the manure composting process, Zhang et al. [20] have found that the ammonia emissions decreased by 34.7%. Luo et al. [21] considered that addition 10% phosphogypsum (dry weight) in pig manure with cornstalks composting was effective for
reducing NH₃ emission. However, the physical method has some limits and shortages, such as non-recovery, high cost and limited adsorption capacity [22]. Chemical method has also some shortages, such as high cost, complex reaction, and easy production of secondary pollution. Microbiological method (inoculation of biological bacteria) is as the main technology for reducing nitrogen loss and improve compost product quality in the process of organic waste composting, because it is simple, feasible, inexpensive, non-polluting and efficient [23, 24]. The addition of domesticated cultivation ammonia-oxidizing bacteria inoculation into rice straw and chicken manure composting could ensure the nitrogen fixation and provide several benefits [23, 24]. The specific function of inoculating actinobacteria agent could improve the efficiency of compost [25].

This study was undertaken with the aim to (1) investigate the effects of different microorganisms under the same carbon nitrogen ratio on TN loss in composting, (2) and determine the optimal C:N ratio for specified microorganisms to minimize nitrogen loss. The effects were evaluated based on TN loss rate (T), TN loss reduction rate (L) and seed germination index (GI). This research will help to provide a reasonable method to reduce TN loss in composting.

MATERIALS AND METHODS

Composting materials. Frass of *Tenebrio molitor* and chicken manure were prepared as the main materials, and sawdust was used to mix with the manure to adjust the carbon to nitrogen (C/N) ratio. All wastes were collected from a local farm (Chongqing Bole, China). The initial parameters of the three materials were shown in Table 1.

In the compost process, two kinds of microorganisms, acetic acid bacteria (AAB), compound microbes (CM, mixed by product ester yeast, saccharomyces cerevisiae and molds; bacterial strain ratio, 1:1:1), were inoculated. Those microorganisms were obtained from a local biotech company (Henan Hebi, China). Main bacterial strain content: about 10×10⁸ CFU/g dry matter.

Compost process and sample collection. The experiments were divided into two stages. The stage 1 was to add different microorganisms with the same compost materials. The C/N ratios of 20:1 compost materials were obtained by mixing sawdust, frass and manure. The mixtures were treated separately by the following methods: A1 group, the mixtures without the addition of CM and acetic acid bacteria (as control group, PS); A2 group, the mixtures with the addition of 0.5% of CM (PS + 0.5% CM); A3 group, the mixtures with the addition of 0.5% of acetic acid bacteria (PS + 0.5% AAB); A4 group, the mixtures with the addition of 0.5% of CM and 0.5% of acetic acid bacteria (PS+0.5% CM+0.5% AAB). Base on the results of the stage 1, the experiments of the stage 2 were to optimize combination by changing the C/N ratio under the same conditions. The treatment groups B1-B5 represented the C/N ratio 15, 20, 25, 30 and 35, respectively.

Compost materials were put in special small rectangle compost reactor with size of 24cm ×14cm ×17cm. Total 2.5 kg raw compost materials were mixed in the reactor. Water was added to achieve an initial moisture content of 60% (w/w) of the compost mixture. To avoid anaerobic compost, the mixtures were turned upside down manually every 3 days. The sampling and weighing time was specified at the beginning and termination of the compost process. Each one was repeated three times in parallel. Solid samples (a total of approximately 50g) were collected at the center and four corners of the compost vessel by five-point sampling method.

Analytical methods. A temperature sensor connected to a data logger was inserted into the composting vessel to automatically record the composting temperature per half an hour. Moisture content of the samples (W) was determined by moisture meter (Sartorius, MA35, Germany). Some fresh samples were directly used for the determination of NH₄⁺-N, NO₃⁻-N, TC, TN and GI.

Ammonium nitrogen and nitrate nitrogen. Based on indophenol blue colorimetric method, ammonium nitrogen (NH₄⁺-N) was extracted from the fresh samples with 2 mol/L KCl solution at 200 rpm shake for half an hour. After filtering, ammonia nitrogen content was determined by absorbance at 625 nm with a UV spectrophotometer (Techcomp, UV1102, China). And nitrate nitrogen (NO₃⁻-N) was extracted with 25% HCl solution at 200 rpm shake for half an hour, then the nitrate nitrogen content was determined by absorbance at 210 nm.

### TABLE 1

Parameters of composting materials*

<table>
<thead>
<tr>
<th>Material</th>
<th>TN (g·kg⁻¹)</th>
<th>TC (g·kg⁻¹)*</th>
<th>C/N</th>
<th>Water content (wt %)</th>
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<tbody>
<tr>
<td>Frass</td>
<td>48.74±1.02</td>
<td>619.24±1.15</td>
<td>12.70</td>
<td>14.96±0.88</td>
</tr>
<tr>
<td>Chicken manure</td>
<td>32.64±0.86</td>
<td>428.32±1.23</td>
<td>13.12</td>
<td>15.79±0.69</td>
</tr>
<tr>
<td>Sawdust</td>
<td>1.62±0.39</td>
<td>971.35±1.75</td>
<td>600.48</td>
<td>22.17±0.92</td>
</tr>
</tbody>
</table>

*Mean value and standard error are shown (n = 3); *TC- total carbon, TN - total nitrogen.
TN and TC. TN was measured by Kjeldahl method. Fresh samples (about 0.5 g) were mixed with CuSO4·5H2O (0.1g), K2SO4 (3 g) and H2SO4 (10mL) to digest in a graphite digestion instrument (Halon, SH420F, China). Then the Kjeldahl nitrogen meter (Halon, K1100F, China) was used to determine TN content after the samples in the digestive tube were cooled. Determination of TC content was tested by the potassium dichromate (K2CrO7) titration according to the Chinese national standard (NY 525-2012). The TN loss rate (T) could be calculated according to Eq. 1.

\[
T_i(\%) = \left(1 - \frac{N_i \times M_i}{N_0 \times M_0}\right) \times 100\%
\]  

Where, M0 and Mi (kg) represent the initial and the final compost dry mass, N0 and Ni (g·kg⁻¹) represent TN content in initial and final compost dry mass, i represents the serial number of different treatments.

The TN loss reduction rate (L) could be calculated according to Eq. 2.

\[
L_i(\%) = \left(1 - \frac{T_i}{T_{ck}}\right) \times 100\%
\]  

Where, CK represents the control group, T represents TN loss rate.

Seed germination index (GI). The seed germination test was broadly used to evaluate compost quality and assess the toxicology of sample [26, 27]. The sample and water were mixed by the solid-liquid ratio at 1:10, 1:20 and 1:30 (w/v), respectively. After stirring for 5 min and standing for 30 min, the supernatant was collected to determine GI. 25 mung bean seeds were evenly distributed on filter paper in Petri dishes (diameter 10 cm) and moistened with 10 mL compost extract (deionized water as a control group). The dishes for each sample were incubated at 27°C for 96 h. Each sample under the same conditions was measured three times in parallel, and the average value was calculated. The number of germinating seeds and the root length were measured and GI values were calculated according to Eq. 3.

\[
GI(\%) = \left(\frac{S \times l}{S_{ck} \times l_{ck}}\right) \times 100\%
\]  

Where, $S$ and $S_{ck}$ are the average germination percentage of the experimental group and the control group, respectively; $l$ and $l_{ck}$ are the average length of seedling in the experimental group and the control group, respectively.

Statistical analysis. Three parallel samples were taken in each experiment group and the results were shown as mean ± standard deviation. Origin 9.0 software was used for all data analyses.

RESULTS AND DISCUSSION

Compost temperature. Pile temperature is one of the most principal parameter of compost, which can indicate the performance, microbial activity, metabolic intensity and the degree of maturity of compost. The changes in the compost temperature for two different composting experiments are shown in Fig. 1. The temperature of those treatments had similar variation with the curve of typical temperature of compost: (i) mesophilic phase: where mesophilic bacteria and fungi degrade simple compounds; (ii) thermophilic phase: aerobic degradation of the organic matter; (iii) cooling phase and (iv) mature phase [28]. Due to the small scale of the experiment, composting thermophilic phase lasted only about 30 h. However, regardless of the addition of bacteria, all the composting treatments increased quickly after 20 h and reached thermophilic temperature (>50°C), which ensured deactivation of potentially pathogenic microorganisms. In Fig. 1a, the pile temperature of A3 (PS+AAB) was always higher than that of other three groups during the mesophilic and thermophilic phases. During compost, the compost materials were...
turned pile when temperature showed a decreasing tendency. In Fig. 1b, the temperature of B4 (C/N=30) was generally lower than that of the other groups in the first 40 h, then it reached the highest peak value of 63.4°C after turning pile. At the later period of the compost process, the temperature gradually decreased to ambient levels and this marked the beginning of the mature phase of compost.

**Different inoculating microbes. Nitrogen conservation effect.** The extractable nitrogen (NH$_4^+$-N and NO$_3^-$-N) contents are shown in Fig. 2. The changes of NH$_4^+$-N was in accordance with the pattern tendency during composting process. The content of NH$_4^+$-N in the initial state was low, then the NH$_4^+$-N content of all piles were rapid increased during thermophilic phases, due to the fact that ammonia-oxidizing bacteria (AOB) transformed organic nitrogen into ammonia nitrogen. The peak value of ammonia nitrogen in the A4 group (PS+CM) was shifted behind the other groups due to thermophilic phases behind other groups (Fig. 2a). Nitrate nitrogen content showed a downward tendency in the process (Fig.2b), which is most likely derived from the inhibition action of AAB and CM to AOB reduced the rate of nitrification directly [23, 24].

The transformation sequence of nitrogen was ammonification (decomposition of nitrogenous organic matters), nitrification (transformation from NH$_4^+$-N to NO$_3^-$-N), denitrification (NO$_3^-$-N reduction to N$_2$) and assimilation (NH$_4^+$-N is transformed into organic nitrogen via microbial action) [29, 30]. Mixing manure with inoculating microbes reduced the total N loss from 32.59% for A1 (CK) to 19.79%, 28.82% and 28.28% for A2, A3 and A4, respectively (Fig. 3). The fact showed that all three treatments in favor of reduction of nitrogen loss. Especially, TN loss reduction ratio (L) reached 39.29%. CM might play a vital role in the process of ammonification or denitrification for decreasing nitrogen loss through inhibiting NH$_3$ or N$_2$ volatilization.

**Seed germination index.** Seed germination index (GI) was an important prediction parameter for evaluating the maturity of compost and the toxicity

![FIGURE 2](image2.png)

**FIGURE 2**
Changes of (a) NH$_4^+$-N and (b) NO$_3^-$-N of four piles during composting

![FIGURE 3](image3.png)

**FIGURE 3**
TN loss ratio (T) and TN loss reduction ratio (L) changes during the compost.
A1: PS; A2: PS+CM; A3: PS+AAB; A4: PS+CM+AAB

![FIGURE 4](image4.png)

**FIGURE 4**
Seed germination index of different inoculating microbes.
A1: PS; A2: PS+CM; A3: PS+AAB; A4: PS+CM+AAB.
FIGURE 5
TN loss ratio (T) tendency during the compost of mixtures with different C/N ratios.
B1- B5: C/N=15, 20, 25, 30 and 35, respectively.

FIGURE 6
Seed germination index changes at different concentration levels.
B1- B5: C/N=15, 20, 25, 30 and 35, respectively.

of the product for the plant. If GI value was less than 50% compared with the control group, it could be considered that the fertilizer was not completely decomposed and had phytotoxicity. GI value of the high compost maturity should be more than 80% [31]. As shown in the Fig. 4, the GI differences between the treatments at three concentrations were quite pronounced. When the ratio of solid to liquid was 1:10, GI value was generally lower than the other two, which was attributed to the high nitrogen content in the raw materials and the high concentration of nutrient substance in the filtrate to inhibit the growth of plants. For the ratio of solid to liquid 1:20, the difference in the GI value of A2-A4 was not obvious. The GI value of A2, highest in the four treatments, exceeded 50% (at 1:10 and 1:30), demonstrating that it was non-toxic to plants.

Base on the analysis and discussion above, A2 group (add CM into the compost materials) was selected as following experiments.

Different C/N ratio. Nitrogen conservation effect. Nitrogen loss rate (T) increased with increasing C/N ratio, reaching a peak (25.31%) at a C/N ratio of 30 and then began to decline (Fig. 5). So C/N ratio was very important factor for nitrogen conservation of compost.

Seed germination index. The B1 group (C/N=15) had the lowest GI value in all three-concentration levels of 5 different treatments (Fig.6), which meant that the low C/N ratio had a high nitrogen fixation rate, but not suitable for plant growth. Generally, the most effective strategy for reduction of nitrogen losses in composting of organic wastes was to add CM into the compost materials and to adjust the C/N ratio to 20.

CONCLUSIONS

In this study, we investigated the effect of the acetic acid bacteria (AAB), compound microbes (CM), the synergetic effects of the two (AAB+CM), and C/N ratio on nitrogen loss during organic waste composting. Composting materials of addition CM resulted in a decrease in TN loss reduction rate (39.29%) compared with the control group. CM was more efficient than AAB and AAB+CM in reducing TN loss. CM could reduce TN loss by 32.59% to 19.79% in mesophilic composting. The C/N ratio of 20 was proved to be the most favorable condition in the nitrogen conservation effect in the organic waste composting of adding CM. On the basis of the results of this study, CM has the potential in compost to reduce nitrogen loss, improve the nutritional value of the compost product, and increase economic and environmental benefits.

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GENOTOXICITY OF MUSSEL EXTRACTS COLLECTED FROM RED SEA COASTS IN SAUDI ARABIA ON HUMAN CULTURED LYMPHOCYTE CELLS

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ABSTRACT

The present study investigated the cytotoxic and genotoxic effects of mussel extracts in human peripheral lymphocytes. The mussels were obtained from three sites in Red Sea coasts of the KSA. The sampling areas receive industrial effluents with different degrees of contamination. The study was performed using two mutagenic biomarkers: the micronucleus test (MN test) and the Halo test (apoptotic frequency). After 72h of exposure to five mussel extract doses, significant dose-dependent relationships were observed. The predominant effects registered were the induction of MN, the increase in cell apoptosis frequencies and the decrease in cell division rates. The MN mean frequencies and apoptosis frequencies registered ranged from 4 to 67% and from 2 to 42%, respectively. The mussel extracts collected from the industrial area induced significant genotoxic effects in human lymphocytes even with a low dose (100 μL). This result might suggest the presence of potentially mutagenic substances in this area.

KEYWORDS:
Genotoxicity, Micronucleus test, Apoptosis, Mussel extracts, Red Sea, Saudi Arabia.

INTRODUCTION

Along the Red Sea coasts, the principal threats to marine ecosystems are human land-based activities. These include urbanization and coastal development industries, refineries, quarrying activities and oil manufacturers. Yanbu is a Red Sea coastal city known for the large number of coastal construction projects (recreational facilities, hotels and restaurants) which have been built in the last few years. The major industrial activities (petrochemical and power plants, oil refineries, food industry) are located in Yanbu Al-Sinayah. As a consequence, severe risks and significant destruction of marine ecosystems might be detected [1]. Furthermore, a power and desalination complex was implanted in Yanbu to provide drinking water for the general population and the industrial facilities. This complex could have substantial impacts on marine ecosystems due to thermal pollution and to the elevated levels of salt and chlorine in the returning waters [2].

Marine ecosystems and key habitats may also be widely damaged by suspended fine materials and pollutants ensuing from the different coastal activities. Moreover, the sedimentation of these polluting materials might cause the suffocation of marine species, especially benthic communities (sea grass beds, coral reefs and mangroves), and might have adverse effects on the surrounding ecosystems. Consequently, the productivity of marine ecosystems would decline [3, 4].

The chemical analysis of environmental pollutants provided limited information on substances present in the environment, and no data was provided on the relationship between the contaminant exposure and the biological effects in aquatic organisms [5]. In environmental studies, in situ bio-monitoring was considered as one of the direct approaches for environmental investigations. The response of this approach was observed directly on the exposed organisms [6, 7]. Several studies have already identified, detected and quantified these toxins in bivalve mollusks [8-11].

Recently, studies of marine toxins released from industrial polluting discharges into the environment have become considerably important. Shellfish, mussels in particular, are well-suited for biomonitoring studies since they are mostly sessile, widely distributed, easily handled and relatively tolerant to xenobiotics [12]. The mechanisms of pumping and filtering of these organisms means they can concentrate various water compounds that the consumer eats in turn.

Mutagenic effects are considered the most worrisome, as well as the potential dangers of chemicals in seafood on human health, if one considers the consequences of a mutation, either on chromosomes or on the genes [13, 14].

The assessment of the genetic damage caused by several environmental contaminants could be conducted through the application of the micronucleus test. This assay consists in counting cells with one or more cytoplasmic nuclei of reduced size associated with the main nucleus. The MN arises when complete chromosome or chromosomal fragments
fail to incorporate into the daughter cell nucleus during the cell division and remain in the cytoplasm throughout the life cycle of the cell [15].

The present study aimed to investigate the genotoxic effects of mussel extracts on cultured human lymphocyte cells using the apoptosis frequencies and the MN assay as bio-monitoring tests for environmental contamination.

**MATERIALS AND METHODS**

**Mussel collection.** Mussel samples (30 mussels) with a mean shell length of $51.6 \pm 2.3$ mm were hand collected during January – March 2015 in Red Sea coasts in Yanbu City. The samples were transported to the Department of Biology in the Faculty of Sciences in Taibah University, Yanbu, in a cooler at 4°C and conserved at -28°C until their analysis.

Three sampling sites were selected according to the pollution levels. The industrial area of Yanbu Al Sinaiya (IA) is located 20 km southeast of Yanbu City. It is the area of all the major refineries and petrochemical installations. The Yanbu Al Bahr (YB) zone is a major Red Sea commercial port in the Al Madinah province of western Saudi Arabia. The natural harbor is protected by the mainland to the northeast and by coral reefs to the southeast. The Yanbu industrial seaport is considered the largest petrochemical, oil and exporting complex on the Red Sea (15 kilometers of coastline). The port consists of 7 terminals with 25 berths, a harbor service (cargo and container handling equipment), and marine support facilities. The port handles crude oil from the Eastern Province delivered through the east-west channels.

The Al Sharam zone (Sh) is located approximately 25 Km from the city center and is considered a touristy area.

**Mussel toxin extraction.** In the laboratory, the fouling organisms were removed from the interior of the shells and cleaned with distilled water. The extraction process was done according to the method described by Yasumoto and Kanno [16] with slight modifications: 10 g of peeled and drained mussels were extracted with 30 mL of heated methanol. The extraction was repeated three times. After filtration and evaporation of the methanol, 5 mL of milliQ water were added to the residue. The extraction was repeated three times with 10 mL of ether. The aqueous phase was extracted 3 more times with 15 mL of a mixture of water-butanol (5v/1v). The butanol phase was evaporated, and the residue was dissolved in cold acetone (4°C). The range of selected doses was chosen according to the induced reduction of cell proliferation; as a consequence, the highest dose tested produced enough high frequencies of binucleated cells to attain the 1000 binucleated cells required for the scoring of each extract dose.

**Micronucleus test.** Blood samples (10 mL) were obtained by venipuncture with heparinized syringes containing sodium as anticoagulant from two healthy, non-smoking female donors aged 28 and 25 years. The samples were taken in the morning of the day of the experiment to avoid possible confusing dietary effect factors. A portion of the blood samples was retained as a control while the rest were exposed to different doses of the mussel extracts.

The micronucleus test was conducted as previously described by Ennaceur et al. [17]. Briefly, human blood cell cultures (duplicate cultures for each donor) were established by adding 0.5 mL of whole blood to 4.5 mL of an RPMI 1640 medium, 15% heat-inactivated fetal calf serum, antibiotics (1% penicillin/streptomycin) and L-glutamine. 1% of phytohemagglutinin (PHA, Gibco) was added for lymphocyte stimulation. The cultures were incubated for a total of 72 h at 37°C. 24h after cell culture, different mussel extract doses were added (50, 100, 200, 400, and 600 µL). The controls used were solutions without mussel extracts. 44h from the start, cytochalasin B (Cyt-B) with a final concentration of 6 µg/mL was added to arrest cytokinesis [18]. The cultures were then centrifuged and treated with KOH at 4°C for 2-3 mins. A second centrifugation was performed and an acetic acid/methanol solution was added (3: v/v). Two slides were prepared for each culture. Slides were stained with Giemsa (10%) for 10 mins, coded and the slide analysis was performed by two different observers.

A total of 1000 binucleated cells were examined for each specimen under the light microscope (Leitz Wetzlar Germany, oil immersion lens, 100/1.25). The criteria for the identification of the micronuclei (MN) used were as described by Fenech [19]: no connection with the main nucleus, same color and intensity as the main nucleus, and an area smaller than one-third of the main nucleus. MN frequency was calculated as follows: MN % = (Number of cells containing micronucleus / Total number of cells counted) x 1000. Additionally, 500 lymphocyte cells were scored (for each mussel extract concentration) to calculate the percentages of cells with one to four nuclei. The cytokinesis-block proliferation index (CBPI) was determined as described by Surralles et al. [20]. To measure the genocytotoxicity of the tested mussel extracts, two parameters were used: the frequency of binucleated cells with micronuclei (BNMN) (biomarker of genotoxicity) and the cytokinesis-block proliferation index (measure of the cytotoxic potential of the tested extracts).

The statistical test applied to determine the statistical differences between the treated cultures and the controls was the one-tailed Fisher exact test. A p-value of ≤ 0.05 was considered as indicative of statistical significance.

**Apoptosis.** The percentages of apoptotic lymphocyte cells were estimated using the method...
described by Singh [21]. Each lymphocyte culture was distributed among seven different slides (5 slides for the different mussel doses, one slide for the control and one slide for the solvent), which were processed as follows: ten 10 µL aliquots of the cell suspension mixed with 85 µL of Low Melting Agarose (LMA—0.7%) in PBS (37 ºC) were added to the coated slides (previously dipped in 1% Normal Melting Agarose). The slides were covered with coverglass and placed at 4 ºC for 40 mins until the agarose layer solidified. A third agarose layer was added to the slides in the same way. After agarose solidification, slides were placed in a lysing solution (NaCl, Na2EDTA, Tris–HCl, Triton X-100 and DMSO) in a staining jar at 4 ºC in the dark for at least one hour. Then, a neutralization fetal buffer was used to wash the slides. Finally, slides were stained, fixed in absolute ethanol and labeled with DAPI (4’,6-diamidino-2-phenylindole). Two different observers then evaluated the slides in order to be carefully coded. The slide observation was done under the fluorescence microscope. 500 cells/slides were analyzed for a total of 1000 cells/cultures. According to the intense autolysis of genomic DNA, apoptotic cells were characterized by nuclear remnants that resemble pinheads and were surrounded by very large DNA halos. Necrotic cells were characterized by circular, faint halos and were not considered in the count.

RESULTS AND DISCUSSION

Human blood lymphocytes were used in the present study since they are easy to collect and to culture. They constitute an excellent model widely used in cytotoxic and genotoxic studies.

No significant genetic effects or mortality were recorded for the cultured lymphocytes in the control solutions. In addition, no statistically significant differences were observed between controls and the solvent solution (p<0.01) for all applied tests.

In general, genotoxic and cytotoxic effects were observed in all the cultures treated with mussel extracts collected from the three studied areas (Figure 1). These effects had different statistical significance and showed a dose-dependent increasing trend (p<0.01). In fact, mussel extracts interfered actively with lymphocyte DNA, as indicated by the high percentage of MN after 72 hours of exposure. Moreover, the mussel extracts obtained from the samples collected in the industrial zone (IA) seemed to act at low doses in human lymphocyte cells. In fact, significant increases in the MN frequency (p<0.01) were observed, mainly for the 100 µL dose (25% in IA; 17% in YB and 11% in the Sh zone).

With the highest dose of 600 µL, clear DNA damage was observed and high frequencies of MN (67% in IA; 57% in YB and 46% in Sh zone) were detected in the lymphocytes when compared to the controls (p<0.01). Furthermore, a dose-dependent increase (p<0.01) was observed in the percentages of apoptotic lymphocyte cells cultured in mussel extracts collected from the three studied sites (Figure 2).

The highest percentage of apoptosis was detected in cultures treated with mussel extracts from the industrial area of Yanbu al Synaia when compared to the other sites (apoptosis frequencies ranged from 8% with a dose of 50 µL to 42% with a dose of 600 µL). It was found that a dose of 200 µL of the extract collected from the IA was capable of producing an apoptosis frequency about seven times higher than the controls and five times higher than the extracts obtained from the other two zones.

Because toxins are found in all aquatic ecosystems, a deep understanding of the risks caused by these chemicals on marine organisms is needed. Nowadays, the application of bio-tests in aquatic ecosystem biomonitoring studies becomes of considerable interest. Many genotoxic studies were performed on marine invertebrates (mainly mussels) to detect aquatic pollution and therefore, possible risks of mutation and cancer.

![FIGURE 1](image)

**FIGURE 1**

Variation of the means of MN frequencies calculated for human lymphocytes for control, solvent and treated samples with mussel extracts collected from the three studied areas (** for p<0.01 and * for p<0.05)
In the present study, the micronucleus test and the Halo assay (apoptotic frequency) were applied to investigate the in vitro effects of mussel extracts collected from three zones in the Red Sea waters on human lymphocytes. It was found that mussel extracts had a clear genotoxic and apoptotic effect when added to lymphocyte cell cultures at moderate doses (100 μL). Similar results were also reported by Nasser et al. [22]. The authors demonstrated that mussel extracts collected from two sites in Morocco were cytotoxic and inhibited cellular macromolecule synthesis. Moreover, they showed that mussel extracts damaged the DNA of human intestinal cells (CaCo-2). Binelli et al. [23] studied the effects of the antibacterial agent Triclosan in hemocytes of the freshwater bivalve Zebra mussel. They found that the tested pollutant caused a significant increase in all of the genotoxic tests performed as well as a clear destabilization of lysosomal membranes, hence demonstrating that the investigated chemical was potentially dangerous for aquatic ecosystems.

In order to assess the effect of mussel extracts on lymphocyte cell division, the cytokinesis-block proliferation index was measured (Figure 3). A decrease in the CBPI was observed depending on the mussel extract dose collected from the three sampling zones. In fact, the decrease in the cell division rate can be easily explained by the elongation of the G2 phase of the cell cycle, which is necessary to repair the damage induced in the DNA.

The comparison of the genotoxic, cytotoxic and apoptotic effects of mussel extracts from the three studied zones showed that the lowest effects were observed at a dose of 100 μL for samples from IA.
This is less evident with the same amount of mussel extract from Yanbu al Balad where a significant high MN frequency (52%), significant high apoptosis effects (36%) and low CBPI (1.2) were observed at a dose of 400 μL. As regards to the al Sharam zone, the cytogenetic effects and the apoptosis frequencies were significant with an extract dose of 600 μL.

These observations could be explained by the presence of toxic compounds in the samples collected from the industrial zone. Yanbu Al Sinaiyya is the most densely populated region and is considered one of the busiest coastal zones in Yanbu City. Its coastal ecosystems have come under intensive stress and pollution due to local pressure and rapid urban development.

The marine pollution of the Yanbu coasts might be the result of industrial contamination by a variety of toxic pollutants, the discharge of local domestic and industrial wastewaters, and the disposal of contaminated discharges from the refineries and petrochemical installations.

Although the three studied sites showed different levels of pollution, the results demonstrated a strong dose-dependent effect. The cytokinesis-block proliferation index, which is a measure of the cell cycle speed, decreased when the dose of mussel extracts increased. The CBPI provided an idea on the average lymphocyte replication depending on the dose administered. The frequencies of cells with a nucleus were directly proportional to the dose, while the frequency of cells with 2, 3 and 4 cores was inversely proportional. Statistical analyses showed a significant dose-dependent effect on the induction of micronuclei formation and cell apoptosis in human lymphocyte cells and a decrease in the rate of cell division. In fact, the present study was able to show, through the use of two genotoxic tests, the presence of potentially mutagenic substances in mussel extracts from Red Sea waters. However, various factors lead to difficulties in the evaluation of the genotoxicity of these substances such as the heterogeneity of the human diet, the low contaminant levels and the instability of some substances.

CONCLUSION

To our knowledge, the present study was the first genotoxic study conducted in a Red Sea coastal city of Saudi Arabia to evaluate the genotoxic and cytotoxic effects of mussel extracts on human lymphocytes. The results described can be used as an indicator of human health risks after consuming contaminated seafood. The present findings highlight the strong and urgent need to conduct a detailed chemical analysis of environmental pollutants and their sources in Red Sea waters and to perform studies on their effects on a longer life expectancy and human health.

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EFFECTS OF GA3 AND H2SO4 PRETREATMENTS ON GERMINATION OF EASTERN STRAWBERRY TREE (ARBUTUS ANDRACHNE L.) SEEDS

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ABSTRACT

This study was carried out to determine the effects of some pretreatments including soaking in 400, 600, 800, 1000 ppm GA3 for 24 hours and concentrate (96%) H2SO4 for 1, 3, 5 and 7 minutes on seed germination and to investigate how to overcome dormancy of Arbutus andrachne L. seeds. The seeds were sown at 24±1°C under darkness in laboratory, at 24±1°C in the greenhouse and under open field conditions. The statistical approach was a randomized complete block design with three replications. Germinated seeds were observed periodically during 90 days to determine germination percentages. The highest germination percentages were obtained from the seeds soaked in 800 ppm GA3 and sown in the laboratory, soaked in 400-800 ppm GA3 and sown in the greenhouse and soaked in 400 ppm GA3 and sown under open field conditions both open field conditions and in the greenhouse with 94.7%, 86.0% and 26.7%, respectively. On the other hand, the lowest germination percentages were obtained from the control seeds which were sown in the laboratory and greenhouse conditions (0.7% and 8.0%) for GA3 treatments. It can be said that the effect of H2SO4 pretreatments on germination of the seeds does not have positively.

KEYWORDS:
Arbutus andrachne, germination, condition, GA3, H2SO4

INTRODUCTION

The genus Arbutus L. includes about 11 species of small trees distributed from the West coast of North America, Central America, the Mediterranean region, Europe, Northern Africa and parts of Middle East [1, 2, 3]. Three species of Arbutus genus are distributed in the Mediterranean region: A. unedo, A. andrachne and their natural hybrid A. x andrachnoïdes [2, 3]. Arbutus andrachne is an evergreen shrub or a small tree with edible fruits. Its fruits are sweet and can be eaten as fresh or dried [4]. It has rich mineral contents such as K, Ca, and P, and sugar in the form of fructose and glucose [5]. Its small trees are usually less than 4 m high, and the wood is used for several purposes including making carved spindles, stools and small furniture [6]. Arbutus andrachne has potential use as a landscape plant because of its ornamental features, which include winter fruiting and smooth, cinnamon-red bark, peeling in long paper strips [7]. Arbutus species could be used in Mediterranean parks and gardens contributing to high biodiversity and sustainability. They could also be used for reforestation in Mediterranean regions because of their potential for sprouting after fires, because they may have their aboveground burnt to ground level without major damage to the roots [7, 8, 9]. In addition, a selection program was carried out with the aim of selecting strawberry tree genotypes of high quality from the native strawberry tree population [10].

Clonal propagation of these species by cuttings is difficult [11, 12]. In the horticultural and forestry practice, the plants are propagated mainly by seed [7]. Seeds of many woody plant species cannot germinate even if they are sown under the optimal moisture, oxygen and soil conditions [13, 14]. This problem is called dormancy and its causes are hard and impermeable seed coat, immature or dormant embryo, absence of endosperm and thick, fleshy seed cover [15, 16]. There is great deal of variation in germination ability of seeds even within the same species. Poulsen [17] reported that dormancy among and within seed lots of the same species varies with provenance, crop year and individual trees.

There is various germination obstacles and rather confusing information concerning the ecophysiology of Arbutus sp. seed germination resulting in propagation difficulties [7, 18, 19, 20, 21]. There have been some studies to determine different methods and techniques to overcome seed dormancy in Arbutus species. Generally pretreatments such as submersion in hot water, mechanical or chemical scarification, and hot aeration are specifically used for seed coat dormancy while the cold and warm stratifications are usually applied to dormancy caused by restrictions at the embryo level [22, 23]. Among these methods and techniques, especially cold stratification, submersion in GAs, KNO3,
H2SO4 and Polystimulin with different doses and durations are well-known ones used to increase germination percentage of Arbutus seeds [3, 24, 25, 26, 27, 28].

The aim of this study was to examine the influence of soaking in GA3 for 24 hours (400, 600, 800, 1000 ppm) and concentrate (96%) H2SO4 (1, 3, 5 and 7 minutes) pretreatments on dormancy of Arbutus andrachne seeds.

MATERIALS AND METHODS

Ripe fruits were collected from Arbutus andrachne individuals in October 2013, in Fistikli-Artvin located in the north eastern part of Turkey (200 m, 41°13’ N, 41°47’ E). The seeds were separated from the fruit material, rinsed with tap water, dried in the shade (24±1°C, 50% humidity) for 2 days and stored at 5±1°C in plastic bags.

The following pretreatment applications were used to determine their effects on germination percentage (GP), and mean germination time (MGT) of A. andrachne seeds:

- Soaking in GA3 for 24 hours (400, 600, 800 and 1000 ppm),
- Submersion in concentrated (96%) sulphuric acid for 1, 3, 5 and 7 minutes,
- Control (no treatment).

After the treatments, the seeds were sown in trays under open field conditions, in the greenhouse (24±1°C, 50% humidity) and in the laboratory (24±1°C, under darkness) at the end of the autumn 2013. The pots used both in the greenhouse and open field were filled with growing medium peat and perlite (3:1). The petri dishes were used in the laboratory condition. The experimental design was a randomized complete block with three replications for each treatment and 50 seeds were sown in each replication. The number of germinated seeds (evaluation done according to ISTA Rules (1993)) were recorded on the 4th, 7th, 10th, 14th and in every week (7 days) after the 14th-day counting. The below formula was used when determining mean germination time [29]:

\[ MGT = \frac{(n_1 \times t_1) + (n_2 \times t_2) + (n_3 \times t_3) + \cdots + (n_i \times t_i)}{T} \]

Where;
- \( MGT \): Germination rate
- \( n \): Number of days for each counting of germinated seeds
- \( t \): Number of germinated seeds at each counting day
- \( T \): Total number of germinated seeds

The whole experiment lasted for 90 days when it was observed that the seeds stopped germinating. Data from the treatments were analyzed by the SPSS statistical software after ar-sinus transformation was applied to GP values to meet ANOVA assumptions. The ANOVA and Duncan tests were used to compare treatment groups to find out whether they showed any statistically significant differences with significance level (α) set at 0.05.

RESULTS AND DISCUSSION

Statistical analyses showed that the highest arithmetically germination percentage (94.7%) for among all the GA3 treatments was obtained from the seeds soaked in 800 ppm GA3 for 24 hours and sown in the laboratory conditions (Table 1). The highest arithmetically germinations were 86% in 600 ppm and 400 ppm GA3 treatment in the greenhouse and 26.7% for 400 ppm GA3 under open field (Table 2 and Table 3). According to Demirsoy et al. [28] and Tilki [26] reported that GA3 pretreatment ranging from 300 to 1200 ppm was successfully overcome dormancy in A. unedo seeds. Tilki and Guner [19] also implied that 200-800 ppm GA3 treatment greatly increased germination percentage of A. andrachne seeds at 24°C in dark. Similar findings were reported by Smiris et al. [24], Pipinis et al. [3] as they found out that Arbutus unedo seeds in GA3 resulted in good germination. The lowest germination percentage (0.7%) was obtained from the control seeds in the laboratory, while the lowest germination percentages were determined from control seeds in the greenhouse and 600 ppm GA3 treatments under open field conditions, respectively 8.0 and 17.7% (Table 1, Table 2 and Table 3). In addition, Olmez et al. [18] reported that low germination percentages in control seeds (1.1-4.2%) and soaked in 250 ppm GA3 for 20 minutes (5.4-15.5%) and sown both greenhouse and open field conditions.

When the H2SO4 treatments for 1 to 7 minutes were considered, while the highest germination percentages were obtained from control seeds under open field and greenhouse conditions, respectively, 25% and 8%, it was 6% for the seeds soaked in H2SO4 for 3 minutes and sown in the laboratory (6%). The better germination results were determined from GA3 pretreatments than H2SO4 all three (laboratory, greenhouse and open field) conditions. It could be said that gibberellins were more effective than concentrated (96%) H2SO4 for good germination results of the seeds. On the other hand, results belong to GA3 pretreatments on seeds before sowing were significantly increased the germination percentages compared to control. We obtained 26.7-94.7% germination percentages from GA pretreatments during germinations (Table 1, Table 2 and Table 3). Olmez and Arslan Olcum [25] implied that the germination percentage were 44.4% and 75.5% in sea buckthorn seeds soaked in H2SO4 for 2 minutes under greenhouse and open field conditions while we could not obtain in good germination results for Arbutus andrachne seeds. According to McDonald [21], some Arbutus menziesii seeds did not germinate easily unless the strong embryo dormancy was broken by cold stratification. It can be
TABLE 1
Results of Statistical Analyses Showing the Relationship of the GP and MGT with Different Treatments in the Laboratory

<table>
<thead>
<tr>
<th>GA3 Pretreatments</th>
<th>F-Ratio</th>
<th>GP (%)</th>
<th>F-Ratio</th>
<th>MGT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.823*</td>
<td>78.0b</td>
<td>13.344*</td>
<td>13.7b</td>
</tr>
<tr>
<td>400 ppm</td>
<td>0.7a</td>
<td>10.0a</td>
<td>13.7b</td>
<td></td>
</tr>
<tr>
<td>600 ppm</td>
<td>86.7b</td>
<td>13.7b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>88.0b</td>
<td>15.3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 ppm</td>
<td>94.7b</td>
<td>15.3b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H2SO4 Pretreatments</th>
<th>F-Ratio</th>
<th>GP (%)</th>
<th>F-Ratio</th>
<th>MGT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>0.0a</td>
<td>11.976*</td>
<td>0.0a</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.7a</td>
<td>0.0a</td>
<td>0.0a</td>
<td></td>
</tr>
<tr>
<td>7 min</td>
<td>0.6a</td>
<td>10.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>6.0b</td>
<td>21.0c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Pretreatments, significantly different at α= 0.05

TABLE 2
Results of Statistical Analyses Showing the Relationship of the GP and MGT with Different Treatments in the Greenhouse

<table>
<thead>
<tr>
<th>GA3 Pretreatments</th>
<th>F-Ratio</th>
<th>GP (%)</th>
<th>F-Ratio</th>
<th>MGT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.048*</td>
<td>80.0b</td>
<td>67.268*</td>
<td>21.7b</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>84.7b</td>
<td>21.3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 ppm</td>
<td>86.0b</td>
<td>23.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 ppm</td>
<td>86.0b</td>
<td>22.0b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H2SO4 Pretreatments</th>
<th>F-Ratio</th>
<th>GP (%)</th>
<th>F-Ratio</th>
<th>MGT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>0.0a</td>
<td>145.540*</td>
<td>0.0a</td>
<td></td>
</tr>
<tr>
<td>7 min</td>
<td>1.3a</td>
<td>42.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>1.3a</td>
<td>35.0b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>6.7b</td>
<td>34.0c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.0b</td>
<td>44.7c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Pretreatments, significantly different at α= 0.05

TABLE 3
Results of Statistical Analyses Showing the Relationship of the GP and MGT with Different Treatments under Open Field

<table>
<thead>
<tr>
<th>GA3 Pretreatments</th>
<th>F-Ratio</th>
<th>GP (%)</th>
<th>F-Ratio</th>
<th>MGT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 ppm</td>
<td>31.395*</td>
<td>20.1b</td>
<td>1.500</td>
<td>13.0</td>
</tr>
<tr>
<td>800 ppm</td>
<td>25.0c</td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>26.7c</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H2SO4 Pretreatments</th>
<th>F-Ratio</th>
<th>GP (%)</th>
<th>F-Ratio</th>
<th>MGT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>1.3a</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>1.3a</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 min</td>
<td>4.0b</td>
<td>15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>4.3b</td>
<td>15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25.0c</td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Pretreatments, significantly different at α= 0.05

said that, cold stratification method should also be tried to solve germination problem of *Arbutus andrachne* seeds besides chemical scarification. When mean germination times of the seeds were took into consideration the seeds had the highest arithmetically germination percentage in the laboratory (94.7%) had mean germination time of 15 days (Table 1). Mean germination time of the control seeds was better (10 days) than GA3 treatments in the laboratory, but germination results of the seeds were the worst (0.7%). In the greenhouse condition, both germination percentage (8.0%) and mean germination time (45 days) were worse than the results of GA3 treatments (Table 2). 400 ppm GA3 treatments gave the best germination percentage (26.7%) and
mean germination time (13 days) results under open field conditions (Table 3).

Consequently, among all the pretreatments applied to the Arbutus andrachne seeds, soaking in 800 ppm GA3 for 24 hours resulted in the highest germination percentages (94.7%) in the laboratory, and soaking in 600 ppm GA3 for 24 hours sowing in the greenhouse gave the better results than using H2SO4 and sowing in the greenhouse gave the better results than using H2SO4 and sowing in the greenhouse.

REFERENCES


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DETECTING LONGTERM TRENDS OF VEGETATION CHANGE AT LOCAL SCALE THROUGH TIMESERIES IMAGE ANALYSIS: A CASE STUDY IN INNER MONGOLIA, CHINA

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ABSTRACT

Analysis of long-term change trend of vegetation covers faces several challenges. First, the change of vegetation covers exhibits remarkable cyclical oscillations and disturbances, which often mask the long-term trend. Second, the remote sensing image based time-series analysis is often complicated by the lack of long-series ground reference data. In addition, current remote sensing based vegetation change analysis focuses on continental, national or regional scales. Local scale analysis of vegetation changes is often neglected. In this paper, we developed a synthesized analysis framework, the domain adaptive and classified smoothing (DACS), to tackle these limitations. We integrated domain adaptive learning with the classified maps of vegetation groups (VGs) to derive the ground reference data supporting the image-based time-series analysis and to support local scale spatial analysis. We applied empirical mode decomposition model to extract long-term trends of VGs NDVI changes. We employed univariate polynomial regression to examine the long-term change trends of VGs and to identify their spatial and temporal patterns. We tested this new algorithm by analyzing the SPOT-VEG NDVI 10-days composites from 1999 to 2007 in Wulate, Inner Mongolia, China. The findings confirmed that the newly developed DACS method effectively captured the long-term trends of NDVI change over various VGs and revealed that the regional trend of NDVI change differed from the local trends of change of different VGs. DACS is suitable to other image sources, such as MODIS and Landsat images, and can be applied in other regions for local scale time-series analysis of vegetation cover changes.

KEYWORDS:
Domain adaptive learning, EMD, time series, trends analysis, univariate polynomial regression, vegetation cover

INTRODUCTION

Vegetation is one of the most critical elements of terrestrial ecosystems and plays an important role in material cycling and energy flow [1-4]. Spatio-temporal variations in vegetation growth can affect the terrestrial carbon cycle and other biochemical processes [5], thus explaining why the study of its dynamics is an emerging issue in the field of environmental analysis. Nowadays, the global environment is facing a variety of challenges that threaten the sustainable development of human societies [6]. Vegetation change dynamics are important indicators for assessing these eco-environmental changes. Long time series data of vegetation cover are often used to detect dynamic vegetation changes and extract change characteristics and patterns [7-9].

Studies of long-term change trend of vegetation cover are challenged by many factors. Firstly, the changes of vegetation cover contain remarkable cyclical oscillations and abrupt disturbances, which often mask and distort the long-term trends that we would like to identify [10]. Seasonal changes are cyclical impacts of climate change on vegetation growth. However, the seasonal changes of either vegetation cover or climate are interwoven with long-term yearly changes (of precipitation and temperature) and sudden short-term events (of flooding, drought, hot wave, cold front, etc.). The long-term and short-term changes can also attribute to human, policy or socioeconomic factors. Therefore, the cyclical changes of vegetation cover have been distorted by the long-term and short-term coupled climate-policy-socioeconomic interactions [11].

Secondly, it is well recognized that the long-term trends of vegetation cover change vary at multiple scales. Studies of spatial variability and pattern of vegetation change are well-informed traditions in ecological and geographical sciences. Factors affecting the density and richness of species at one scale may not have similar impact at other scales [12]. The temporal trends of vegetation cover change often show noticeable spatial heterogeneities [13]. In
addition, the ecological factors and processes that impact vegetation cover change interact across scales. The investigation of cross-scale interactions can shed insights on a wide range of multi-scaled problems, such as, ecosystem evolution, and land-use and land-cover change [14].

Thirdly, current ecological and environmental models or analytical methods are often built on time-averaged or cumulative data or the comparisons between minimum, average and maximum values of observations. They are not capable of separating long-term trends from cyclical fluctuations and abrupt changes or capturing temporal dynamics or regional patterns of ecosystem change [11, 15].

Fortunately, in recent years, the land use and land cover data from the Earth observation satellites have continuously increased and become accessible to the public, leading to the explosion of satellite image time series (SITS) analyses and applications [16]. The SITS based techniques are time-saving and economically efficient for land cover change detection and vegetation mapping over a large area [17, 18]. It has been long agreed that remote sensing techniques have provided unique insights to global environmental change research during the past decades [19]. Remote sensing data products become an important and very useful data sources in monitoring the changes of globe vegetation coverage. These data have significantly improved our understanding of intra- and inter-annual variations in vegetation from a regional to global scale in the past three decades [20]. Especially, NDVI imagery data which were acquired by different remote sensors are the most widely used in many research fields, such as vegetation dynamic monitoring, ecological environment evaluation, spatial distribution and pattern analysis, and dynamic evolution [7].

In this paper, we attempted to develop a new SITS technique to detect long-term trends of vegetation change. In particular, this new technique was synthesized from several SITS algorithms, advanced signal processing and noise removing techniques, and statistical analysis to extract long-term trends of vegetation change. We also applied this innovative method to investigate the long-term trends of change over one vegetation type and its ten groups in Wulata County, Inner Mongolian Autonomous Region, China, to reveal the distinct trends of change at local scale.

MATERIALS AND METHODS

The Research Method. Satellite image time series analysis of temporal trends of change is by no means a new research front. The traditional approaches of image-based time-series analysis include either a simple combination of discrete image processing or classification at a sequence of times [21], or an interpolation technique to construct a time-series dataset between several classified images or vegetation maps interspersed over a period [22]. However, the recent SITS algorithms are based on continuously stacked time-series images due to the increased availability of satellite images and the advancement of computational algorithms. These newly developed SITS methods can be categorized into three groups. Similarity measurement between consecutive image pairs is the first group. This group of algorithms computes quantitative measures of similarity to classify and detect the changes of ecosystem characteristics between the stacked images of the study area. Lhermitte and his colleagues [23] did a comprehensive overview and quantitative comparison of eight commonly used similarity measures, ranging from Manhattan, Euclidean, and Mahalanobis distance measures, to correlation, Principal Component Analysis (PCA) and three Fourier based similarity measurements.

The second group includes the time-series data smoothing techniques. Fourier analysis, asymmetric Gaussian model, double logistic model, the Whittaker filter, and discrete wavelet transform are five commonly adopted techniques for smoothing multi-temporal satellite sensor observations aimed at deriving an appropriate annual vegetation growth cycle and estimating phenological parameters. Atkinson and his colleagues [24] compared the first four methods by using Level 3 Medium Resolution Imaging Spectrometer (MERIS) and Terrestrial Chlorophyll Index (MTCI) data composited at eight day intervals from 2004 to 2006 and over the Indian sub-continent. These four models illustrated varied accuracy levels in the context of deriving the main characteristics of vegetation seasonal changes, although all techniques yielded consistent results over a large portion of the study area.

The domain adaptation algorithms belong to the third group. The main innovation of this group is the concept of domain adaptation, which assumes that the training data are available only for a source domain but not for the target domain although they are related [25], and takes advantage of the available knowledge of the source domain in order to infer a classification of the related target domain for which a priori information is not available [26]. A transfer or adaptation learning happens each time when you infer the knowledge of a source domain to a target domain. In other words, the transfer of knowledge across domains is similar but not the same because each time unique characteristics of the target domain should be taken into consideration.

This paper presents a novel approach for detecting the long-term trends of vegetation cover change by synthesizing a time-series data smoothing algorithm with a domain adaptation method and by applying this new algorithm onto a stacked NDVI time-series data. We call this new approach “Domain Adaptable and Classified Smoothing” algorithm – DACS. The main goal of DACS is to differentiate
the variations of the change trends at finer local scales (i.e., the vegetation groups). It is worth pointing out that the majority of current vegetation coverage studies on the basis of remote sensing have focused on investigating the total change trend over a large area or a region. Less effort has been found to probe in depth into the variations among different vegetation groups. Additionally, as discussed above, NDVI time-series data contain yearly, seasonal and abrupt changes and, hence, are neither linear nor stationary. It is inevitable for this new method to be capable of removing the noises and in particular the cyclical disturbances of NDVI time-series in order to detect the long-term trends of change.

DACS is designed to consist of three main components: (1) iterative classification and adaptation; (2) smoothing; and (3) nonlinear fitting. The classification process is interwoven with the adaptation procedure (Fig. 1). DACS starts from a classified plant group image layer (a raster or grid dataset), which is called the first source domain. The time-series NDVI values on the source domain are known and are used to compute monthly Maximum Value Composites (MVC). MVC minimizes the effects of cloud cover, the variability of the scanning angle, sun angle, aerosols, and water vapor, which are the factors causing some residual noise [27–29]. MVC selects the maximum NDVI during each month as the pixel value of each dataset. MVC is computationally formulated as the following function,

\[
NDVI = \max_{i,j} \{NDVI_{i,j}\} \tag{1}
\]

Where \(i = 1, 2 \ldots 12; \ j = 1, 2, 3; \ NDVI_{i,j}\) is the \(j\)th day value in the \(ith\) month; and \(NDVI_{i}\) is MVC of the \(ith\) month.

We confine the months of interest to the growing season of each year, May to September in general. The highest monthly MVC in a growing season (usually occurring in August) is called MaxVC of NDVI, while the lowest monthly MVC in the growing season (often happening in May) is called MinVC. We further compute the average values of MaxVC and MinVC for each vegetation group on the first source domain. Next, we find and compute the average values of MaxVC and MinVC for the second source domain.

It is expected that the values of MaxVC and MinVC differ between two source domains and the areas that various vegetation groups (VGs) occupy on two source domains are varied (Fig. 1). The annual change rates of the values of MaxVC and MinVC and the areas of different plant groups are computed according to the following equation, respectively,

\[
R_i = \left(\frac{V_2}{V_1}\right)^{\frac{1}{n}} - 1 \tag{2}
\]

Where \(R_i\) is the change rate of the value of either MaxVC, MinVC, or the area of \(ith\) VG; \(V_1\) is the value of MaxVC, MinVC, or the area of \(ith\) VG on the 1st source domain; \(V_2\) is the value on the 2nd source domain; \(n\) is the number of the target domains between the two source domains.

**FIGURE 1**

The design of Domain Adaptable and Classified Smoothing – DACS – algorithm
Afterwards, the Ri values of MaxVC and MinVC are applied to a target domain of the ith VG (in the longitudinal order of the target domains) to determine the area of the ith VG. The newly determined area of the ith VG is then checked with the corresponding Ri value of the area change rate. If they are consistent, the allocation of change is done. When they are not consistent, minor adjustments to the Ri values of MaxVC, MinVC, and the area of ith VG need to be made to minimize the discrepancies. It is important to point out that the final products of the integrated classification and adaptation procedures are the boundaries of all plant groups over the study area for each of the consecutive years.

The time-series smoothing technique being adopted in this paper is a new signal noise deduction method called the Empirical Mode Decomposition (EMD) [30]. As a time domain signal analysis technique, it is not the same as Fourier method and the discrete wavelet transform described earlier. EMD can be adapted for analysing non-linear and non-stationary data sets.

The principle of EMD is to decompose the signal into a group of similar sinusoidal signals and trend signal functions, which are defined by the signal itself and named the intrinsic mode functions (IMFs), and a residue [31, 32]. In the process of decomposing the original signal, the original time scale is gradually decreasing. In other words, the time scale in the first IMF is coarser than the original. As a result, as the order of the IMFs increases, their corresponding frequencies gradually decrease, and the residual component has the lowest frequency. The residual decomposition can be treated as a monotonic function according to the convergence criteria of the EMD [33]. EMD of one-dimensional signal can be expressed as [34]:

\[ x(t) = \sum_{i=1}^{imf} imf_i(t) + r_n(t) \quad \text{Eq. 3} \]

Where \( imf_i(t) \) is the IMF and \( r_n(t) \) is a monotonic residual function. The reconstructed signal is composed of the signal components of IMF, and corresponds to the definition given in equation (3). In the process of decomposing the original signal, the original time scale is gradually decreasing. In other words, the time scale of the first IMF is coarser than the original. As a result, as the order of the IMFs increases, their corresponding frequency gradually decreases, and the residual component, \( r_n(t) \) has the lowest frequency. The residual decomposition \( r_n(t) \) can be treated as a monotonic function according to the convergence criteria of the EMD. Thus, its cycle is greater than the recording length of the original signal, and so \( r_n(t) \) is the trend term.

It is important to note that the EMD algorithm is applied to the original time-series (ten-day) NDVI observations that are averaged over each VG. Although the NDVI value is the average over a VG, i.e., the average of all cells belonging to that VG, the ten-day time series are kept, which is different from the iterative classification and adaptation procedure that is computed by years. We employ the EMD method for two purposes: removing the noises from the time series NDVI data prior to analysis, and diagnosing the variations of ten-day NDVI change trends in different VGs and in whole study area of WZQ respectively. In short, we attempt to have a clear understanding of the dynamic changes of various types of vegetation at the VG scale.

The third process of DACS is to apply univariate polynomial regression (UPR) to analyze the residues of the entire study area and various VGs. UPR is a form of linear regression in which the relationship between the independent variable \( x \) and the dependent variable \( y \) is modelled as a \( n \)th degree polynomial [35], which fits well with most EMD derived residues of NDVI time series in graphical forms of non-linear curves because the residue from EMD is usually treated as a monotonic function [34]. UPR is incorporated into DACS to visualize the change trends of NDVI and reveal the variations of the change trends among different VGs.

**Study Area.** The Inner-Mongolia grassland is the third largest in the world and supports the world’s largest population of sheep and goats, and the fourth largest population of cattle [36]. At present, it is considered to be an important timber and livestock region in China. The main portion of Inner-Mongolia is characterized as a typical continental climate of rare precipitation and frequent drought, with frequent snow hazards in winter and sand storms in spring. In the past decades, it has experienced dramatic climate change, which has been more pronounced than most other areas in China [37-39]. For this region, many remote sensing data archives have been processed to examine various aspects of vegetation cover change dynamics [40–42], degradation of grassland [22]; phenology patterns [36]; driving forces of vegetation changes [43–45]; and the impacts of climate change [37, 46–47]. We chose Wulate Zhong Qi (WZQ) located in the north-central Inner Mongolia as the case study area (Fig. 2). There are three considerations of supporting the selection: (1) WZQ is situated 107°16’ to 109°42’ E and 41°07’ to 41°28’ N, and is one of the representative desert steppe zones in Inner-Mongolia grassland; (2) We have done intensive field sampling in this area from our prior projects and thus have a large set of ground truthing data that can be used as the training and testing samples to assess our results; and (3) In the past three decades, the typical steppe and meadow steppe grassland in Inner Mongolia has been extensively studied, but scarce effort has been made to examine the vegetation changes of desert steppe grassland. WZQ is about 23098 square kilometers with the Yinshan Mountain lying across the southern border from east to west. Topographically, WZQ is divided into three parts: the southern is the piedmont plain, the central part is the mountainous
region, and the northern is the hilly plateau. There are significant geographical differences and transitional changes between the different landscapes. The study area is extensively covered by the temperate desert steppe, including, plain and hilly temperate desert steppe, mountain temperate desert steppe, and gravel steppe-desert.

Data Sources. SPOT VGT NDVI. SPOT VEGETATION 10-day Synthesis Archive (SPOT VGT-S10) products with a spatial resolution 1 km × 1 km from 1999 to 2007 (total 324 images) were used in the study. The SPOT-4 VEGETATION (VGT) sensor was launched on April 1998 and provided measurements of land surface reflectance in the visible and the infra-red domains. The data used in this paper were a series of 10-day composite images (S10 products) of the VGT sensor [48]. The data were stored in a digital number format (0-255) for convenient storage. The NDVI values were calculated using the following formula developed by the image processing and archiving center, VITO, Belgium (http://www.vgt.vito.be/):

\[
\text{NDVI} = \frac{0.004 \times \text{DN} - 0.1}{
\]

Ground-truth data and other additional data. For this study, our research group collected an array of datasets. The first one was the 1999 vegetation classification map (scale:1/1000000), which was used to create the first source domain plant group boundary data set. The second dataset included 305 ground vegetation samples in the summer of 2004, 82 quadrat surveys in July, 2006 (100mx100m) and 305 ground vegetation samples in the summer of 2009 taken at the same locations as those in 2004. The ground samples and surveys were roughly evenly distributed over 10 plant groups across the study area, including the data items of grassland type, vegetation type, vegetation group (VG), productivity, grazing intensity, and location (Fig. 2). The survey and ground sampling data were used to map the boundaries of different vegetation groups, which were used to define the classified source domains in the years of 1999, 2004, 2006 and 2009. The classification of ten different VGs was based on the new classification system that was adopted by the Institute of Botany of Chinese Academy of Sciences in 2011 (Fig. 3) [50]. The boundaries of VGs in 1999, 2004, 2006 and 2009 were used to generate the classified source domains in the corresponding years. These boundaries and the related Max-VC and Min-VC data were used to derive the bounding areas of the classified target domains according the research design (Fig. 1). The boundaries of both classified source and target domains were finally used to compute the EMD curves for each of the ten VGs.

FIGURE 2
The study area – Wulatezhongqi, Inner Mongolia
FIGURE 3
The map of ten vegetation groups (VGs) in 2007

TABLE 1
The average ranges of MaxVC and MinVC values of ten VGs in the study area

<table>
<thead>
<tr>
<th>Vegetation groups (Categories)</th>
<th>Range of NDVI Value</th>
<th>Plant community</th>
<th>Area (Sq. Km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.05~0.1</td>
<td>temperate steppe-desert type rangeland Gobi Desert - bunch grass group</td>
<td>4202</td>
</tr>
<tr>
<td>C2</td>
<td>0.1~0.11</td>
<td>temperate steppe-desert type rangeland - Gravel desert steppe subclass-bunch grass group</td>
<td>5598</td>
</tr>
<tr>
<td>C3</td>
<td>0.11~0.14</td>
<td>temperate steppe-desert type rangeland - Gravel desert steppe subclass-Shrub and grass group - Caregana</td>
<td>5909</td>
</tr>
<tr>
<td>C4</td>
<td>0.14~0.154</td>
<td>temperate steppe-desert type rangeland - Gravel desert steppe subclass - Shrub and grass group - Caregana microphylla</td>
<td>6085</td>
</tr>
<tr>
<td>C5</td>
<td>0.154~0.170</td>
<td>temperate steppe-desert type rangeland - Plain and hilly temperate desert steppe subclass - Shrub and grass group - Cleistogenes squarrosa and Carex pediformis</td>
<td>3289</td>
</tr>
<tr>
<td>C6</td>
<td>0.170~0.190</td>
<td>temperate steppe-desert type rangeland - Plain and hilly temperate desert steppe subclass (including part of the mountain steppe desert subgroup) - Artemisia group or gravel desert steppe subclass-Bunchgrass group-Gobi needle</td>
<td>2116</td>
</tr>
<tr>
<td>C7</td>
<td>0.190~0.22</td>
<td>Temperate desert steppe - Plain and hilly temperate desert steppe subclass - Artemisia group or gravel desert steppe subclass-Bunchgrass group-Gobi needle</td>
<td>1782</td>
</tr>
<tr>
<td>C8</td>
<td>0.22~0.30</td>
<td>Temperate desert steppe - Plain and hilly temperate desert steppe subclass - Bunchgrass group - Stipa breviflora Artemisia frigida</td>
<td>1174</td>
</tr>
<tr>
<td>C9</td>
<td>0.30~0.60</td>
<td>Temperate desert steppe - Mountain temperate desert steppe subclass - Shrub grass group-Convolvulus and Witch Hazeri (including cultivated land)</td>
<td>1013</td>
</tr>
<tr>
<td>C10</td>
<td>0.60~1</td>
<td>Forest and Cropland</td>
<td>22</td>
</tr>
</tbody>
</table>
RESULTS

Inter-annual NDVI changes during 1999-2007. Applying the DACS algorithm, we computed the MaxVC and MinVC values for ten VGs during 1999 – 2007. Although these values showed some variations between years, the average ranges of MaxVC and MinVC values were reported in Table 1. Moreover, the MaxVC and MinVC values on the source domains were applied to the target domains to obtain the MaxVC and MinVC values and the corresponding boundaries of ten VGs. The boundaries of ten VGs in 2007 was mapped in Figure 3 as an illustration. The areas of ten VGs in 2007 were also included in Table 1.

FIGURE 4
The map of MaxVC from 1999 to 2007
Moreover, the MaxVC values from 1999 to 2007 were mapped in Figure 4. The validation tests were conducted on the basis of two known source domains in 2004 and 2006. The accuracy levels were around 87.5 – 92.5 percent over these two years. The MaxVC values from 1999 to 2007 clearly illustrated the spatial distribution of the inter-annual NDVI changes and the different vegetation groups from 1999 to 2007. It was noticed that the vegetation cover conditions were improved slightly over the whole study area during 1999-2007. Especially, in the northwest High Plains steppe desert area where was originally occupied by bunch grass group, significant improvement was observed. Nearly half area over this region, the MaxVC values increased from level one to level two since 2000. However, in other regions no obvious changes were observed except that some areas showed slight fluctuations.

The annual changes of MaxVC were mapped on Figure 5. The red areas were the severe recession areas of NDVI attenuation over 0.15. In general, the recession rates of NDVI attenuation ranged from 0.05 to 0.15. On Figure 5, severe recessions were noticed between 2000 and 2001 in the central and western plateau grasslands and between 2004 and 2005 in the southeast hills and mountains. The green color indicated an increase of vegetation index values, for instance, in 2000, 2002 and 2007. The white color illustrated no or slight changes of vegetation index values, ranged from 0.005 to -0.005. In summary, over the study area, the vegetation covers suffered severe recessions in 2001, 2004, and 2005.

EMD filtered long-term trends of NDVI change and analysis. The SPOT-VEG NDVI 10-day composite scenes during the 9-year period were stacked into 324 bands and classified into ten VGs according to their annual MaxVC and MinVC values. Afterwards, the EMD method was applied to the 324 NDVI time series over the whole study area and over each of the ten VGs, respectively. In other words, the EMD method was employed to remove the noises caused by cloud cover, the variability of the scanning angle, sun angle, aerosols and water vapor in order to extract the long-term trends of change. As a result, the NDVI time series were decomposed into a set of IMFs and the residue. The EMD-filtered NDVI time series of the entire study area were shown in Figure 6. The noises were removed through iteratively applying IMFs \( f_1, f_2, f_3, f_4, f_5, f_6 \). After six rounds of iteration, a trend line was obtained, which was called the residue \( r \). The EMD filtering procedures for the ten VGs were the same as the EMD filtering for the entire study area and thus the iterative IMFs of the ten VGs were omitted.
DISCUSSION

Over the entire study area, the filtered NDVI represented the intra annual variations of vegetation cycles, whereas the residue revealed the trend embedded in the original time series [34, 51-52]. The regional trend of NDVI temporal change ($r$) was depicted on the left panel of Figure 7 (the left) and the residue and its polynomial fitted line on the right panel. We found that the NDVI slowly increased from 1999 to 2007, and the average NDVI value over the entire study area increased from 0.113 of 1999 to 0.128 of 2007. The result was similar with the research findings by Zhang [29], and Miao, et al. [53].

10-day series of NDVI values over 10 different VGs in the study area were decomposed by EMD. The NDVI temporal trends ($r$) from 1999 to 2007 were displayed in Figure 8. The polynomial curves of the temporal trends were also extracted and depicted in the same figure. As shown in Figure 8, the NDVI trends of 10 VGs were very complex and different in both temporal and spatial dimensions. The decomposed signals clearly illustrated four different types of NDVI temporal change trends during the nine years: (1) VGs of C1, C2, C4, C8 and C9 displayed an increasing trend; (2) C3 and C7 exhibited a changing trend of rising first and declining later; (3) C6 revealed a noticeable fluctuating but generally declining trend; and (4) C5 and C10 showed no or little change.

The fitted polynomial quadratic curves from the EMD residual values confirmed the aforementioned findings. First, the 2-degree polynomial curves fitted extremely well with the increasing and stable (little or no change) trends, such as those curves of the VGs of C1, C2, C4, C8, C9, C5 and C10. The $R^2$ values were higher than 0.99. Second, the polynomial curves fitted well for the curvilinear (the rising-first and declining-later) trend and the corresponding $R^2$ were around 0.90. Third, the 2-degree polynomial function was not working with VG C6, and its multiple curled curve generated a poor $R^2$, 0.0461. The negative slope of C6 indicated that the NDVI change of VG C6 was gradually declining due to the overgrazing, reclamation and intensive human interferences, which were validated by our field sampling trips.

On the basis of the long-term trends of NDVI increase, decrease and fluctuation, we remapped the NDVI changes by VGs in Figure 9. We found that the areas of NDVI increase (57.9%) were larger than the areas of NDVI fluctuation (24.7%), which were larger than the areas of NDVI decrease (6.8%). This finding confirmed that the general trend of NDVI
change over the study area was the increase. From Figures 8 and 9, we derived that the NDVI value for the first vegetation group (C1) increased from 0.076 to 0.088 while C2 from 0.077 to 0.113 during the period 1999-2007. They together occupied about 31.42% of the whole study area. Those regions were typical arid and sub-arid regions located in the high plains of the Gobi Desert with low-height vegetation cover, which suffered noticeable desertification in the past but was improved during the study period. In the southern and south-central regions, the vegetation cover conditions were better, but some patches were seriously degraded too.

In addition, the green color presented the areas where apparent improvement happened to vegetation conditions, mainly covering the C4, C8 and C9 areas located in the southern part of the Yinshan Hetao region, Plain Piedmont Alluvial Fan region, and the southeast of hilly steppe and desert steppe region. They occupied about 26.5% of the whole study area.

FIGURE 8
Local trend of NDVI temporal changes by VGs and UPR curve fitting
The yellow color represented the fluctuating areas, which occupied a noticeable portion of the study area, and was about 24.7% of the study area. The vegetation groups included C3 and C7. They were mainly temperate desert steppe, and some were the temperate steppe desert vegetation located northwest of the plateau region. According to the fitted trend curves, the vegetation cover conditions were gradually improving during 1999-2003. However, the NDVI values began decreasing from 2004.

The red color areas indicated the serious degradation regions (Figure 9) and were mainly located in the southeastern plains and mountainous temperate desert and Wengen area. Stipa breviflora, microphylla children, and Gobi Stipa were the primary vegetation plants. These areas used to be the natural and traditional grazing territories with good living conditions. However, they were severely degraded because of heavy grazing. These areas occupied about 6.78% of the study area. By analyzing the fitted curve (Fig.7: C6), and the ground survey data, we concluded that these areas began to decrease in the eastern region since 2003, and in the southeastern region since 2004.

The white areas were about 10.6% of the total area, which represented the stable regions, located in the south-center regions. They were mainly occupied by the shrub, forest and cropland.

**CONCLUSIONS**

The goal of this study was to develop an easy-to-apply image-based time-series analysis of local vegetation covers to identify long-term change trends and to analyze their spatial and temporal variations. We validated this analytical approach by applying it to the time-series SPOT VGT products data in the WZQ during 199-2007. We can draw several conclusions from this case study.

First, the synthesis of the domain adaptive learning algorithm, the EMD method and the polynomial regression worked well to reveal the long-term trends of NDVI change and their local (spatial) variations between different VGs. The domain adaptive learning was realized in two ways, by dividing the study area into sub-areas (i.e., 10VGs over this case study according to the vegetation community types), and by incorporating the information from the source domains to interpolate the boundaries over the target domains between the source domains. The former method was integrated to identify local or spatial variations. The later approach was designed to use the available sampling data in the known years to derive the information needed in the unknown year in order to construct a complete time-series data over the study period. The case study confirmed that the adaptive learning over local vegetation groups worked well. In other words, interpolating the available vegetation samples in the known years to the unknown years worked when these samples were confined within fine spatial scales. No
doubt, the accuracy of the temporal interpolation (or adaptive learning) would be affected by the frequency (number) of the known years out of the time-series, the spatial scale of the subject of interest, and the intensity of the change of the study subject [54].

Moreover, the EMD decomposition was performing well to remove the noise and extract the changing trends of VGs. It is worth pointing out that EMD was recently applied in remote sensing applications although it had long been used in signal noise deduction. In particular, the adoption of EMD in series image processing has been new endeavors. In addition, the univariate polynomial regression fitted curves worked well to visualize the trends of change of NDVI by VGs. The case study outcomes confirmed that the newly developed DACS method effectively captured the long-term trends of NDVI change over various VGs and revealed their local variations. The domain adaptive learning, EMD and the polynomial regression were well integrated to support the research design for identifying long-term change trends and analyzing their temporal variations and spatial patterns. The DACS method can be extended to the time-series analysis of vegetation cover changes at local scale from other image sources, such as MODIS and Landsat images, or in other regions.

Second, the case study revealed that the general regional trend of NDVI change differed from the local trends of change of different VGs that resided in the study region. Although, the regional vegetation cover condition was improved during the study period of 1999 - 2007. However, the vegetation covers of local VGs showed different changing trends: improved, degraded, fluctuated and unchanged, inconsistent with the general regional changing trend. Spatially, the southern Yin Shan, the southern Foothills, the alluvial fans and the Yellow River irrigation areas had good vegetation coverages and most of the regional vegetation covers were improving from 1999 to 2007. In the northwestern and the northern parts of the Gobi Desert region, although some improvement was observed in the past nine years the average NDVI values were still below 0.1 and displaced severe desertification. In the central regions, a large stretch of vegetation cover showed fluctuating and no significant improvement was found in this study period. In the eastern and southeastern mountainous and hilly plains, the NDVI exhibited clear degradation trends. Several previous studies in this region defined the areas with NDVI < 0.1 as “the sparsely vegetated areas” and with NDVI > 0.1 as “the vegetated areas” [38]. Therefore, the ecosystems in the region were still in an extremely fragile state.

This finding has important policy implications. The study area represents a unique climatic zone with the growing season precipitation less than 150 mm, the dry zone in Inner Mongolia of China. This area is known as the desert steppe. It is commonly agreed that the ecological systems in this area are very fragile and the grassland deterioration caused by overgrazing or other human interferences is almost irreversible. For the same reason, the grassland grazing exclusion policy has been enacted since 2004 [43]. However, the grazing exclusion policy has not taken into consideration of local variations of grassland growth and resilience to the climate change. As a result, the ecological effect of the policy on grassland recovery has not been optimized or universally achieved [55]. No doubt, the policy could better help vegetation recovery if it was implemented by incorporating local or micro environments for plant growth as well as local responses to global climate change and variability. Future implementations of grassland ecosystem management measures must consider local plant growth variations and heterogeneous responses to climate changes in order to maximize costs/benefits and to achieve long-term ecosystem sustainability.

Third, although the DACS method was effective revealing that the NDVI changing trends of various VGs differed from the general trend of the entire study area, there were rooms for future improvement. For instance, in our study, only SPOT VET products were used to analyze the changing trends of VGs. Longer time-series of remote sensing data, especially the image data available in recent years should be tested in future research, such as MODIS NDVI products. Another effort in future could take into global climate into consideration to analyze how the changing trends of VGs in a local environment differ from the long-term trend of global climate change. In other words, it will be interesting to investigate how local vegetation groups or different plant communities respond to global climate change and whether there are any spatial and temporal variations of the responses of local VGs to climate change. Finally, the coupled natural and human interactions should be examined in the context of driving factors or key drivers of local vegetation changes.

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FLOOD DAMAGE ASSESSMENT METHOD: GIS BASED APPROACH

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ABSTRACT

This paper presents a method for the estimation of the expected property damage in the case of flood risk realization in the territory of the province of Vojvodina. The analysis is focused on floods which are the result of surface water overflow. The emphasis is on the way of determining direct tangible damage which can occur on endangered residential buildings in the zone of interest. The presented approach includes the analysis of possible danger of flood water, exposure and vulnerability of the observed building in the case of a flood wave of the defined recurrence interval (probability of occurrence). The next step is determining flood depth, maximum possible damage and degree of damage to the residential unit of interest. The simulation of spreading of the flood wave was performed by means of GIS techniques. The aim of this paper is determining the monetary value of possible damage in the flood depth function.

KEYWORDS:
Damage assessment, Spatial analysis, GIS, Flood, Flood depth

INTRODUCTION

Climate change, as a change of average weather conditions, has been recognized as a major risk that the today’s society is exposed to. Strong effects of climate change which have been evident around the world over the past decades are a threat to sustainable development and community resilience, hindering socio-economic prosperity and increasing poverty. Data show that losses from natural disasters tripled between 1980 and 2016 [1]. Additionally, floods have been recognized as a natural hazard that affects more and more people every year, so flood hazard analysis is becoming increasingly necessary [2]. Over the last twenty years, flooding accounted for 47% of all weather-related disasters, affecting 2.3 billion people [3]. Accordingly, reduction of vulnerability of communities to the effects of floods and other extreme weather events has become the focus of activity of the international community as one of the priorities.

Vulnerability to extreme weather events is defined as the capacity level of a natural or social system to cope with a specific hazard, as a result of the impact of environmental change or extreme events, depending on their nature, structure and exposure to hazard, as well. Hazards affect communities of different levels of preparedness, resistance and resilience. Certain communities are more vulnerable to the effects of extreme weather than others. Small rural communities in transitional societies are extremely vulnerable to the impact of extreme weather [4]. This is the case with small rural communities in the province of Vojvodina, too.

As Vojvodina abounds in different types of water bodies (surface water and groundwater), small rural communities have always been at risk of flooding. It is believed that 46% of the whole territory of Vojvodina is at risk of flooding [5], with the majority of river valleys used for agriculture. The consequences of flooding in small rural communities are reflected in lower income, or even absence of income from the yield, and in property damage (house, contents, farm, tools and machinery), leading to the progression of poverty and increase in the vulnerability of the affected households. Bearing in mind that floods which once occurred every hundred years now occur much more frequently [5], it is clear that adequate measures must be taken in order to reduce the vulnerability of small rural communities to the effects of this extreme weather event.

Hydrotechnical measures have long been seen as the basic instrument for the reduction of vulnerability to floods. However, the contemporary holistic approach to risk management, apart from adequate hydrotechnical measures, emphasizes the financial resilience of a social community as the key component of efficient reduction of vulnerability to extreme weather [6]. Accordingly, financial instruments, such as insurance, have been recognized as an important mechanism for the increase of resilience of poor and developing countries. However, data show that insurance is unaffordable for the most vulnerable parts of the society [7], which is the case for Vojvodina, too. In order to make insurance more affordable for the poor, it is necessary to establish insurance schemes which would enable determining a more affordable insurance premium.
of flood depth, three individual building types, two
classes of building quality, three classes of contamination and three classes of private precaution [14]. The model was derived from actual data surveyed after the 2002 flood event in the Elbe and Danube catchments [14]. The FLEMOps model has been further developed for different flood types [15,16].

Haque et al. [17] developed damage functions for Dhaka City. The damage functions were developed from a survey of 430 properties. The survey was conducted using a systematic stratified random sampling method based on the flood depth and structure of the building [17]. Hasanzadeh Nafarí et al. [18] derived a new flood loss function for Italian residential structures (FLF-IT), on the basis of empirical damage data collected from a flood event in the region of Emilia-Romagna at the beginning of 2014. The function was developed based on a new Australian approach (FLFA) which represents the confidence limits that exist around the parameterized functional depth–damage relationship [18]. The FLFA method [19] is based on a simplified synthetic approach called the sub-assembly method, proposed by the Hazus technical manual [20]. This method measures the extent of losses for each stage of floodwater and suggests a flexible curve that accounts for the variability in the characteristics of structures [18].

In addition to flood depth as the main impact parameter, some models also integrate additional parameters such as flow velocity [e.g. 21], contamination [e.g. 14], the duration of flooding [e.g. 11] or the recurrence interval [e.g. 16]. The method presented in this research is based on the use of function that relates property damage to flood depth.

**MATERIALS AND METHODS**

**Study area.** Vojvodina is an autonomous province of the Republic of Serbia. The province is characterized by ethnic and religious diversity (more than twenty-six nationalities and ethnic groups, and at least five religions) [22]. According to the 2011 census, the average age of the population is 41.8 years, indicating the dominance of the elderly population and, by implication, increased socio-economic vulnerability. As 81% of the whole territory of Vojvodina consists of arable land [23], agriculture is the dominant industry and the main source of livelihood. According to the Census of Agriculture, there are 147,624 agricultural holdings in the province of Vojvodina [23]. Out of the total number of holdings, 1% of these are owned by legal entities and entrepreneurs, whereas the remaining 99% are family farms [23].

Vojvodina is characterized by even distribution of settlements (a great number of large and medium-sized urban and rural settlements) of compact type. Small rural households in Vojvodina are
numerous and belong to a very vulnerable socio-economic group (extreme poverty and depopulation). Bearing in mind that the average size of used agricultural land in Vojvodina amounts to 10.9 ha per farm, and that 65% of farms are smaller than 5 ha [23], it is clear that commercial production is practically non-existent on such small surfaces and the production is focused on satisfying the individual needs of households. These households are exposed to high income risk due to several factors: growing competition in the domestic and world market, bimodal structure of agriculture (gradual abolition and nationalization of agricultural holdings), lack of opportunities for employment and generating external income, devastation of rural areas and lack of adequate institutional support. In such a difficult situation, it is clear that small rural communities in Vojvodina are vulnerable even without the additional burdens caused by extreme weather.

Hydrological data. Long-duration or intense rainfall, as well as melting of snow in the upper course of a river are the major causes of flooding. Floods normally occur when the level of surface water, in the conditions of waterlogged soil, exceeds the capacity of the river bed. Floods which are the result of rivers overflowing their banks are mostly river floods in low-lying areas, and the major characteristic of floodwater is its depth, i.e. the level that the floodwater reaches.

Floodwater depth is influenced by geomorphologic features of the flooded area and hydrological characteristics of the river flow in question, mainly the water level. Water level is defined as the difference between the water level Z and a reference elevation level Zo. The reference elevation level Zo is fixed with a known height. Water level is measured in relation to the reference elevation level Zo and is expressed in centimeters. The level Zo is usually located slightly below the lowest point of the stream bottom, so that the water level has positive values [2].

Wide lowlands usually flood in spring or early spring, depending on the temperature in the upper course of the river, i.e. snow melt in the higher zones of the basin. In river flows with smaller basins floods occur in the periods of long-duration and heavy rainfall, mostly in spring or autumn, which is also determined by weather conditions in the basin.

In the territory of Vojvodina, the largest floodplains are the valley of the Tisa River (2,800 km²), the Sava River (around 2,243 km²) and the Danube (around 2,070 km²) [24]. Floods in the valley of the Tisa occur due to the gentle river gradient, type of bedrock and wide alluvial plain [24]. Floods in the Sava and the Danube floodplains are caused by precipitation, but also by the coincidence of flood waves of their tributaries [24].

The Republic Hydrometeorological Service of Serbia, responsible for observing and measuring water potential, has a wide network of stations for observing and measuring surface water. On the River Tisa, there are five active reporting hydrological stations: Novi Knezevac, Senta, Novi Becej, Branka Novi Becej GV, Titel [25]. Two out of five reporting stations on the Sava are located within the territory of Vojvodina: Jamena and Sremska Mitrovica [25]. There are thirteen reporting stations on the Danube, six of which are within the territory of Vojvodina: Bezdan, Apatin, Bogojevo, Backa Palanka, Novi Sad and Slankamen [25].

At the hydrological stations, water level is observed and measured by a staff gauge, a limnigraph and/or digitally. Water level is observed at 7 a.m. winter time and 8 a.m. summer time (6 a.m. according to UTC-Universal Time Coordinated). At twenty-four stations, water level is also recorded at 7 p.m. winter time and at 8 p.m. summer time (6 p.m. according to UTC) [26].

At the stations where water level is recorded by means of a limnigraph or digitally, the mean daily water level is reported. At the stations where water levels are observed twice a day, the mean daily water levels for both times are given, whereas at all the other stations, water levels observed at 7 a.m. and 8 a.m. are reported. At the hydrological stations where analogue or digital recording of the water level functioned during the whole year, the time of the recorded monthly extremes is also reported [26].

Topographic data. A digital elevation model is today considered a standard method of representing a terrain’s surface digitally. A terrain’s surface is represented by means of a mathematical model which is based on the use of a raster (grid of squares) or a triangular irregular network (TIN). These models are created based on the known positions and heights of points, and specific lines (structural lines and break lines) of the terrain’s surface. DEMs enable us to see every point of the observed area in three spatial coordinates, i.e. to see its latitude, longitude and elevation. By means of DEMs, using an algorithm analysis of morphometric relief features, a new way of observing spatial relations and relief features has been created, enabling a much faster, more rational and better quality analysis of spatial data which make up topographic plans.

The contemporary methods of collecting DEM data are based on the use of total stations, GPS devices and aerophotogrammetry. Aerophotogrammetry and subsequent digital photogrammetric processing, as well as making measurements from photographs, ensure an optimum relationship between the requirements for the quality of data on the one hand, and efficiency and effectiveness, on the other. After procession, the data are exported.
into the desired CAD (dwg, dxf, dgn, etc.) or GIS (shp, mif, gml, etc.) format.

Economic data. Flood damage is usually divided into direct and indirect damage which is further divided into tangible and intangible damage [16]. This paper focuses on direct tangible damage. Direct tangible damage refers to losses which are the result of direct physical impact of the flood on the exposed property. A standard approach to the assessment of direct tangible damage is based on the use of the appropriate damage functions.

Damage functions are an important element in the process of modelling complex relationships between the given hazard, exposure and degree of damage to the endangered entities. The established analytical expressions describe the nature of the relationship between one or more parameters of the analyzed hazard (e.g. flood depth, wind speed, soil compaction due to lack of water, etc.) and the consequent occurrence of tangible damage.

The contemporary methods of assessing tangible consequences of floods are mostly based on functions which express the relationship between flood depth and property damage [8]. Numerous parameters which to a certain extent affect the damage are not included in the analyses because their quantitative effect is mostly unknown.

The largest number of functions created for the estimation of the expected damage at the given flood depths express the expected property damage as relative damage. This implies that possible damage is expressed as part of the total value of the property that is being assessed. Quantitative damage is described by means of weight coefficients with values ranging from 0 (no damage) to 1 (total damage) or by means of the corresponding degree of damage (0-100%).

Expressing the degree of damage in the form of equivalent monetary value requires prior determination of maximum possible damage. Maximum possible damage is the highest level of damage that can occur under the assumption that total damage is possible but unlikely. Thus, it could be said that the maximum possible damage is equal to the estimated monetary value of the endangered property. Monetary value of property can be expressed in two ways. The first way refers to expressing the property value at its original price, whereas the second way refers to expressing the present depreciated value of property.

The method proposed in this paper consists of five steps: Locating the object of interest, Hazard analysis, Exposure analysis, Vulnerability analysis, Estimating possible property damage, Expected property damage.

Locating the object of interest. At the very beginning, it is necessary to determine the position of the object of interest in space. This can be done by entering the address or geographic coordinates of the given object. The geographic coordinates are determined by means of the Google Earth system.

Hazard analysis. After the position of the object has been identified, it is necessary to determine whether it is in danger of flooding, i.e. whether a hazard exists. This is determined using a statistical analysis of maximum annual water levels of the river flow in whose basin the object of interest is located. The analysis of maximum annual water levels was carried out by means of RStudio software. Based on the obtained data, indicators of flood risk, the probability of the occurrence of the event and the recurrence interval of flooding were determined.

The probability of the occurrence of floods is mostly expressed as the annual exceedance probability p(x), i.e. the probability of the annual maximum exceeding the value of x of the random variable X (water level). The recurrence interval (in years) T(x) is the reciprocal value of this probability and represents the expected number of years in which the value x of the random variable X (water level) will occur at least once or will be exceeded at least once.

In accordance with the recommendation from the European Flood Risk Management Directive of the European Parliament and Council (FRMD-Flood Risk Management Directive 2007/60/EC), the analysis included floods with recurrence intervals of around 100 years (floods with medium probability of occurrence) and floods with high probability of occurrence with recurrence intervals of 10-50 years. Extreme floods whose recurrence interval is greater than 100 years (floods with low probability of occurrence) were not included in the analysis because such extreme events were not recorded in the investigated area.

With the aim of achieving a high degree of accuracy of the obtained results, the analysis has to include a time series of maximum annual water levels observed during a period of at least twenty years. This investigation included eighty-nine sequential maximum annual water levels which were observed at the hydrological stations of the Republic Hydrometeorological Service of Serbia, located in the territory of Vojvodina.

If the hazard analysis shows that the object of interest is not within the flood zone, i.e., that there is no hazard, the given object will no longer be analyzed. Thus, it is necessary to return to the first step, which is locating the next object which is the subject of analysis.

Exposure analysis. After it has been determined that the danger of hazard exists in the observed area, it is necessary to determine whether the object of interest is exposed to the hazard. GIS was used for the determination of exposure indicators.
The most significant exposure indicators include distance from the river, terrain elevation and the elevation of the first slab of the object of interest.

In order to determine what exactly is exposed to the dangers of flood water, a digital elevation model of the terrain (DEM) for the observed area has to be constructed. IMAGINE VIRTUALGIS software was used as a tool for constructing the DEM. The model was created by vectorization and georeferencing of analogue topographic maps of the area of interest. Ortophotos of the observed area were draped over the DEM. The format used for ortphoto images is geoTIFF format, which is on a scale of 1:5000 with the coordinates in the Gauss-Krüger system and was manually georeferenced.

The boundaries of flood zones were determined using visualization and simulation techniques. Visualization and simulation of spreading of the flood wave were performed using the Quantum GIS application.

If the elevation of the first slab of the object of interest is above the flood level, the object is not exposed to the effect of the hazard and will no longer be analyzed.

**Vulnerability analysis.** When flooded objects or objects exposed to flooding are concerned, it is necessary to determine how vulnerable these objects are to floods. The most significant indicator in the assessment of vulnerability is the depth of flooding. Apart from flood depth, important vulnerability indicators include the age of the object of interest, usable area, and its structure.

Flood depth was determined by means of the Geoinformation system by relating the elevation of the building to the specific value of the water level. In order to obtain a multi-layer data presentation, we used QGIS by means of which we connected the spatial image with the database of buildings (age of buildings, usable area, structure, etc.) so that it is possible to obtain the desired information about every building marked in the picture in the table. In the chosen zone of interest, a PostgreSQL database was used as a spatial database, version 8.4/2009 with spatial extension PostGIS in the 1.3.6/2009 version.

As vulnerability is measured indirectly and in retrospect, based on the occurred damage, the quantification of damage implies the assessment of property damage.

**Estimation of possible property damage.** The method proposed in this paper includes direct tangible damage inflicted to residential objects expressed in the flood depth function. This refers to possible structural damage which can occur due to effects of flood water. Damage to contents was not analyzed.

For the estimation of damage, it is necessary to collect data concerning flood depth in relation to the specific value of water level, age, usable area of the building and monetary value of the square meter of the building.

Information concerning the age (year when the object was built) and usable area of buildings was gathered in a field survey. The data about the current price of a square meter of real estate were obtained from the National Mortgage Insurance Corporation of the Republic of Serbia.

When expressing the maximum possible damage, the changeable value of property in time was taken into account, i.e. the depreciated value of property. According to the property tax law of the Republic of Serbia, the value of property can fall as a result of property depreciation up to 1% a year using the method of proportion, up to the maximum of 40%, starting from the end of each calendar year in relation to the year in which the property was built, or in which the property was last reconstructed.

The expected degree of damage to the structure of a residential building was determined by means of IKSR depth-damage function which was developed by German insurance companies [5]:

\[ y = 6.9x + 4.9 \]

where the dependent variable \( y \) is the expected degree of damage expressed as percentage, and the independent variable \( x \) is flood depth in meters. The function was derived based on the empirical data about property damage of earlier floods.

**Expected property damage.** The monetary equivalent of the expected damage is obtained by multiplication of the maximum possible damage and the determined degree of damage.

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**RESULTS AND DISCUSSION**

The proposed method was verified on the example of a randomly chosen residential building which is located in the flood zone of the village of Begec. Begec is a rural settlement within the municipality of Novi Sad, located in the district of South Backa of the province of Vojvodina (Figure 1). Its latitude is 45° 14'07'' N and its longitude is 19° 37'14'' E. It occupies an area of 43.8 km² on the left bank of the river Danube. Its elevation ranges from 76.07 m to 81.12 m above the level of the Adriatic Sea. The village of Begec was chosen for investigation because of its specific location, topography of the surroundings and demographic characteristics typical of small rural communities of Vojvodina.

Based on the 2011 census, the population of Begec is 3,325. Out of the whole population, 2,665 are adults. Elderly population is dominant (men 50-54 years old, women 45-49 years old). The average age of the population is 39.6 years (men 38.4, women 40.8). The village numbers 1,034
households. The average number of household members is 3.23. Population density is 76/km². Agriculture is the dominant industry and the major source of income.

Historical data mention two big floods in the territory of Begec and its surroundings. The first flood occurred in 1926 at the water level of 661 cm when 1,700 ha of arable land were flooded. The second one occurred in 1965 at the water level of 778 cm when 4,400 ha of arable land were flooded. It was the consequence of the collapse of the embankment near the village of Celarevo, 1,283 km upstream from the mouth of the river Danube, along the 88.89-89.19 km stretch of the embankment.

Statistical analysis of data about maximum annual water levels of the Danube recorded at the hydrological station of Novi Sad and at other stations as well, in the period from 1919 to 2008, showed that the extreme values were recorded exactly during the flood wave in 1965. The maximum water level at the station of Novi Sad was 778 cm. The elevation marked '0', above which the water level at the station Novi Sad is measured is at the elevation of 71.73 m. The maximum or absolute height of the Danube in Novi Sad was 71.73 m + 7.78 m = 79.51 m. That is the absolute maximum which was recorded once in eighty-nine measurements. Accordingly, the absolute height of 80 m above sea level is considered a 10-year flood. The absolute height of 78 m above sea level is considered a 10-year flood. These values were determined by means of data processing of maximum water levels using the RStudio application. Since all three values are higher than regular flood defense (400 cm) and emergency flood defense (700 cm) on the Danube near Novi Sad, it is obvious that the hazard exists. The annual probability of the occurrence of 100-, 20- and 10-year floods is 0.01, 0.05 and 0.1, respectively.

Flood simulation for the three specific probabilities of occurrence was performed using Quantum Gis software (QGIS). The required input for simulation is the DEM of the territory of Begec, as well as the map of Begec loaded into QGIS by means of OpenLayers plug-in (Google physical Map was used). A topographic map of the Military Geographical Institute of Serbia from 1970 was used for the analysis of the topography of the terrain and creation of the DEM. While importing the DEM into the QGIS project, MGI 1901/Balkans zone 7 was used as a reference coordinate system.

After giving commands for the presentation of parts of the terrain lower than the specific values of water levels for 10-, 20- and 100-year floods, three vector layers were created. The Figure 2 and Figure 3 show the polygons of the worst possible scenario (100-year flood) and scenario with high probability of occurrence (10-year flood), respectively. For arable land visualization, the CORINE Land Cover vector maps were used (clc06_c211-non-irrigated arable land and clc06_c212-permanently irrigated land) (Figure 3).
The polygon of 100-year flood shows that 43% of the municipality of Novi Sad is potentially endangered, while 10-year flood potentially affect 32% of the municipality's territory.

The created polygons of specific water levels are input data for the spatial analysis of exposure of residential buildings to possible floods. For the purpose of exposure analysis, a vector layer was created which, using position vectors of points, shows the residential buildings of the settlement. By draping the vector layer of residential buildings over vectors of floods of the specific water levels, it was determined which the buildings would be flooded in the case of realization of a 10-, 20- or 100-year flood.

The building chosen for the verification of the model is 76.77 m above sea level. Relating the elevation of the chosen building to the specific values of water levels, the expected flood depths of 1.23 m, 1.83m and 3.23m were determined, respectively.

Using the damage function of German insurance companies, it was determined that the expected degrees of damage to the structure of the object of interest at the given flood depths are 13.39%, 17.53% and 27.19%, respectively. In order to express the degree of damage to the structure of the residential building of interest in monetary units, the depreciated value of the building was determined. The depreciated value of a building is the value of the new building reduced by a certain degree of depreciation. The value of a new building is expressed as the product of the usable area and the price of the square meter of the usable area (€/m²). It was determined in the field that the average usable area of the residential buildings in the zone of investigation is around 90 m², whereas the age of buildings ranges from 50 to 80 years. According to the data from the National Mortgage Insurance Corporation of the Republic of Serbia, in the fourth quarter of 2017 the average price of a square meter in the territory of Begec was 513.50
euro. Thus, the average value of a new building is 46,215 euro.

Since the object of interest was built 75 years ago, in accordance with the regulations of the property tax law of the Republic of Serbia, the depreciation rate is 40%. Relating the depreciated value of the object of interest to the determined degrees of damage, a monetary equivalent of the expected damage is obtained, amounting to 3,712.91 euro, 4,860.89 euro and 7,539.51 euro in this case.

CONCLUSION

With the emergence of GIS technologies, more favorable conditions have been created for the creation and representation of natural and spatial features of relief. With the development and introduction of GIS technologies, a great number of conditionally non-spatial data assumed spatial meaning. This paper described a GIS-based flood damage assessment method. Research showed how GIS technologies can be used for flood damage assessment. Results indicated that expected damage to structure is strongly related to the floodwater depth. There is a linear relationship between deeper depth and greater damage of a structure. In this sense, GIS enables to determine an price of risk which is equivalent to the level of risk. Accordingly, method verification confirmed necessity of geoinformation technologies utilization in the field of damage assessment.

REFERENCES


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RESEARCH OF CONDITIONS FOR MITIGATION OF FLOOD RISK IN VOJVODINA, SERBIA

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ABSTRACT

The main goal of this paper is to analyze conditions for mitigation of risks with catastrophic consequences, especially floods. Respondent’s sense of vulnerability to flooding was analyzed by the influence of independent variables such as Education, Meteo-alarm and Hydro-alarm. Answers were obtained from a total of 490 respondents using questionnaire consisted of various parts and items given in the form of question with offered multiple answers where respondents provided answers at the five-degree Likert scale and evaluation scales. The study provides insight into the actual awareness of population about the existence and potential of use existing mechanism for reduction of risk, prevention and reaction in situations of flood crisis. The results clearly indicate the conclusion that non-structured mitigation of flood risk depends on the education of people endangered by floods, their estimation of their own vulnerability and protection they have from all relevant factors.

KEYWORDS:
Flood, risk, prevention, meteo-alarm, hydro-alarm

INTRODUCTION

Floods are one of the most frequent of all weather-related natural hazards [1]. Generally, floods occur as a result of rainfall patterns changes in different parts of the world, climate changes due to global warming, as well as changes in natural vegetation cover [2]. As such, floods may result as a consequence of gradual increases of water level, abundant rains, specific detrimental event (e.g. hurricane caused flood) or as a consequence of continuous occurrence of rain over a longer period of time. Therefore, floods are defined as unusual amounts of water directly affecting environment inducing changes on the water quality both, on its chemistry (e.g. suspended sediments, reloading of contaminants) and the aquatic communities [3] or as partial or complete inundation of land areas which are otherwise dry [4].

Most frequently, flooding occurs due to out-pour of surface flows caused by characteristics of basin (geologic material, morphology, vegetation and the manner of field usage) as well as non-regulated riverbed. Also, floods can be very frequent due to impact of torrents on downstream and due to rise of underground waters levels. Sudden appearance of maximum discharge in river bed with high concentration of hard phase is known as torrential flood [5]. Primarily, floods endanger people and material goods located near water surfaces (oceans, seas, lakes and rivers) but can endanger more distant areas (e.g. during hurricane).

Having in mind negative consequences of floods on human lives and property [6, 7] and environment as well, there is an increase in demand for flood risk forecasting and protective measures. In general, risk concept consists of the following elements: the event that might occur, it’s environmental context, the damage it might provoke and the incertitude level with respect to the probability of occurrence or extent of damage [8]. According to international standard, risk is defined as a combination of the probability of an event and its consequences [9]. Qualitatively, risk is proportional to the product of risk occurrence probability and risk occurrence consequence.

Flood consequences and flood risk forecast.
Floods are among the most typical hydrological risk phenomena. Flood risk represents the potential damage of hydrological processes to population, goods or environment, where these processes can be expressed quantitatively or qualitatively [10]. According to official reports, 90% of the total natural disaster mortality is water related, while 99% of flood mortality between 1975-2001 (nearly a quarter of a million people) originates from low income population [11]. Coastal areas of Europe are also exposed to the risk of flood. The total value of material goods in coastal areas of Europe in width of 500 meters of coast, including beaches, agricultural land and industrial facilities, is estimated at 500-1,000 billion euros [12]. In 2014 there were 3,064 fatalities as a results of floods while insurance companies lost USD 2,162 million for damage compensation requests [13].

In Great Britain, research show that the flood risk in the next hundred years will significantly increase as well as the number of endangered peo-
ple from 1.6 million currently to 2.3 and 3.6 million by 2080 [14]. Estimates show that in England 1.7 m homes and 130,000 commercial properties are at risk from river or coastal flooding [15]. Another analysis provided forecast of increased falls ranged 10-20 % in 30-year mean values for the period 2071-2100 compared to the means of period 1961-1990 [16].

Implementation of flood risks reduction approach based on the application of natural mechanisms such as restoration of natural habitat i.e. rivers in their natural states [17] represent a sustainable solution for future. This approach is based on the flood risk management within riverbeds and not by limitation of rivers by dams and embankments as well as allowing flooding of rivers in yielded areas with application of influence reduction measures via planned use of land and use of more robust materials. Examples of sustainable solutions of minimizing flood risks via sustainable flood management techniques in Europe are applied in practice such as geomorphic adjustment of river Rhine meanders aimed to protect Netherlands from floods [18]. Regarding cost-effectiveness, it is estimated that the sustainable flood management techniques are ten times more efficient compared to traditional engineeringly based approach [19].

The aim of this paper is to analyze conditions for non-structured mitigation of flood risk that includes a variety of additional pre-flood and post-flood mitigation activities such as preparedness, response, legislature, financing, environmental impact assessment, etc. In this way, by analyzing preliminary data gathered during research, results can provide guidelines on how to, in one hand, improve education of people in endangered areas regarding floods and available national services such as meteo-alarm and hydro-alarm and to, in other hand, provide better management and practices of mitigation regarding floods.

MATERIALS AND METHODS

The subject of the research are attitudes of respondents toward dangers of floods as well as locating of their locus for floods prevention. The problem of this research is to disclosure of relevant predictors of exact responsibility for prevention of flood risks from the perspective of relevantly most endangered population.

Research procedure. In the first part of the research, employees of insurance companies (IC) responded to a short questionnaire. Out of 343 online questionnaires sent to e-mails of respondents, answers were obtained from 159 respondents (response rate=44.83%). Since nine respondents were expelled from processing, the sample of 150 IC employees was formed. Field research formed the final sample of 490 respondents from the areas threatened by floods at the territory of Vojvodina (North Serbia) which indirectly or directly have experience with floods.

Research instrument. The research was conducted using specific questionnaires created for the purposes of this study. The content of the questionnaire was created on the basis of the review of literature on measures of non-structured mitigation. The fundamental assumption based on literature review is that there is a linear connection between the estimation of respondent vulnerability and their motivation for insurance.

Before the beginning of the research, a pilot version of the questionnaire for respondents from endangered areas was formed and distributed to the sample of 100 respondents. By item analysis the questions reducing reliability of the questionnaire were excluded so the final version of the questionnaire was formed and distributed to respondents. The questionnaire consists of various parts and items were given in the form of question with offered multiple answers - questions where respondents provided answers at the five-degree Likert scale and evaluation scales as well.

The questionnaire designed for IC employees consisted of four statements which were estimated on the scale from 1 to 10.

Reliability of both questionnaires is high: the questionnaire used to interview inhabitants of endangered areas is very reliable - Cronbach's alpha=0.899 so the data obtained by examining this sample of respondents is very reliable. The other questionnaire, used for interviewing the IC employees, is ad hoc questionnaire of very high reliability, too: Cronbach's alpha=0.836. Since the research was conducted at the representation sample of 490 respondents including random sample of 150 employees of different IC (Table 1), in this manner two independent panels of respondents were obtained. The sample of respondents from the areas threatened by floods is greatly uniform according to gender, age, and education of respondents so that these features of samples did not affect importantly the obtained research results.

The data from the Table 1 clearly indicates that the respondent sample is greatly uniformed according to gender and place of residence which provide us with objectivity of obtained research data. The age of respondents is also well balanced so these parameters cannot influence reliability of obtained data. It is very interesting that graduates are dominant in this research, both from village and from the city, which would potentially have a positive impact on the credibility of obtained data. Finally, we can see that the majority of respondents are living in near proximity to the river which further contributes to the reliability of the obtained data.
RESEARCH RESULTS

Attitudes of respondents toward dangers of floods, preventive measures, responsibilities and flooding in general are given in Fig. 1 and Table 2.

The obtained results (Fig. 1) clearly indicate the fact that our respondents are not sufficiently introduced and familiar to the concepts of meteo-alarm and hydro-alarm. This condition of insufficient education additionally aggravates the situation regarding floods. It is interesting to notice that this lack of information is more dominant with respondents from urban areas.

The greatest number of respondents unequivocally locates the responsibility of the state for flooding in Serbia during 2014 (Table 2) and clearly believes that it is not a problem that should be solved on a local or provincial level. It is interesting to notice that local structures are also considered responsible for these events, but not to the extent such as state, i.e. republic structures.

TABLE 1
Characteristics of respondents according to place of residence

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Village</th>
<th>City</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender of respondents</td>
<td>Male</td>
<td>91 (40.6%)</td>
<td>133 (59.4%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>123 (46.2%)</td>
<td>143 (53.8%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>214 (43.7%)</td>
<td>276 (56.3%)</td>
</tr>
<tr>
<td>Age of respondents</td>
<td>Up to 20</td>
<td>2 (50.0%)</td>
<td>2 (50.0%)</td>
</tr>
<tr>
<td></td>
<td>From 20 to 30</td>
<td>82 (50.3%)</td>
<td>81 (49.7%)</td>
</tr>
<tr>
<td></td>
<td>From 30 to 40</td>
<td>65 (43.0%)</td>
<td>86 (57.0%)</td>
</tr>
<tr>
<td></td>
<td>From 40 to 50</td>
<td>34 (30.6%)</td>
<td>77 (69.4%)</td>
</tr>
<tr>
<td></td>
<td>From 50 to 60</td>
<td>26 (47.3%)</td>
<td>29 (52.7%)</td>
</tr>
<tr>
<td></td>
<td>over 60</td>
<td>5 (83.3%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>214 (43.7%)</td>
<td>276 (56.3%)</td>
</tr>
<tr>
<td>Respondents education</td>
<td>Primary school</td>
<td>0 (0.0%)</td>
<td>1 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>52 (38.0%)</td>
<td>85 (62.0%)</td>
</tr>
<tr>
<td></td>
<td>Higher school or college</td>
<td>46 (47.4%)</td>
<td>51 (52.6%)</td>
</tr>
<tr>
<td></td>
<td>Graduate studies</td>
<td>73 (47.7%)</td>
<td>80 (52.3%)</td>
</tr>
<tr>
<td></td>
<td>Master</td>
<td>40 (43.0%)</td>
<td>53 (57.0%)</td>
</tr>
<tr>
<td></td>
<td>PhD</td>
<td>3 (33.3%)</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>214 (43.7%)</td>
<td>276 (56.3%)</td>
</tr>
<tr>
<td>Type of water surface in near proximity</td>
<td>None</td>
<td>1 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>Lake</td>
<td>3 (33.3%)</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>Channel</td>
<td>17 (34.7%)</td>
<td>32 (65.3%)</td>
</tr>
<tr>
<td></td>
<td>Sea</td>
<td>1 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>River</td>
<td>192 (44.7%)</td>
<td>238 (55.3%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>214 (43.7%)</td>
<td>276 (56.3%)</td>
</tr>
</tbody>
</table>

FIGURE 1
Responses regarding Meteo-alarm and Hydro-alarm according to place of residence

TABLE 2
Responsibility for floods in 2014 according to place of residence

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Responsibility of state</th>
<th>As</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village</td>
<td>4.94</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>City</td>
<td>4.79</td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.86</td>
<td>490</td>
<td></td>
</tr>
<tr>
<td>Responsibility of province</td>
<td>Village</td>
<td>2.13</td>
<td>214</td>
</tr>
<tr>
<td>City</td>
<td>2.12</td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.12</td>
<td>490</td>
<td></td>
</tr>
<tr>
<td>Responsibility of local government</td>
<td>Village</td>
<td>4.61</td>
<td>214</td>
</tr>
<tr>
<td>City</td>
<td>4.29</td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.43</td>
<td>490</td>
<td></td>
</tr>
<tr>
<td>Responsibility of population</td>
<td>Village</td>
<td>2.21</td>
<td>214</td>
</tr>
<tr>
<td>City</td>
<td>2.30</td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.26</td>
<td>490</td>
<td></td>
</tr>
</tbody>
</table>
The obtained data unequivocally indicate that respondents in rural areas feel significantly more endangered by floods and protected than those in the city. Respondents from urban areas believe that there is a lower level of flood protection by the state than those in villages (Table 3).

The influence of independent variables to the sense of vulnerability to flooding was examined by unilateral variant analysis. It may be indicated that the level of education as well as other independent variables (knowledge of Meteo-alarm and Hydro-alarm) affect the level of sense of flood vulnerability of respondents at the level of the state. The direction of the impact is as follows: the higher level of education the greater impact it is, and such trend refers also to the understanding of meteo-alarm and hydro-alarm concepts (Table 4).

**TABLE 3**
Flood protection level according to place of residence

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Number</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village</td>
<td>214</td>
<td>6.36</td>
</tr>
<tr>
<td>City</td>
<td>275</td>
<td>5.63</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>489</strong></td>
<td><strong>5.94</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Number</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village</td>
<td>214</td>
<td>5.30</td>
</tr>
<tr>
<td>City</td>
<td>275</td>
<td>4.68</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>489</strong></td>
<td><strong>4.95</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>Number</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village</td>
<td>214</td>
<td>1.88</td>
</tr>
<tr>
<td>City</td>
<td>275</td>
<td>2.11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>489</strong></td>
<td><strong>2.01</strong></td>
</tr>
</tbody>
</table>

**TABLE 4**
Influence of independent variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Freedom levels</th>
<th>Main square</th>
<th>F</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td>5</td>
<td>17.651</td>
<td>3.181</td>
<td>.008</td>
</tr>
<tr>
<td>Meteo-alarm</td>
<td>1</td>
<td>254.123</td>
<td>45.799</td>
<td>.000</td>
</tr>
<tr>
<td>Hydro-alarm</td>
<td>1</td>
<td>30.483</td>
<td>5.494</td>
<td>.020</td>
</tr>
</tbody>
</table>

**TABLE 5**
Responses of IC employees

<table>
<thead>
<tr>
<th>Question</th>
<th>Number</th>
<th>As</th>
<th>StdDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>On a scale of 1 to 10 estimate vulnerability of people from floods</td>
<td>150</td>
<td>7.49</td>
<td>2.170</td>
</tr>
<tr>
<td>On a scale of 1 to 10 estimate the level of need for new product</td>
<td>150</td>
<td>7.35</td>
<td>2.092</td>
</tr>
<tr>
<td>On a scale of 1 to 10 estimate the level of mitigation with new product</td>
<td>150</td>
<td>6.95</td>
<td>2.441</td>
</tr>
<tr>
<td>On a scale of 1 to 10 estimate the level of flood protection</td>
<td>150</td>
<td>6.85</td>
<td>2.438</td>
</tr>
</tbody>
</table>

The obtained data unequivocally indicate that respondents in rural areas feel significantly more endangered by floods and protected than those in the city. Respondents from urban areas believe that there is a lower level of flood protection by the state than those in villages (Table 3).

The influence of independent variables to the sense of vulnerability to flooding was examined by unilateral variant analysis. It may be indicated that the level of education as well as other independent variables (knowledge of Meteo-alarm and Hydro-alarm) affect the level of sense of flood vulnerability of respondents at the level of the state. The direction of the impact is as follows: the higher level of education the greater impact it is, and such trend refers also to the understanding of meteo-alarm and hydro-alarm concepts (Table 4).

**Attitudes of IC employees.** Employees completed a short online questionnaire which consisted of only four questions and the fundamental purpose of this questionnaire was their awareness of the need of introduction of a new product which provides exclusively flood insurance.

The obtained data indicate that IC employees are aware of people’s flood vulnerability (As=7.49) as well as the need for introduction of a new product which would refer only to the flood insurance (As=7.35). Also, we may see that it is evident to them that this manner would lead to risk mitigation but also of the awareness that other relevant factors would have to participate in this process in order to increase the actual flood protection of the people (Table 5). It is interesting that standard deviations with all answers are very high (over 2) which indicates to great variations in these estimations, which is somewhat clear considering the policy of IC on these questions so far.

**SEM Analysis.** For the testing purposes of obtained values, results were subject of Structural Equation Modelling - SEM analysis [20]. Two independent panels of respondents were integrated. The dependent variable is non-structured flood risk mitigation whereas independent variables of flood threat, education of population and responsibility for protection, protectiveness and introduction of a new product of IC.

Basic parameters of SEM analysis indicate that the proposed model is statistically important and new relevant parameters indicating significance of analysis within the limits which make this model acceptable. Table 6 presents the review of these parameters.

Values from the Table 6 explicitly shows that non-structured mitigation mostly depends on the introduction of a new product since this independent variable has the biggest path coefficient (0.144), the importance itself is smaller than 0.001 and the size of effect is the greatest.

Further, the independent variable of responsibility is isolated and its parameters are very similar to the variable of introduction of a new product (Table 7). Regardless of the fact that other inde-
pended variables have statistically important impact on mitigation of these two independent variables according to all relevant criteria, they have the biggest and the most important impact.

The independent variable of vulnerability has a negative path coefficient and this finding suggests that the bigger the vulnerability evaluation the smaller non-structured mitigation is.

**DISCUSSION**

The low level of education of respondents regarding floods is very concerning. This is a problem that requires special attention because results show that many endangered respondents do not know what meteo-alarm and hydro-alarm are. The education which would lead to mitigation should be on a medium level because over-education does not lead to mitigation, but it decreases it on the contrary (Fig. 2). Information and education of population are necessary preconditions for efficient implementation of flood defense. Apart from that, the additional improvement of professionals is needed, including the issue of flood protection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Size</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average path coefficient (APC)</td>
<td>0.273</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average R-squared (ARS)</td>
<td>0.203</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average adjusted R-squared (AARS)</td>
<td>0.201</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average block VIF (AVIF)</td>
<td>1.091</td>
<td></td>
</tr>
<tr>
<td>Average full collinearity VIF (AFVIF)</td>
<td>2.443</td>
<td></td>
</tr>
<tr>
<td>Tenenhaus GoF (GoF)</td>
<td>0.429</td>
<td></td>
</tr>
<tr>
<td>Sympon’s paradox ratio (SPR)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>R-squared contribution ratio (RSCR)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Statistical suppression ratio (SSR)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Nonlinear bivariate causality direction ratio (NLBCDR)</td>
<td>1.000</td>
<td>Acceptable if &gt;= 0.7</td>
</tr>
</tbody>
</table>

**TABLE 7**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Path coefficient</th>
<th>Importance</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responsibility</td>
<td>0.129</td>
<td>&lt;0.001</td>
<td>0.016</td>
</tr>
<tr>
<td>New product</td>
<td>0.144</td>
<td>&lt;0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>Vulnerability</td>
<td>-0.112</td>
<td>0.003</td>
<td>0.013</td>
</tr>
<tr>
<td>Protection</td>
<td>-0.090</td>
<td>0.012</td>
<td>0.011</td>
</tr>
<tr>
<td>Education</td>
<td>0.097</td>
<td>0.008</td>
<td>0.012</td>
</tr>
</tbody>
</table>

**FIGURE 2**

Education and mitigation relation
The basis of future development of flood protection is the identification of social and individual responsibility in relation to flood risk. Namely, it is necessary to determine which risks have social features so that they have to be taken care of by the state and which have private features so the individuals living in areas with flood potential should take care about them. At the same time, it should be aimed at establishment of righteous relations between state measures and actions trusted to the care of individuals, paying attention to financial possibilities of both sides. In any case, the understanding so far that flood protection is exclusively the responsibility of the state and it must ensure safety of all inhabitants of river coastal areas regardless of their frequently irresponsible behavior, failure to adhere to conditions, limitations and construction prohibition, must be changed gradually.

It is interesting to notice that the sense of protection dramatically increases the mitigation and it's obvious almost linear relation of these two variables. However, it is more probable that the respondents feel very unprotected (Fig. 3).

Introduction of a new product of flood insurance would have to be incremental innovation with wider effects on society, not just on appearance improvement of IC or increase of their profit. Fig. 4 shows that the relation of mitigation and introduction of a new insurance product is not linear but by increase of probability of its occurrence the level of mitigation increases, too.
The proper distribution of responsibility in the sphere of flood would overcome the reactive approach to crisis situation so far and transfer to anticipatory approach to this problem would also significantly increase mitigation of flood risk. This approach would certainly greatly reduce the sense of flood vulnerability of population and therefore return their trust in all relevant institutions dealing with this problem. Surely, it is also necessary to increase the quantity of structural investment into protection in order to have the existing condition.

CONCLUSION

The results of research clearly indicate the conclusion that non-structured mitigation of flood risk depends on the education of people endangered by floods, their estimation of their own vulnerability, protection they have from all relevant factor which should deal with this problem and adequate distribution of responsibility of all relevant factors. Also, mitigation of flood risk depends also on the insurance companies which should introduce a completely new product in all endangered zones: flood insurance.

Also, it is necessary to work on the education of people in endangered areas, to clarify all concepts concerning flood and primarily tools such as meteo-alarm and hydro-alarm, to boost the feeling of solidarity toward people. At the same time, it is necessary to create a clear strategy regarding flood protection and prevention at the state level, which must be clearly implemented and supported and implemented also locally and to realize the synergy of the state, population and insurance companies as the most important factors in protection and prevention, which has not been the case so far. Finally, affirmative campaign on the state level should be implemented, which would be based on above mentioned basis.

ACKNOWLEDGEMENTS

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EFFICACY AND SAFETY OF PROBIOTICS ON FUNCTIONAL CONSTIPATION: A META ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Department of Integrated Traditional Chinese and Western Medicine, West China Hospital of Sichuan University, Chengdu, 610041, China

ABSTRACT

Existing literatures of Randomized Control Trials (RCTs) were reviewed in the study to ascertain the efficacy and safety of probiotics for functional constipation. Cochrane Library, PubMed, EMBASE, and Clinical Trials.gov databases were searched for controlled trials from the earliest record in each database to October, 2017. Stool frequency, stool consistency and the incidence of adverse events were analyzed as endpoints. Seven RCTs involving 450 patients met the inclusion criteria. The endpoints include stool frequency, stool consistency and the incidence of adverse events. Compare with placebo used, probiotics associated with higher stool frequency compare with placebo used in functional constipation patients (SMD = 0.97; 95% CI = 0.31–1.64; P <0.00001; I² = 89%) after 4 week treatments. There are no difference between probiotics and placebo used in functional constipation patients on stool consistency (SMD = -0.22; 95% CI = -1.40–0.96; P <0.00001; I² = 96%) and adverse events (RR = 0.87; 95% CI = 0.65–1.14; P =0.72; I² = 0%) after 4 week treatments. Probiotics can increase the stool frequency of patients of functional constipation compared with similar adverse events and stool consistency versus placebo after 4 week treatments.

KEYWORDS:
Functional constipation, Meta-analysis, Probiotics

INTRODUCTION

Functional constipation with the clinical manifestations of defecation difficulties, excrement dry, defecation endless and reduced defecation frequency. The disease incidence ranges from 0.7 to 29.6% globally and it was estimated that functional constipation will reach 3.2 million visits from the estimates of medical centers in the United States in 2012 [1, 2]. Although functional constipation does not affect the life expectancy, but it reduces the quality of life of patients and increase medical costs [3, 4]. Traditional treatments for functional constipation include changing life and eating habits, consuming cellulose, lubricating the intestine, applying laxatives, leavening agents, etc., but about one in three patients do not receive the desired effect [5]. Probiotics are organisms that have a beneficial effect on the host and the most common probiotics is lactobacillus and bifidobacteria. Probiotics engraftment in intestine, and its metabolites of lactic acid, acetic acid, short chain fatty acids and other organic acids can reduce intestinal pH value, increase the intestinal peristalsis, shorten the colon through time, thus has certain therapeutic effect for constipation [6]. With the interest in probiotics adjunct to lactulose in treating constipation is growing, the efficacy and safety of the probiotics is conventional. In 2005, Benninga et al. [7] conducted a pilot study in which reported that probiotics had positive effects on constipation that increase defecation frequency and decrease the frequency of stool incontinence. However, another study by Bu et al. [8] state that there are no significant difference in the efficacy between probiotics and placebo.

Our meta-analysis is undertaken to evaluate the efficacy and safety of probiotics used in patients with functional constipation after 4 week treatments.

METHODS

Data Sources and Searches. We searched PubMed, Cochrane Library, Clinical Trials.gov databases and EMBASE from database inception until October 2017 with the keywords of “functional constipation”, “probiotics”, “synbiotic” and “bifidobacterium lactis”. Randomized controlled trials was utilized as a sensitive filter for the search. References from literatures were hand-searched for additional articles which were not identified in the searching of database.

Study Selection. The following inclusion criteria were applied: (1) patients associated with functional constipation; (2) randomized controlled trials of probiotics versus placebo or probiotics adjunct to common therapy; (3) clinical outcomes
were reported (such as stool frequency, stool consistency and the incidence of adverse events; (4) the treatment time is 4 week. The literature with the most comprehensive data was included if there were duplicate studies from the same trial and similar outcomes were reported. Reviews, meta-analyses, editorials, observational studies, and studies in which it was not possible to assess the outcomes or lacked a control group were excluded. The meta-analysis was complied with Preferred Reporting Items For Systematic Review and Meta-analysis (PRISMA) [9].

Data Extraction and Quality Assessment. Data were extracted from the relevant sources by two independent investigators and disagreements was resolved by consulting a third investigator to achieve consensus. We collected baseline demographic characteristics of the patients (sample size, age and sex in the experimental and control group) from eligible studies. Data of the following events in patients were abstracted: stool frequency, stool consistency and adverse events. Quality access information of each study was clarified as low, unclear, high by evaluating the following seven characters: generation of random sequence, concealment of allocation, blinding of participants and outcome assessment, incomplete outcome data, selective outcome reporting and other issues according to Cochrane Handbook.

Data Analysis. Risk ratio (RR) and the 95% confidence intervals (CIs) was used to measure the difference of binary outcomes. Standard mean differences (SMDs) and the 95% CI was used to measure the differences in continuous outcomes. Statistically significant was considered when two-sided P<0.5 and random-effect model was used for pooled estimate. Publication bias were assessed with Begg's test. RevMan 5.2 software (Nordic Cochrane Centre, Cochrane Collaboration, 2013) was used for data analysis and sensitivity analysis was performed by Stata 11.0 (StataCorp, College Station, TX, USA).

RESULTS

Search results. We identified a total of 691 articles with 7 trials [8, 10-15] that satisfied our inclusion criteria and details of selection procedure depicted in Figure 1. There are 233 patients were randomized to probiotics group, and 217 patients were randomized to control group. Baseline characteristics of included studies are detailed in Table 1. Quality assessment characters are presented in Figure 1 and Figure 2. All the included were associated with a low risk of random sequence generation, incomplete outcome data and selective outcome reporting. However, eight trials were with an unclear risk of allocation concealment, other bias, blinding of participant and outcome assessment.

TABLE 1
Baseline characteristics of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Age</th>
<th>Male/Female</th>
<th>Treatment</th>
<th>Treatment Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bu</td>
<td>T C T C T C</td>
<td>T C</td>
<td>T C</td>
<td>Lcr35, 8 × 10^8 c.f.u./day</td>
<td>Placebo 4 week</td>
</tr>
<tr>
<td>2007</td>
<td>18 9 3.1±1.2 2.9±1.2 10/8 4/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khodadad</td>
<td>2010</td>
<td>37 29 5.9±2.2 6.9±2.4 15/14 13/24</td>
<td>Liquid paraffin+Symbiotics</td>
<td>Liquid paraffin+Placebo</td>
<td>4 week</td>
</tr>
<tr>
<td>Sadeghzadeh</td>
<td>2014</td>
<td>24 24 6.1±2.4 6.3±1.9 14/10 10/14</td>
<td>Lactulose+Protexin</td>
<td>Lactulose+Placebo</td>
<td>4 week</td>
</tr>
<tr>
<td>Tabbers</td>
<td>2011</td>
<td>79 80 7.0±3.4 6.5±3.1 42/37 41/39</td>
<td>Probiotic Minas Frescal cheese plus Bifidobacterium lactis Bi-07plus Bifidobacterium lactis Bi-07</td>
<td>Placebo 3 week</td>
<td></td>
</tr>
<tr>
<td>Favretto</td>
<td>2013</td>
<td>15 15 37.5±14 .4 40.8±12.8 / /</td>
<td>Minas Frescal cheese without the addition of probiotics</td>
<td>30d</td>
<td></td>
</tr>
<tr>
<td>Mazlyn</td>
<td>2013</td>
<td>30 50 31.8±9.4 31.7±9.4 8/42 14/36</td>
<td>Milk with LcS Lactobacillus plantarum and Bifidobacterium breve or Bi dobacterium lactis</td>
<td>Placebo 4 week</td>
<td></td>
</tr>
<tr>
<td>Ojetti</td>
<td>2014</td>
<td>20 20 34.5±16 36.7±14 9/11 7/13</td>
<td></td>
<td>Placebo 4 week</td>
<td></td>
</tr>
</tbody>
</table>

T: treatment group
C: control group
FIGURE 1
Flow chart of literature retrieval and selection

SFIGURE 1
Risk of bias summary
Clinical results. Stool frequency. There are seven clinical investigations and 450 patients with 233 patients randomized to probiotics arm and 217 patients randomized to placebo arm reported the endpoint of stool frequency. Compare with placebo used, probiotics associated with higher stool frequency compare with placebo used in functional constipation patients (SMD = 0.97; 95% CI = 0.31–1.64; \( P <0.00001; I^2 = 89% \)) after 4 week treatments as shown in Figure 2.

Stool consistency. There are three clinical investigations and 307 patients with 153 patients randomized to probiotics arm and 154 patients randomized to placebo arm reported the endpoint of stool consistency. There was no difference between probiotics and placebo used in functional constipation patients after 4 week treatments when random effect used (SMD = -0.22; 95% CI = -1.40–0.96; \( P <0.00001; I^2 = 96% \)) as shown in Figure 3.

Adverse events. There are four clinical investigations and 352 patients with 184 patients randomized to probiotics arm and 168 patients randomized to placebo arm reported the endpoint of stool consistency. The most common adverse events of probiotics are vomit and nausea. There was no difference between probiotics and placebo used in functional constipation patients after 4 week treatments when fixed effect used (RR = 0.87; 95% CI = 0.65–1.14; \( P =0.72; I^2 = 0% \)) as shown in Figure 4.

Sensitivity analysis. Sensitivity analysis was conducted by excluding each individual study and we found similar result was found when excluded each individual study which indicate that the outcome of our analysis is stable. Details shown in Figure 5.

DISCUSSION

This meta-analysis includes 450 patients who under probiotics therapy with 237 patients and 213 patients under placebo therapy within 7 RCTs. In this meta-analysis, we found that there are no significant difference between probiotics therapy and placebo on endpoints of stool frequency, stool consistency and adverse events for functional constipation patients after 4 week treatments.
FIGURE 2

Forest plot of stool frequency

FIGURE 3

Forest plot of stool consistency

FIGURE 4

Forest plot of adverse events

FIGURE 5

Sensitivity analysis
Probiotics is a new kind of therapy for functional constipation and there are series of clinical trials reported the clinical use of probiotics. Probiotics were reported associated with potential positive activities for constipation in children. Studies conducted by Benninga et al. [7], Sadeghzadeh et al. [11] and Bekkali et al. [16] reported that probiotics may be a new option of constipation medical therapy and it had positive effects of defecation frequency increasing and the stool consistency. However, studies by Bu et al. [8] demonstrated that there are no significant difference between probiotics group and control group on the efficacy of treatment in children with chronic constipation. Another double-blind RCT conducted by Tabbers et al. [12] showed that probiotics increased the stool frequency without serious side effects. And in the study of Saneian et al. [17] which stated that probiotics can improve symptoms of constipation in children with no side effects. Our meta-analysis included the newest clinical investigations of probiotics used in patients with functional constipation and indicated that patients under 4 week probiotics associated with higher stool frequency and same adverse events compare with placebo which is similar with the meta-analysis conducted by Eirini Dimidi et al. [18] Compared with that meta-analysis, we included more clinical investigations about children and unified the use time of probiotics which may be more meaningful for clinic. There are also some limitations of this study. Firstly, due to the limited number of sample sizes, the power of our meta-analysis was restricted. Secondly, the different kind of probiotics used in difference investigations may have affected our outcome. Finally, several of the trials we included lack of detailed descriptions of allocation concealment and blinding that might have affected the quality of our analysis. Therefore, more rigorous, large-sample and internationalized trials are needed to confirm our results.

CONCLUSION

Probiotics can increase the stool frequency of patients of functional constipation compared with similar adverse events and stool consistency versus placebo after 4 week treatments.

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REFERENCES


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AN INVENTORY OF FISHING GEARS USED IN THE NORTH EASTERN MEDITERRANEAN AND CHANGES FROM 2008 TO 2018

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ABSTRACT

This study aims to find out varieties and amounts of fishing gears used in the north-eastern Mediterranean, and compare them with those used ten years ago. The work was conducted in 20 fishing harbours in Mersin, Adana and Hatay provinces of Turkey in 2008 and 2018. Numbers of vessels using each specific gear and average amounts of each gear in a vessel were obtained by interviews with either head of the relevant fishery cooperatives or with at least ten years experienced fishermen in each harbour. Technical specifications of each gear as well as fisher’s problems regarding structure and operation of them were also recorded.

Results show that in total 35 different gear types have been used in the area. The most commonly used fishing gears are trammel nets, gill nets, thick and thin long lines, hand lines, pots, demersal trawls and purse seines. Despite used by small scale fishers, cumulative amount of some of these gears are rather significant. Among them total lengths of sole trammel nets for example reaches up to 1697 km which is 2.6 times longer than the length of the coast lines of these three provinces. Amounts of the gears used in 2008 and 2018 have shown significant changes. Reasons for the changes and role of applied regulations in sustainability of the commercial fishing activity in the north-eastern Mediterranean are discussed.

KEYWORDS:
Fishing gears, inventory, north-east Mediterranean

INTRODUCTION

With increasing concern about the excessive fishing inputs in world fisheries, the issue of managing fishing capacity was formally raised in 1997 by the FAO COFI [1]. The catching capacity of fishing fleets can be described as the product of the fishing effort and the combined efficiency of the fishing gear and the fishing vessel, as well as the skills of the crew [2]. Despite a long history of intensive commercial fishing, even the basic information such as varieties and amounts of fishing gears used in world fisheries is rather limited in literature. However, due to changes in the amounts of the natural stocks and exploitation patterns, types, amounts and in some cases operation of the fishing gears may change.

Fisheries in the north-eastern Mediterranean have multi species and multi gear nature [3]. Number of the marketed species captured in the area exceeds sixty [4]. This number can be as high as thirty only in one demersal trawl haul [5]. Area is very dynamic in terms of species composition due to Lessepsian migration [6] and intensive fishing [4].

Fishing gear restrictions and species bans implemented for the sustainability of the resources and fisheries, naturally forces fishers to modify and/or change their vessels, gears and operations in this area. Therefore, there is a need to keep a record of an inventory of actively used gears to be able to monitor the shifts in fishing practices. Official records, particularly in the case of small scale fisheries, usually do not meet this need mainly because some leisure fishing vessels hold commercial fishing licence while some unlicensed vessels or leisure vessels act as commercial fishers. Moreover, these operational changes are rather dynamic both spatially and temporally.

This study investigates the varieties and amounts of the fishing gears used in the north-eastern Mediterranean where the fisheries has a multi species and multi gear nature. The aim also extends to find out the changes in the types and amounts of the gears used in past ten years. Then this information is related to the regulatory changes implemented during this period.

MATERIALS AND METHODS

Field investigations were conducted in 20 fishing harbours in Mersin, Adana and Hatay provinces of Turkey (Figure 1), where total length of coast lines is 656 km. Fishing gears were inspected, photographed and measured at the harbours and operational information as well as problems regarding their use were obtained from fishers. Numbers of vessels using each specific gear and average amounts of each gear in a vessel were obtained by
interviews with either head of the relevant fishery cooperatives or with at least ten years experienced fishermen in each harbour. Only the gears of the vessels belonging to visited harbours and actively involved in commercial fishery were investigated and counted. The gears belonging to vessels based elsewhere or the vessels officially licensed but commercially inactive for various reasons were excluded from the study. The investigations were first conducted in 2008, repeated in 2018 by paying particular attention to the changes in the same harbours.

RESULTS

A total of 35 different gear types were found to be used in the north eastern Mediterranean commercial fisheries. These can be first grouped in two; gears operated by large and small scale vessels. All the purse seines and demersal trawls are used by vessels over 12 m overall length (OAL), while almost all the gill and trammel nets, hooks and pots are operated by vessels smaller than 12 m OAL. Purse seiners and trawlers may occasionally use other gears with seasonally obtained special permissions. A great majority of the small scale vessels use a variety of gill and trammel nets, and longlines depending on the season and presence of the target species in their operational grounds. Purse seines and trawlers often shift their departure and landing ports within the north east Mediterranean, and occasionally work in the Aegean and western Mediterranean coastal waters of Turkey. However, depending on amount of quota, up to 30 large purse seiners, bigger than 30 m OAL, registered in other ports come to north east Mediterranean targeting blue fin tuna (Thunnus thynnus) in late spring for a month. Other than tuna purse seiners, some Egyptian, Syrian and Italian demersal trawlers and pelagic long liners are reported to operate outside 12 miles Turkish coastal waters. However, information on numbers and operational capacities of these vessels are not available.

Despite 35 different gear types were identified in field investigations, some of them were used by only few vessels and in low amounts. Table 1 shows types of gears used by vessels based in the north-east Mediterranean harbours, numbers of the cooperatives and vessels which they have been used, and their total amounts in 2008 and 2018. The table excludes the gears operated by less than 10 vessels. The gears listed in the table are grouped in six main categories: Purse seines, demersal trawls, gill nets, trammel nets, hooks and pots. Figure 2 shows the changes in the total amounts of gears used in each of these categories where purse seines and demersal trawls assumed to use only one gear, although they may have more than one for different target species and/or fishing grounds but use only one gear at each operation.

Presently 35 purse seiners based in the North eastern Mediterranean ports are operating in the area. This number has not shown a significant change since 2008. Although some purse seiners are small (12-15 m in total lengths) and operate their gears by hand without mechanisation and allowed to fish with a smaller net in shallower areas, majority of them are large scale vessels (up to 35 m in length), haul in the gear with winches and power blocks and are banned to fish at shallower than 24 m depth. Despite their targets change seasonally, the main target species is sardine (Sardina pilchardus) and bunt of the most of purse seines are made of 12 mm mesh size accordingly. Purse seining has a 5-month seasonal closure starting from 15th April, but some large vessels may be given special permission for catching several species of
tunas in certain areas and periods during the seasonal closure [7]. Purse seiners report the biggest problems with their operations as prohibition of use of artificial lights during their operations, and 24 m depth limitation which pushes them offshore, particularly off Karatas, where the fishing ground is rather shallow, and 24 depths is reached over 5 miles from the shore.

**TABLE 1**

<table>
<thead>
<tr>
<th>Fishing Gear</th>
<th>Number of the Cooperatives</th>
<th>Number of Vessels</th>
<th>Total Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
<td>2018</td>
<td>2008</td>
</tr>
<tr>
<td>Purse Seine</td>
<td>2</td>
<td>5</td>
<td>34</td>
</tr>
<tr>
<td>Demersal Trawl</td>
<td>7</td>
<td>10</td>
<td>220</td>
</tr>
<tr>
<td>Bonito Net*</td>
<td>11</td>
<td>11</td>
<td>181</td>
</tr>
<tr>
<td>Blue Fish Net*</td>
<td>2</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Sardine Net*</td>
<td>1</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Red Mullet Net*</td>
<td>6</td>
<td>6</td>
<td>63</td>
</tr>
<tr>
<td>Monofilament</td>
<td>3</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>Dentex Net*</td>
<td>5</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Sole Net**</td>
<td>13</td>
<td>15</td>
<td>357</td>
</tr>
<tr>
<td>Prawn Net**</td>
<td>13</td>
<td>15</td>
<td>310</td>
</tr>
<tr>
<td>Red Mullet Net**</td>
<td>3</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>Grey Mullet Net**</td>
<td>1</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Monofilament</td>
<td>2</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Common Pandora Net**</td>
<td>1</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Cuttle Fish Net**</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Reef Net**</td>
<td>1</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Melozma Net**</td>
<td>9</td>
<td>8</td>
<td>103</td>
</tr>
<tr>
<td>Thin Longline</td>
<td>16</td>
<td>19</td>
<td>414</td>
</tr>
<tr>
<td>Thick Longline</td>
<td>18</td>
<td>17</td>
<td>679</td>
</tr>
<tr>
<td>Handline</td>
<td>5</td>
<td>3</td>
<td>205</td>
</tr>
<tr>
<td>Vertical Line</td>
<td>1</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Trolling Line</td>
<td>2</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Pot/Trap</td>
<td>6</td>
<td>3</td>
<td>100</td>
</tr>
</tbody>
</table>

**FIGURE 2**

Changes in the total amounts of gears used in each main categories of fishing gears used by Turkish north eastern Mediterranean fishing vessels
Demersal trawls are the only towed nets commercially used in the area. Their numbers have shown about 5% reduction since 2008. Despite many trawlers were sold following implementation of buyback programme started in 2012, some fishers bought inactive vessels from elsewhere and continue trawling in the area. Demersal trawl gears used in these grounds are relatively similar in size, the most common one being 600 meshes (44 mm) around the mouth conventional Mediterranean type trawls which have 44 mm codend mesh size and rigged with either single or double ground gears used on soft and hard bottoms, respectively [8]. Although lands more than 30 commercial species [9], red mullet (*Mullus barbatus*) and green tiger prawn (*Penaeus semisulcatus*) make up more than half of the income in north-eastern Mediterranean demersal fisheries [5]. Trawlers report that there is need to modernize presently used demersal trawl gears, particularly otter boards for more efficient fishing. Similar to purse seining, there is a 5-months summer seasonal closure from 15th April, and near shore area restrictions for trawling in the north-eastern Mediterranean. However, special permissions are usually given until 15th July for outside territorial waters, 12 miles from the coast. Trawling is permitted only for vessels larger than 12 m OAL. However, illegal trawling locally called “şebekê” by small vessels with high engine power in near shore closed areas and before the beginning of the legal trawling season is among one of the main complains of the fishers.

Almost all the other gears than purse seine and trawls are operated by small scale fishing boats, smaller than 12 m OAL, but typically 6-8 m OAL and 20-60 HP engine power. These boats operate several types of gears depending on the season and location. Table 1 shows the number of the vessels using different types of gears, both in 2008 and 2018. Gears which are used in less than 10 vessels are excluded from this Table. As can be seen from the Table 1, six types of gill nets and nine types of trammel nets are commonly used by more than ten vessels. These nets are usually named with the target species, their technical specifications vary and can be found with technical drawings in Özbilgin et al. [4;3]. Among these 15 types of gill and trammel nets total amounts of sole and prawn nets are almost ten folds higher than all the others, and their use has shown an increase since 2008. Almost all the gill and trammel nets used in the area are rigged with a factor of 0.5, and a pack of un-rigged netting is 200 m in length. Therefore, ten units of rigged gill or trammel nets can be assumed as 1 km in length.

There are two main types of longlines being used in the area; thin and thick longlines. While thin long lines operate near shore and use small hooks and mainly target near minimum landing size sparid species, thick longlines mainly target white grouper (*Ephinephelus aeneus*) for which a species ban was implemented in August 2016 [7]. Therefore, a significant reduction both in number of the vessels and amount of the hooks are detected in 2018 (Table 1). The other types of fishing with hooks recorded in this study are not intensive, most of the times conducted by commercial fishers involved in fisheries as a secondary occupation. Most of them sell their catch and hold professional licence but usually act like leisure fishers who has other incomes.

Pots in comparison to many other fishing gears are known to be more selective but less efficient fishing gears (10). In the north east Mediterranean fisheries they are called ‘kafes’ which means ‘cage’. They are constructed by thin wire meshes mounted on iron frames, and usually target groupers. Despite pots were not illegal, capture of groupers by pots had long been forbidden. In 2016, use of pots in the Turkish Mediterranean fisheries forbidden [7]. Therefore, their number in 2018 has shown a significant reduction in comparison to 2008. But they are reported to be used by few fishers in 2018 surveys. Pots are usually used in summer months and always remains at sea during the season. Therefore, difficult to be detected by control agencies. Fishers report that as the thin wire meshes perish in 2-4 months, this types of pots do not have a ghost fishing problem in the long term.

**DISCUSSION**

Despite its importance in calculations of fishing or capture capacity, information on numbers of the actively used fishing gears (regardless of them being legal or illegal) and their trends with changing conditions in fisheries is rather limited. Several studies conducted in the area provides data on particular gears [11], and state statistics report registered vessel information. However, due to lack of full list of actively used gear inventory, mechanism of informed decision making is hampered. The data presented in this paper provides such information on gear inventory for the north-eastern Mediterranean based on field inspections and fisherman interviews. Information provided by fishers regarding amounts of the gears can be more, or less than the actual numbers sometimes. The main reasons for reducing the actual figures are “being scared of having to pay additional taxes, and/or being scared of having to face new fishery limitations”, and reason for exaggerating the figures are “expectations of investment, such as better port facilities”.

The north eastern coastal waters of the Mediterranean, particularly Iskenderun and Mersin Bays are among the most fertile fishing areas of the Mediterranean Sea which is under heavy pressure of pollution, alien species, over fishing and illegal fishing activities [12]. Data collected in this study
reflects the present state and the recent changes in the use of commercial fishing gears in these fertile fishing grounds. Although amounts of some gear types have remained relatively similar during the last decade, some of them have shown significant changes since 2008. This study shows that most of these changes are direct or indirect outcomes of management measures aiming at sustainability of the fisheries and resources, despite some other factors such as decreasing abundance of the target species, loss of fishing grounds to other sectors, and insufficient recruitment of young fishers to aging and retired members.

The most significant reductions are observed in monofilament gill and trammel nets, pots and thick long lines. Main reason for these changes were banning of monofilament nets in 2012 [13], banning of pots [7] and species ban for white grouper [7] which is the main target species of the thick long liners. Following the white grouper species ban, many long liners had to stop their conventional fishing activity and started to construct and use sole and prawn trammel nets (Table 1). Some of these boats with powerful engines are also reported to be illegally trawling in nearshore waters, however, data for these activity were not requested as it could be biased. Another significant reduction is seen in the numbers of hooks used by thin long liners. Main reason for this change was reported as increased abundance of alien species puffer fishes (Lagocephalus sp.) in the area. As they cut the lines with their sharp teeth and cause loss of the catch and gear, amount of the hooks used in this fishery have shown a reduction. Another regulation regarding 6000 m maximum length of gill and trammel net limitation has entered into force in 2016 [7], but has hardly shown any significant effect in total numbers of these gears in this area yet, mainly due to lack of strict implementation.

Despite the buyback programmes first implemented in June 2012 for fishing vessels bigger than 12 m [14], and many large scale, wooden, aged vessels were taken out of the fleet, numbers of the active purse seiners remained almost the same and demersal trawlers reduced only about 5%. This reduction was expected to be more pronounced in relatively large scale fishery. However, many owners who sold their boats to the state, bought relatively inactive steel vessels from other parts of the country, and continue their operation in the area, working even more intensively to pay their depts for vessel replacement.

This study shows that the north east Mediterranean is a heavily exploited ground, fished by 35 purse seiners, more than 200 demersal trawlers, 345 km length of gill nets, almost 3000 km length of trammel nets and more than half a million hooks. No comprehensive study of ratio of lost gears in this area is found in the literature. However, Özyurt et al. [15] calculated average effective loss rate per fishing boat for prawn and sole nets as 8.5 % in İskenderun Bay. If this ratio is applied to total amount of the prawn and sole nets estimated in the present study, amount of only these two types lost nets is found as 2349 units annually. This makes 235 km long lost prawn and sole nets, which is longer than a third of the length of total coast lines of these three provinces. This value is too large to be ignored. Additionally, contribution of lost gears to marine pollution has caused great concerns in the area [16]. Therefore, ghost netting potential of the gears used in the area (17) has to be studied in earliest possible time and relative management measures have to be applied.

Information presented in this study, provides not only the present amount of the active gears reported by fishers, but also reflects the response of the fleet to management measures and changes in fishing conditions. Such data is very dynamic and critical for decision makers. Therefore, it needs to be regularly updated, and to ensure the accuracy of the data, close communication and cooperation with active fishers have to be maintained.

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EVALUATION OF YIELD TRAITS, QUALITY PARAMETERS AND SEED YIELD STABILITY FOR LENTIL GENOTYPES IN DIFFERENT LOCATIONS IN TURKEY

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ABSTRACT

Lentil is conventionally grown as a rain fed crop, mostly in the Middle East; it’s a rich source of protein for human feeding. Yield, seed quality, adaptability and chalky spot syndrome of fifteen lentil genotypes and five lentil cultivars were evaluated in the South-Eastern Anatolia of Turkey. Yield and yield traits as well as seed quality such as protein content, seed rate with chalky spotted and germination rate for of all genotypes were varied significantly. Seed yield were varied from 1337 to 2142 kg/ha among the genotypes. While cultivar ‘Sakar’ produced the maximum yield and cultivar ‘BM 848’ produced the minimum seed yield. It was also revealed that genotypes ‘ILL10975’, ‘FLIP2010-94L’ and cultivar ‘Sakar’ was found the most sensitive to cold. Lentil crops with chalky spotted were detected in Adiyaman location and its rate ranged from 42% to 13%. Genotype × environment interaction was significant for seed yield and different stability parameters were computed for genotype adaptability. Ecovalance (W²i) and stability variance (σ²i) were low value in genotypes ILL 3375 and Kafkas, but Firat 87 had high values.

KEYWORDS:
Chalky spot syndrome, lens culinaris, lentil, seed quality, stability

INTRODUCTION

Lentil (Lens culinaris Medik.) is traditionally grown as a rain fed crop, particularly in the Middle East and four major lentil producing countries in decreasing order are India, Canada, Turkey and Iran [1]. Turkey mainly produces red lentil, and it is a very important pulse crop in South-eastern Anatolia of Turkey, and Sanliurfa, Diyarbakir and Mardin is the leading lentil producers [2]. Lentil is important source of protein, minerals and vitamins in human diet [3]. Furthermore, its mineral- and carbohydrate-rich straw provides valuable animal fodder [4]. Lentil has mostly been influenced by global climate changing, particularly, due to rainfall and environmentally variability. Seed yield is the primary objective in most lentils breeding programs. Seed yield is a result of the interaction between genetic and environmental conditions. Lentil cultivation is affected by the climate conditions in rainfed conditions, and rainfall is an important environmental condition in arid and semi-arid areas. Lentil genotypes are low yielding due to low rainfall and environment sensitive genotypes. So, it is essential for plant breeders to identify specific genotypes adapted or stable to environment. A significant genotype × environment interaction for grain yield can limit efforts in selecting superior genotypes for both new cultivar introduction and improved genotype development [5]. A number of models for the statistical measurement of the stability have been proposed by several researches [6, 7, 8, 9], but researchers reported that single method could not adequately explain cultivar performing across environments [10]. Finlay and Wilkinson [6] used the coefficient of regression (b) as a stability parameter; they reported the regression coefficients can be used to describe the response of different cultivars to environments. The coefficient of variation [9] is used to select cultivars that have both high yield and low variance (a small among-environment variance). Wricke’s ecovariance [7] suggested using genotype environment interactions for each genotype as a stability measure. Shukla [11] used stability variance of genotypes for defining stability of a genotype.

The chalky spot syndrome in lentil caused by the lygus bugs a serious seed-quality problem in lentil cultivation in Southeast Anatolia of Turkey [12]. Akkaya [13] revealed Piezodorus lituratus and Dolycoris baccarum feeding on red lentil caused chalky spot injury. The grain buyers, in the southeast Turkey, couldn’t have detected the real ratio of chalky spots visually and often overestimate the damage because of less public consciousness in domestic markets few years ago. Seeds with chalky spot have
pitted, crater like depressions in their seed coats. Injured areas usually appear discoloured and chalky. Lygus bugs feeding on developing lentil pods and seeds have been identified as the primary cause of chalky spot [14, 15]. Therefore, the objective of this study was to study the stability parameters for yield, seed quality and chalky syndrome in lentil genotypes under rainfed conditions.

MATERIALS AND METHODS

Description of the Study Area. Experiments were conducted at three different locations (Diyarbakir, Adiyaman and Kiziltepe/Mardin) of South-eastern Anatolia of Turkey in 2015-2016 growing season under rainfed conditions. The locations were selected to sample climatic and conditions likely to be encountered in lentil growing throughout Turkey and to vary in rainfall, temperature and other agro-climatic factors. Meteorological data for three locations were given Figure 1.

Experimental Design and Treatments. Fifteen lentil genotypes (Lentil International Drought Tolerance Nursery, Lentil International Elite Nursery- Drought Tolerance Nursery from ICARDA and local genotypes) and five lentil cultivars, namely (Firat 87, Kafkas, Cagil, Sakar and Ozbek) were used in the experiments. Experiments were laid out in a randomized complete block design with four replications at all locations. The experiments were managed according to local practice.

The appropriate pesticides were used to manage insects, weeds and diseases, and appropriate fertilizers were used according to recommended levels usual for the environment. The seed were sown with a seed rating 300 seed/m² and plot size was 4.8 m². Sowings were performed November 20 in Diyarbakir, November 21 in Adiyaman and November 22 in Kiziltepe/Mardin locations. Plots were fertilized with 30 kg N/ha and 50 kg P₂O₅/ha in planting.

Measurement and stability analyses. The harvested plot size was 3.2 m² and seed yield (kg) was recorded from each net plot area. Ten randomly chosen competitive plants from each of plot for recording seed quality analysis for fresh weight, hydration capacity and index, dry and fresh volume, swelling capacity and index, protein content, mean sieve rate and 100 seed weight were made in Field Crops Central Research Institute, Department of Quality and Technology Management.

Chalky spot syndrome in lentil genotypes was tested by visually. Chalky spotted seed ratio was determined in 200 g grain sample per plot as split whole seed. Samples were divided as damaged and non-damaged, weighed and counted. Seed samples were tested for germination speed and power. 50 seed samples for germination tests were counted and placed in petri dishes in three replications. Tests were performed at 23±2°C and daily light. Seeds were considered germinated when the radicle had extended for at least 2 mm. Germinated seeds were counted at 24 h intervals until end of 10 days. Germination speed and power was calculated at end of 5 days and 10 days after incubation, respectively.

Statistical analysis. The collected data was statistically analyzed following the regression coefficient (bi) was computed according to Finlay and Wilkinson [6] for the estimated to measure the stability and adaptability. The deviations of the regression coefficients (bi²) were computed according to Eberhart and Russell [8]. Ecovalence (W²i) as suggested by Wricke [7] was computed to further describe stability. A low ecovalence (W²i) value

FIGURE 1
Meteorological data of environments tested
indicates high relative stability. The stability variance ($\sigma^2$) of genotypes was determined according to Shukla [11]. The stability was measured by combining use of coefficient of variation (CVi) and mean yield [9].

RESULTS AND DISCUSSION

Mean values of yield and yield components of winter red lentil genotypes grown Diyarbakir, Adiyaman and Kiziltepe locations over locations were given in Table 1. Genotype, location and genotype x location interaction was significant for plant height, number of pods per plant, 1000 seeds weight and seed yield. Accordingly this significant variation might be due to the influence of climatic condition. The variation in temperatures lead to reduction in pod set in chickpea by reducing pollen viability and pollen production per flower [16]. It was observed a significant difference was attributed to the genotype, location and genotype x environment interaction and as well as, in several studies on different crops, significant differences in yield among genotypes [17, 18, 19, 20].

Yield and yield components. Plant height was ranged from 30.0 cm (ILL 3375) to 37.0 cm (Firat 87). Although ILL 3375 produced the lowest plant height as well as, it had response the tendency to lodging, thus the variety had completely lodging in last growth stage. Local varieties, BM 798 (tall) and BM 848 (short) produced the maximum lodging (Table 1). The plant height was ranged from 32.8 cm in Adiyaman and Kiziltepe to 34.4 cm in Diyarbakir. The pods with seeded were counted for the calculation of number of pods per plant. Number of pods per plant ranged from 13.1 pods/plant in BM 848 to 25.58 pods/plant in FLIP2009-55L. Although BM 848 and BM 798 achieved a lot of flower, they produced the minimum pods due to bacterial wilt caused plant lodging. Diyarbakir and Adiyaman locations were produced the maximum number of pods per plant; Kiziltepe location produced the minimum 15.2 pods/plant. Because the late of maturing genotypes produced a lot of dead flowers in the last week of May in Kiziltepe location. Firat 87 and Ozbek genotypes were severely affected by Fusarium oxysporium in Kiziltepe location. Grain yield ranged from 1241 kg/ha in BM848 to 2408 kg/ha in FLIP2009-55L. FLIP2009-50L, FLIP2011-26L, ILL10975, FLIP2011-49L, Kafkas and Sakar genotypes recorded the maximum grain yield. 1000 seed weight varied from 45.83 g in FLIP2010-19L to 29.83 g in FLIP2011-61L. Similar results have also been reported by other scientists [21, 22]. Kumar et al. [23] reported that plant height, and number of pods per plant varied in the genotypes under different environmental conditions. Bilal et al. [24] recorded that this traits had wide differences.

### TABLE 1

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Plant height (cm)</th>
<th>Number of pods per plant</th>
<th>1000 seeds weight (g)</th>
<th>Seed yield (kg/ha)</th>
<th>Cold damage (1-9)</th>
<th>Plant lodging (1-9)</th>
</tr>
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<tbody>
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<td>FLIP2011-26L</td>
<td>33.8 abc</td>
<td>20.6 cde</td>
<td>43.58 abc</td>
<td>2069 a</td>
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<td>3</td>
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<tr>
<td>ILL 3375</td>
<td>30.0 d</td>
<td>18.4 e</td>
<td>32.17 ij</td>
<td>1929 ab</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>BM798</td>
<td>35.4 ab</td>
<td>14.5 f</td>
<td>36.67 egf</td>
<td>1396 fg</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>BM848</td>
<td>30.8 cd</td>
<td>13.1 f</td>
<td>38.92 d-g</td>
<td>1337 g</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>FLIP2009-50L</td>
<td>32.8 bcd</td>
<td>22.0 cd</td>
<td>39.75 def</td>
<td>2136 a</td>
<td>4</td>
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<td>FLIP2011-19L</td>
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<td>20.6 cde</td>
<td>45.83 a</td>
<td>1905 abc</td>
<td>1</td>
<td>1</td>
</tr>
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<td>FLIP2009-55L</td>
<td>34.8 ab</td>
<td>25.6 a</td>
<td>41.83 bcd</td>
<td>2142 a</td>
<td>3</td>
<td>1</td>
</tr>
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<td>ILL10975</td>
<td>35.6 bc</td>
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<td>4</td>
<td>1</td>
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<tr>
<td>FLIP2011-94L</td>
<td>33.2 bcd</td>
<td>19.8 cde</td>
<td>33.92 hi</td>
<td>1885 a-d</td>
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<td>1</td>
</tr>
<tr>
<td>FLIP2011-61L</td>
<td>30.9 ed</td>
<td>22.6 bc</td>
<td>29.83 j</td>
<td>1388 fg</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>FLIP2010-82L</td>
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<td>19.3 de</td>
<td>36.42 fg</td>
<td>1577 efg</td>
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<td>36.42 fg</td>
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<td>1</td>
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<tr>
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<td>19.1 de</td>
<td>40.75 cd</td>
<td>1637 c-f</td>
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<tr>
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<td>1724 b-e</td>
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<td>3</td>
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<td>Cagil</td>
<td>33.4 bc</td>
<td>20.9 cde</td>
<td>33.33 hji</td>
<td>1876 a-d</td>
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<td>1</td>
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<td>Firat 87</td>
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<td>40.17 cde</td>
<td>1625 def</td>
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<td>2</td>
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<td>37.9</td>
<td>1820</td>
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<td>3</td>
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<td>4.66</td>
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<td>28.06**</td>
<td>37.6**</td>
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<td>Diyarbakir</td>
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<td>38.2 a</td>
<td>2097 a</td>
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<td></td>
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<td>38.0 ab</td>
<td>1808 b</td>
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<td></td>
</tr>
<tr>
<td>Kiziltepe</td>
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<td>15.12 b</td>
<td>37.3 b</td>
<td>1556 c</td>
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<td></td>
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<td>LSD</td>
<td>1.22**</td>
<td>2.0**</td>
<td>0.8**</td>
<td>134.3**</td>
<td></td>
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</table>

** Significant at p<0.01 level. There is no differences between same letter in the same column
Cold damage. Since the temperature values for February 2016 were higher than normal ones, plants grown normal, but lentil plants at seedling stage were adversely affected by the low temperature which lasted for several nights at 0ºC and above at the beginning March. ILL10975, FLIP2010-94L and Sakar genotypes had more sensitive to cold than other genotypes. Sakar had early flowering and maturity, but Firat 87, Kafkas, Ozbek, and local varieties (BM 848 and BM 798) are late flowered and matured. Several researchers have reported that late-flowering genotypes in low-temperature are less influenced than early-flowering genotypes [25, 26, 27].

Seed quality properties. Variances among genotypes for seed quality properties, except for protein content were non-significant. Fresh weight of the seeds ranged from 5.93 g (Ozbek) to 9.29 g (Sakar). Hydration capacity was 0.0238 g/seed (Ozbek) - 0.0449 g/seed (FLIP2011-26L and Sakar), swelling index ranged from 1.72% (Kafkas) to 2.29% (FLIP2011-26L), all genotypes recorded almost two times swelling index. Commercial variety, Ozbek produced lower hydration capacity and index, swelling capacity and index, fresh weight and protein content. The differences among genotypes for protein content were significant, and protein content varied from 26.37% in FLIP2009-50L to 28.76% in BM 848 (Table 2). Varsha and Grewal [28] reported that hydration capacity and index of lentil varieties ranged from 0.018-0.030 g/seed and 0.83-1.05, respectively. Solanki et al. [29] recorded that different generations of three microsperma × microsperma and two microsperma × macrosperma crosses of lentil's significant variation for hydration index (0.740-0.996), 100-seed weight (1.86-4.85 g) and protein value (15.85%-23.75%). Previous investigations have recorded that the changing environment is the influence of seed quality of genotype [30, 31].

Chalky spot syndrome in lentil genotypes. Lentil crops with chalky spotted were detected in Adiyaman location. This damage was no observed from Kiziltepe and Diyarbakir locations. Therefore, evaluation on chalky spot were only made for Adiyaman location. Chalky spotted seed ratio in lentil genotypes ranged from 11.0% to 42.92%. Lygus affected some genotypes at significant level and the ratio was more than 20%. Chalky spot ratio in Firat 87, Sakar (leading cultivars grown in the region), BM 798 and BM 848 (selecting from local varieties) was more 35% (Table 3). The chalky spotted seed ratio in this research was observed higher than reported in the previous literature. Ozberk et al. [14] reported that there was significant difference among the content (%) of chalky spot damage of lentil samples collected from the farmers and ranged from 1.45 to 29%. Germination speed varied from 56.0% to 98.0% and germination rate ranged from 60% to 100%. Although the germination rate in the seeds with chalky spotted was high at first, hypocotyl and epicotyl were weak and short and fungi growth was intensively observed in petri dishes, also, a lot of seeds could not germinate. Differences among genotypes for germination rate in non-damaged seed were no significant.

Stability parameters in lentil genotypes. Genotype × environment interaction was significant for seed yield. The mean grain yield was ranged from 1337 kg/ha in BM 848 to 2142 kg/ha in FLIP2009-55L. Although Firat 87 has a good performance in Southeast Anatolia of Turkey. It has not showed good performance at Kiziltepe due to Fusarium oxysporum. Regression co-efficient (bi) values ranged from -0.6 in FLIP2010-103L to 2.5 in FLIP2010-19L (Table 4). Ten genotypes recorded b value less than one and it produced high yield stability and the response was less to year x location effects. The other ten genotypes recorded a low yield stability as indicated by b value significantly greater than one. These genotypes recorded a greater response to

### TABLE 2

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fresh weight (g)</th>
<th>Hydration capacity (g/seed)</th>
<th>Hydration index (%)</th>
<th>Dry Volume (ml)</th>
<th>Fresh volume (ml)</th>
<th>Swelling capacity (ml/seed)</th>
<th>Swelling index (%)</th>
<th>Protein rate (%)</th>
<th>Sieve rate (mm)</th>
<th>100 seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLIP2009-50L</td>
<td>7.34</td>
<td>0.0355</td>
<td>0.858</td>
<td>53.00</td>
<td>106.25</td>
<td>0.033</td>
<td>2.08</td>
<td>26.37</td>
<td>4.21</td>
<td>3.79</td>
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<tr>
<td>FLIP2009-55L</td>
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<td>0.0395</td>
<td>0.939</td>
<td>53.25</td>
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<td>0.040</td>
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<td>26.49</td>
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<td>0.0372</td>
<td>0.942</td>
<td>53.00</td>
<td>106.90</td>
<td>0.039</td>
<td>2.30</td>
<td>27.84</td>
<td>4.43</td>
<td>4.14</td>
</tr>
<tr>
<td>FLIP2011-61L</td>
<td>6.18</td>
<td>0.0299</td>
<td>0.893</td>
<td>52.50</td>
<td>105.35</td>
<td>0.028</td>
<td>2.14</td>
<td>27.43</td>
<td>3.99</td>
<td>3.19</td>
</tr>
<tr>
<td>FLIP2011-82L</td>
<td>6.37</td>
<td>0.0292</td>
<td>0.652</td>
<td>52.25</td>
<td>104.50</td>
<td>0.023</td>
<td>2.00</td>
<td>27.88</td>
<td>3.94</td>
<td>3.45</td>
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<tr>
<td>FLIP2012-26L</td>
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<td>0.0449</td>
<td>0.982</td>
<td>53.45</td>
<td>107.90</td>
<td>0.045</td>
<td>2.29</td>
<td>27.76</td>
<td>4.48</td>
<td>4.53</td>
</tr>
<tr>
<td>ILL10975</td>
<td>6.46</td>
<td>0.0313</td>
<td>0.886</td>
<td>52.50</td>
<td>105.45</td>
<td>0.030</td>
<td>2.18</td>
<td>27.94</td>
<td>4.07</td>
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<td>105.75</td>
<td>0.028</td>
<td>2.14</td>
<td>27.75</td>
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<td>3.16</td>
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<tr>
<td>BM 848</td>
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<td>0.0357</td>
<td>0.809</td>
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<td>106.35</td>
<td>0.031</td>
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<td>0.0289</td>
<td>0.787</td>
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<td>105.45</td>
<td>0.027</td>
<td>1.98</td>
<td>27.11</td>
<td>4.20</td>
<td>3.43</td>
</tr>
<tr>
<td>Kafkas</td>
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<td>0.0254</td>
<td>0.598</td>
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<td>104.90</td>
<td>0.021</td>
<td>1.72</td>
<td>27.60</td>
<td>3.84</td>
<td>3.43</td>
</tr>
<tr>
<td>Sakar</td>
<td>9.29</td>
<td>0.0444</td>
<td>0.845</td>
<td>53.90</td>
<td>108.00</td>
<td>0.041</td>
<td>2.05</td>
<td>28.14</td>
<td>4.51</td>
<td>4.85</td>
</tr>
<tr>
<td>Firat 87</td>
<td>7.02</td>
<td>0.0337</td>
<td>0.959</td>
<td>52.75</td>
<td>106.25</td>
<td>0.035</td>
<td>2.27</td>
<td>28.91</td>
<td>4.39</td>
<td>3.65</td>
</tr>
<tr>
<td>Ozbek</td>
<td>5.93</td>
<td>0.0238</td>
<td>0.662</td>
<td>52.55</td>
<td>104.90</td>
<td>0.024</td>
<td>1.92</td>
<td>26.97</td>
<td>4.00</td>
<td>3.55</td>
</tr>
</tbody>
</table>
environmental effects. Ecovalance ($W^2_i$) and stability variance ($ı^2_i$) were low value in genotypes ILL 3375 and Kafkas, Firat 87 had high values. The genotypes Kafkas and ILL 3375 produced stable while the genotypes Firat 87 was unstable. The genotypes FLIP2010-94L and FLIP2009-55L according to Francis and Kannenberg [9] stability parameter ($CV_i$) were stable genotypes since these genotypes recorded a low $CV_i$ and high yield. Variety Firat 87 achieved high values of stability parameters, generally, this variety recorded good performance and high yield under investigation conditions. These findings indicated that some lentil genotypes were recorded more sensitive to the small changes in environment while others were recorded more stable. These findings agree with other findings of previous experiments [32, 33, 34].

### TABLE 3
Chalky spotted seed rate and germination rate in lentil

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Seed rate with chalky spotted (%)</th>
<th>Germination speed (%)</th>
<th>Germination power (%)</th>
<th>Germination rate (%)</th>
<th>Germination rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLIP2011-26L</td>
<td>22.30 cde</td>
<td>86.7 bcd</td>
<td>13.0 ab</td>
<td>99.67 a</td>
<td>96.6</td>
</tr>
<tr>
<td>ILL 3375</td>
<td>19.49 cde</td>
<td>92.7 abcd</td>
<td>3.0 de</td>
<td>95.7 abcd</td>
<td>95.3</td>
</tr>
<tr>
<td>BM798</td>
<td>39.76 a</td>
<td>70.0 h</td>
<td>6.0 ed</td>
<td>76.0 fg</td>
<td>91.3</td>
</tr>
<tr>
<td>BM848</td>
<td>35.53 abc</td>
<td>56.0 i</td>
<td>4.0 de</td>
<td>60.0 h</td>
<td>89.3</td>
</tr>
<tr>
<td>FLIP2009-50L</td>
<td>16.06 de</td>
<td>92.7 abcd</td>
<td>3.0 de</td>
<td>96.0 abc</td>
<td>95.0</td>
</tr>
<tr>
<td>FLIP2010-19L</td>
<td>20.00 cde</td>
<td>85.3 cdef</td>
<td>2.0 de</td>
<td>87.3 cde</td>
<td>98.0</td>
</tr>
<tr>
<td>FLIP2009-55L</td>
<td>20.85 cde</td>
<td>71.3 gh</td>
<td>1.7 a</td>
<td>88.3 bcde</td>
<td>96.6</td>
</tr>
<tr>
<td>ILL10975</td>
<td>15.21 e</td>
<td>89.3 abcd</td>
<td>2.0 de</td>
<td>90.3 abcd</td>
<td>99.0</td>
</tr>
<tr>
<td>FLIP2010-94L</td>
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<td>99.0 a</td>
<td>2.0 de</td>
<td>91.0</td>
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</tr>
<tr>
<td>FLIP2011-61L</td>
<td>32.13 abcd</td>
<td>66.0 hu</td>
<td>3.0 de</td>
<td>96.9 gh</td>
<td>94.6</td>
</tr>
<tr>
<td>FLIP2010-82L</td>
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<td>94.7 abc</td>
<td>1.0 e</td>
<td>96.0 abc</td>
<td>100</td>
</tr>
<tr>
<td>FLIP2011-55L</td>
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<td>96.7 ab</td>
<td>3.0 de</td>
<td>100 a</td>
<td>94.6</td>
</tr>
<tr>
<td>FLIP2010-56L</td>
<td>15.05 e</td>
<td>96.0 ab</td>
<td>2.0 de</td>
<td>98.0 ab</td>
<td>94.0</td>
</tr>
<tr>
<td>FLIP2011-49L</td>
<td>13.61 e</td>
<td>90.7 abde</td>
<td>2.7 de</td>
<td>93.3 abcd</td>
<td>93.3</td>
</tr>
<tr>
<td>Kafkas</td>
<td>19.06 de</td>
<td>83.3 def</td>
<td>4.0 de</td>
<td>87.3 cde</td>
<td>96.0</td>
</tr>
<tr>
<td>Sakar</td>
<td>38.46 ab</td>
<td>66.0 h</td>
<td>3.0 de</td>
<td>69.0 gh</td>
<td>94.0</td>
</tr>
<tr>
<td>Ozbek</td>
<td>16.33 de</td>
<td>76.0 fgh</td>
<td>10.0 bc</td>
<td>86.0 de</td>
<td>96.6</td>
</tr>
<tr>
<td>Çagil</td>
<td>23.06 bcd</td>
<td>88.7 abde</td>
<td>0.0 e</td>
<td>88.7 bcde</td>
<td>91.3</td>
</tr>
<tr>
<td>Firat 87</td>
<td>42.92 abc</td>
<td>81.3 efgh</td>
<td>3.0 de</td>
<td>84.3 ef</td>
<td>93.3</td>
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<tr>
<td>Mean</td>
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<td>83.3</td>
<td>4.6</td>
<td>87.9</td>
<td>95.1</td>
</tr>
<tr>
<td>LSD</td>
<td>16.08***</td>
<td>10.3***</td>
<td>4.18***</td>
<td>9.98**</td>
<td>ns</td>
</tr>
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</table>

** Significant at p<0.01 level, There is no differences between same letter in the same column, ns: non-significant

### TABLE 4
Stability parameters in lentil

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Grain yield (kg/ha)</th>
<th>b</th>
<th>Deviation from the regression ($ı^2_i$)</th>
<th>Genotypic variance ($s^2_i$)</th>
<th>Coefficient of variation ($CV_i$)</th>
<th>Wricke's ecovariance ($W^2_i$)</th>
<th>Stability variance ($ı^2_i$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLIP2011-26L</td>
<td>2069</td>
<td>1.1</td>
<td>924.2</td>
<td>1352.2</td>
<td>17.8</td>
<td>927.7</td>
<td>5680.7</td>
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<tr>
<td>ILL 3375</td>
<td>1929</td>
<td>1.1</td>
<td>165.2</td>
<td>972.7</td>
<td>16.2</td>
<td>104.2</td>
<td>4670.0</td>
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<tr>
<td>FLIP2010-103L</td>
<td>1918</td>
<td>-0.6</td>
<td>19.4</td>
<td>274.5</td>
<td>8.6</td>
<td>3817.7</td>
<td>2072.6</td>
</tr>
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<td>9.0</td>
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<td>2142</td>
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<tr>
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</table>
CONCLUSION

It was concluded that, Seed yield were varied from 133.7 to 214.2 kg/da among the genotypes. While cultivar ‘Sarak’ produced the maximum yield and cultivar ‘BM 848’ produced the minimum seed yield. It was also revealed that genotypes ‘ILL10975’, ‘FLIP2010-94L’ and cultivar ‘Sarak’ was found the most sensitive to cold. Lentil crops with chalky spotted were detected in Adiyaman location and its rate ranged from 42% to 13%. Genotype × environment interaction was significant for cation and its rate ranged from 42% to 13%. Genotype stability parameters were computed for genotype adaptability. Ecowalence seed yield and different stability parameters were from 133.7 to 214.2 kg/da among the genotypes.

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CHANGES IN THE CONTENT, UPTAKE AND BIOACCUMULATION OF MACRONUTRIENTS IN GENOTYPES OF MISCANTHUS GRASS. POSSIBILITIES OF USING ASH FROM MISCANTHUS BIOMASS

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ABSTRACT

The aim of this study was to determine the changes in the content, uptake and bioaccumulation of macronutrients in biomass of six genotypes of Miscanthus grass over a period of three years of cultivation. The possibilities of using ash from Miscanthus biomass were also assessed. The field experiment was conducted in the three years in central-western (Poznań) and in the central-northern (Elbląg) part of Poland. Six genotypes of Miscanthus grasses were grown: MG1 (M1) Miscanthus giganteus (as standard), MG2 (M2) (plant code M 116) - hybrid M. sinensis (2x) x M. sacchariflorus (4x), MS3 (M3) (code M 105)- hybrid as above, MS4 (M4) (code M 114)- hybrid as above, MS5 (M5)- M. sinensis, obtained from hybrids 92M012 2012, MS6 (M6)- M. sinensis, obtained from hybrids 92M017 2004. Deep ploughing and harrowing was performed and fertilisation at N-80; P-100 and K-120 kg ha⁻¹. Biomass of genotypes of Miscanthus grass grown in diverse soil and climatic conditions in Poland during three years contained the largest amount of carbon, hydrogen, silica, calcium, followed - in a decreasing order - by K, Mg, N, P, S and Na. The content of SiO₂, Ca, K and N was significantly differentiated in biomass of consecutive genotypes. The Miscanthus biomass yield has been found to be significantly negatively correlated with the total content of Ca, K, Mg and Na. High content of alkaline elements in crude ash obtained from biomass Miscanthus testifies to the possibility of its use in agriculture as liming agent.

KEYWORDS: Yield, macronutrients, uptake, bioaccumulation, ash, Miscanthus

INTRODUCTION

Introduction of new energy plants into cultivation is stimulated by progressing depletion of sustainable resources of fossil fuels in Poland and world-wide [1, 2]. According to the assumptions of the EU Climate Package of 17/12/2008 member states by 2020 should reach a 20% share of energy from renewable sources. The waste product after burning biomass are ashes. On average, from the combustion of 1 ton of Miscanthus’s biomass 55 kg of ash are obtained. In agriculture, plant ashes are frequently used as mineral fertilisers [3, 4]. The fertilisation value of ash depends on the type of combusted material, the type of plant and their fertilisation [5]. A study carried by [2, 6, 7, 8,] indicated an increase in alkalinity, and in the level of macro- and micronutrients in the soil. Indicated the possibility for using the ash from heat and power stations for composting sewage sludge and the obtained composts for soil reclamation [9]. An analysis of the consumption of nutrient uptake and biomass growth during the vegetation period will help to develop principles of fertilisation of the crop, since studies dealing with the issue have been scarce [10, 11]. Being an energy crop, Miscanthus is harvested during the stage of its technological ripeness, usually beginning in October [12]. It is recommended as the best period for studies of the uptake and accumulation of nutrients in the plant biomass. This is the time when there is a balance between the aboveground and the underground parts (mainly rhizomes), to which a certain amount of nutrient is supplied [13]. A delay in harvest can lead to its lodging caused by precipitation (rain, and especially snow) and in washing considerable amounts of potassium and magnesium from its leaves [14]. The findings of other studies [15, 16] have shown that more calcium is accumulated in Miscanthus biomass at the end of its growth, which probably prevents the plants from lodging. With shoots up to 2.5-3.0 m and with heavy leaves, Miscanthus stalks must be strong and flexible [17]. A high content of calcium in its biomass (like in other ash-forming components) increases the amount of
ash, which is important in generating energy from this crop [2, 18, 19, 20]. In terms of its physiological activity, magnesium is similar to calcium, and - in many cases - antagonistic to potassium. According to [21], the content of potassium in biomass harvested in February or March was smaller by approx. 50% than in that harvested in October. The physical and chemical properties of ash from plants lead to the improvement of the structure of heavy soils and the fertilizer components contained in them are easily absorbed by plants [22].

The aim of this study was to determine the changes of the content, uptake and bioaccumulation macronutrients in biomass of six genotypes of Miscanthus grass over a period of three years of cultivation. The possibilities of using ash from Miscanthus biomass were also assessed.

**MATERIALS AND METHODS**

The field experiment was conducted in the three years in central-western (Poznań) and in the central-northern (Elblag) part of Poland. Six genotypes of Miscanthus grasses were grown [17]; most of them were obtained from TINPLANT Gmbhl Klein Wanz (Germany): MG1 (M1) Miscanthus giganteus (as standard), MG2 (M2) (plant code M 116) - hybrid M. sinensis (2x) x M. sacchariflorus (4x), MS3 (M3) (code M 105)- hybrid as above, MS4 (M4) (code M 114)- hybrid as above, MS5 (M5)- M. sinensis, obtained from hybrids 92M012 2012, MS6 (M6)- M. sinensis, obtained from hybrids 92M017 2004.

Rhizomes of the genotypes were planted in May 2007 (10,000 plants per 1 ha), in two cities: Poznań (16°55′ E, 52°25′ N) on experimental fields of IGR-PAN, on sandy leressive, formed from light sandy loam [23]. According to the [24] ochric luvisol. It was a quality class IVa soil, with pHKCl 6.82, total carbon content 9.81 g kg⁻¹ and nitrogen content of 0.80 g kg⁻¹ and medium abundance of available forms of phosphorus, potassium and magnesium; Elblag (19°23′E, 53°47′ N) – the experiment was carried out on typical brown eutrophic soil formed from medium loam [23]. According to the [24] ochric cambic. It was a quality class IVa soil, with pHKCl 5.12, total carbon content 8.54 g kg⁻¹ and nitrogen content of 0.71 g kg⁻¹ and medium abundance of available forms of phosphorus, potassium and magnesium.

Deep ploughing and harrowing was performed and fertilisation at N-80; P-100 and K-120 kg ha⁻¹ as ammonium nitrate (34% N), triple superphosphate (20% P) and potassium salt (50% K) were applied before the plantation was set up.

The three-year experiment was carried out with no plant protection measures and the growth and development of the plants was observed each year. The biomass was harvested during the phase of technological ripeness, in March of each consecutive year.

Samples for tests were taken from the soil and plant material, air-dried and then dried at 105°C in order to determine dry weight. Total carbon, hydrogen and nitrogen by dry combustion using auto analyser series II CHNS/O 2400, Perkin Elmer. Plant samples were ground and analysed at a chemical laboratory. The organic mass was dry-oxidised (in crucibles made of semi-vitreous chinaware material) at 450°C for 6 hours with a gradient increase in the temperature to the desired level. The ash was cooled down and poured over with 5 cm³ HCl in order to decompose the carbonates formed in the oxidation process and to form chlorides of the elements present in the ash. Excess HCl was evaporated on a sand bath. Silica precipitated in the process. HCl (10 cm³ 10%) was added to the crucible again and the contents was transferred through a hard filter (to separate the silica) to a volumetric flask. The solution was made up to mark and the flask contents were used as the working analytic solution, in which the total content of Ca, Mg, K and Na was determined by ICP-AES, on a Perkin Elmer 3200RL apparatus. The assay accuracy was compared with the company’s reference standard solutions. The bioaccumulation factors were calculated as the quotient of the content of an element in the plant to its content in the soil.

The experimental data were analysed with uni- and multivariate statistical methods. In the first step a three factor analysis of variance was applied to examine differences between genotypes of Miscanthus grass with respect to the yield and the presence of macronutrients in the biomass of six clones. Much attention has been devoted to comparing genotypes due to their main effects, stability and adaptability to environments. Methods of statistical analysis of a series of experiments with genotypes, conducted at places over successive years. The analysis is based on the mixed linear model, which is constructed under assumption that the choice of the experimental site within each environment is random. This model allows to test the general hypothesis of no genotype x environment interaction HGE hypothesis of no genotype by environment interaction within places (Poznań, Elblag) and years (1st, 2nd, 3rd) and specially interesting hypotheses concerning main effects of individual genotypes, their interaction with environments and regression of interaction for genotypes on environment. Important information about sensitivity of genotypes to environment were obtained by the regression analyses. In addition figures illustrating main effects and regression lines of interaction for all studying genotypes on environmental deviations have been made for the yield and for each of the chemical elements C, N, H, SiO₂, P, K, Ca, Mg, S and Na. Finally, in order to investigate the relationship between K, Ca, Mg and Na accumulation by...
Miscanthus genotypes and the Miscanthus biomass yield, the multiple regression analyses were applied.

The following was the rainfall in Poznań during the growing season (April-October) in the study years (1st, 2nd, 3rd): 350 mm, 320 mm and 409 mm; the rainfall in Elblag was as follows: 530 mm, 400 mm and 548 mm. The average temperature in the months of the growing season was: in Poznań 15.2°C, 14.30C and 15.30C; in Elblag 15°C, 14.3°C and 14.8°C.

RESULTS AND DISCUSSION

The genotypes, experiment sites as well as interaction between them significantly differentiated the total content of potassium and calcium in the soil (Table 1). Experiment sites significantly differentiated in the content of potassium, phosphorus, calcium and magnesium. The total content of the analysed elements in the soil was at a medium level. Their contents can be presented in the following series of decreasing values: C > Ca > N > P > K > Mg > S > Na.

The dry weight of the Miscanthus biomass (Table 2a and 3) was highly differentiated by the genotypes under study, the experiment site, study years and the interaction of these factors. The yield of Miscanthus grass in Elblag was higher by 18% than in Poznań. Regardless of the experiment site, the highest yield was obtained for the genotype marked as M1 and M2, and the lowest was for the genotype marked as M5 and M6. The study years also had a great effect on the yield of the clones under study expressed as the biomass weight. A similar yields of Miscanthus aboveground biomass was observed [25] on gleysol. In the three years of the experiment, a relationship was observed between the yield in the experiment years and the dry weight yield of the genotypes under study (r = 0.66) and between the yield obtained in the experiment years and the experiment sites (r = 0.99). The content of nitrogen and silica of the Miscanthus grass under study (Table 2a) was significant differentiated depending on the genotypes, experiment site and study year (Table 3). Significantly more nitrogen was found in Miscanthus grass grown in Elblag (mean 7.90 g kg⁻¹ D.M.). The content of silica in biomass harvested in Poznań was significantly higher (by 60%) than in that harvested in Elblag. The content of the remaining elements in the Miscanthus biomass presented in Table 2a did not differ significantly from the influence on the studied factors. The mean potassium content (Table 2b) in Miscanthus grass biomass at all sites was 15.66 g kg⁻¹ D.M. ranging from 5.16 to 40.14 g kg⁻¹ D.M. The genotypes, experiment sites and study years as well as interaction between them significantly differentiated the content of potassium in Miscanthus biomass. An analysis of the potassium content in biomass of the clones under study revealed the highest level (20.88 g kg⁻¹ D.M.) in biomass of the M1 clone, and the lowest level (12.56 g kg⁻¹ D.M.) in biomass of M6. The content of potassium in Miscanthus biomass harvested in Elblag was twice as high as in Poznań (20.55 and 10.76 g kg⁻¹ D.M., respectively) – the opposite to the content of calcium (Elblag: 26.08, Poznań: 35.47 g kg⁻¹ D.M.). Such a high content of this element was caused by a high content of calcium in soil in Poznań, which restricts potassium uptake, because these two elements are antagonists in uptake by a plant. Potassium does not form strong bonds in a plant like calcium and it can move with water within it. Potassium was transferred from the over ground parts to rhizomes at the end of the growing season and it was washed out by rain, mainly from leaves, which is a consequence of increase in their weight with extending cultivation period. The content of potassium in Miscanthus biomass was consistently balanced by the interaction of the experiment site and study years. The calcium content (Table 2b) in biomass of the six Miscanthus genotypes under study varied and ranged from 17.36 to 47.83 g kg⁻¹ D.M., mean 30.78 g kg⁻¹ D.M. The content of this element was affected significantly by the experimental factors: genotypes, conditions at the experiment site, study years and their interaction. The significantly highest content of calcium was determined in biomass of M4 (34.24 g kg⁻¹ D.M.) and M6 (33.52 g kg⁻¹ D.M.), less in M1, M2 and M5 (32.02, 29.91, 19.35 g kg⁻¹ D.M.)

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
</tr>
</thead>
<tbody>
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<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>9.04</td>
<td>0.76</td>
<td>0.51</td>
<td>0.58e</td>
<td>4.53e</td>
<td>0.61</td>
<td>0.38</td>
<td>0.12</td>
</tr>
<tr>
<td>M2</td>
<td>9.58</td>
<td>0.73</td>
<td>0.62</td>
<td>0.72e</td>
<td>4.42e</td>
<td>0.52</td>
<td>0.42</td>
<td>0.10</td>
</tr>
<tr>
<td>M3</td>
<td>9.72</td>
<td>0.84</td>
<td>0.75</td>
<td>0.87e</td>
<td>3.28e</td>
<td>0.48</td>
<td>0.40</td>
<td>0.09</td>
</tr>
<tr>
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<td>9.67</td>
<td>0.79</td>
<td>0.80</td>
<td>0.84e</td>
<td>3.11e</td>
<td>0.52</td>
<td>0.38</td>
<td>0.11</td>
</tr>
<tr>
<td>M5</td>
<td>9.79</td>
<td>0.77</td>
<td>0.68</td>
<td>0.78e</td>
<td>3.04e</td>
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<td>0.45</td>
<td>0.10</td>
</tr>
<tr>
<td>M6</td>
<td>9.80</td>
<td>0.82</td>
<td>0.72</td>
<td>0.82e</td>
<td>3.24e</td>
<td>0.47</td>
<td>0.40</td>
<td>0.13</td>
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</table>

<table>
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<th>Place of research</th>
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<th>n.s.</th>
<th>n.s.</th>
<th>*</th>
<th>n.s.</th>
<th>n.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poznań</td>
<td>9.81</td>
<td>0.82</td>
<td>0.72e</td>
<td>0.38</td>
<td>5.22e</td>
<td>0.67</td>
<td>0.45</td>
</tr>
<tr>
<td>Elblag</td>
<td>8.54</td>
<td>0.71</td>
<td>0.45*</td>
<td>0.38</td>
<td>2.92e</td>
<td>0.45</td>
<td>0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypes x place of research</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
<th>*</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
</tr>
</thead>
</table>

a, b - values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05, n.s.- non significant
29.63 g kg⁻¹ D.M., respectively) and the least in M3 (25.34 g kg⁻¹ D.M.). Significantly more calcium was found in *Miscanthus* grass grown in Poznań (mean 35.47 g kg⁻¹ D.M.), and in Elblag (26.08 g kg⁻¹ D.M.), where the soil pH was lower. The statistical calculations revealed a significant correlation between the *Miscanthus* yield and the calcium content in the plant in consecutive experiment years (r = -0.99). The content of magnesium in biomass of the *Miscanthus* genotypes under study (Table 2b) was highly differentiated depending on the experiment site and study years (Table 3). Significantly more magnesium was found in biomass harvested in Elbląg (mean 6.28 g kg⁻¹ D.M.). The largest amounts of magnesium were found in biomass of M1 and M2 (6.31 and 5.96 g kg⁻¹ D.M.) and the least in M5 (4.66 g kg⁻¹ D.M.). Aerial parts of grasses *Miscanthus sacchariflorus*, *Miscanthus sinensis* and *Miscanthus giganteus* accumulated mostly calcium, potassium and magnesium [26]. The content of sodium in biomass harvested in Elbląg was significantly higher (by 71%) than in that harvested in Poznań (Table 2b). Significant differences in the sodium content in biomass for the experiment site and study years were observed in Elbląg. The content of sodium in *Miscanthus* biomass was differentiated significantly by the experiment site, study years and their interaction (Table 3).

### TABLE 2a

The yields of biomass (t ha⁻¹ D.M.) and macronutrients content in biomass genotypes of *Miscanthus* grass (g kg⁻¹ D.M.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield</th>
<th>C</th>
<th>N</th>
<th>H</th>
<th>SiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>16.59⁺</td>
<td>429.9⁺</td>
<td>6.60⁺</td>
<td>62.90⁺</td>
<td>45.25⁺</td>
</tr>
<tr>
<td>M2</td>
<td>18.94⁺</td>
<td>434.0⁺</td>
<td>6.85⁺</td>
<td>63.15⁺</td>
<td>39.80⁺</td>
</tr>
<tr>
<td>M3</td>
<td>12.64⁺</td>
<td>431.7⁺</td>
<td>5.25⁺</td>
<td>61.75⁺</td>
<td>36.05⁺</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>10.74⁺</td>
<td>430.6⁺</td>
<td>5.30⁺</td>
<td>62.05⁺</td>
<td>38.50⁺</td>
</tr>
<tr>
<td>M5</td>
<td>8.24⁺</td>
<td>430.0⁺</td>
<td>6.55⁺</td>
<td>62.00⁺</td>
<td>45.30⁺</td>
</tr>
<tr>
<td>M6</td>
<td>9.19⁺</td>
<td>428.7⁺</td>
<td>6.65⁺</td>
<td>61.90⁺</td>
<td>41.50⁺</td>
</tr>
<tr>
<td>Place of research</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poznań</td>
<td>11.71⁺</td>
<td>429.6⁺</td>
<td>4.50⁺</td>
<td>62.4</td>
<td>50.50⁺</td>
</tr>
<tr>
<td>Elbląg</td>
<td>13.76⁺</td>
<td>432.0⁺</td>
<td>7.90⁺</td>
<td>62.1</td>
<td>31.50⁺</td>
</tr>
<tr>
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<td>*</td>
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<td>Genotypes x place of research</td>
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<td>*</td>
<td>n.s.</td>
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</table>

a, b, c – values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05, n.s. - non significant

### TABLE 2b

Macronutrients content in biomass genotypes of *Miscanthus* grass (g kg⁻¹ D.M.)

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.46</td>
<td>20.88⁺</td>
<td>32.02⁺</td>
<td>6.31</td>
<td>0.66</td>
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<tr>
<td>M2</td>
<td>0.50</td>
<td>17.15⁺</td>
<td>29.91⁺</td>
<td>5.96</td>
<td>0.63</td>
<td>0.99</td>
</tr>
<tr>
<td>M3</td>
<td>0.42</td>
<td>13.69⁺</td>
<td>25.34⁺</td>
<td>4.84</td>
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</tr>
<tr>
<td>M4</td>
<td>0.53</td>
<td>14.64⁺</td>
<td>34.24⁺</td>
<td>5.10</td>
<td>0.48</td>
<td>0.73</td>
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<tr>
<td>M5</td>
<td>0.48</td>
<td>15.03⁺</td>
<td>29.63⁺</td>
<td>4.66</td>
<td>0.51</td>
<td>1.22</td>
</tr>
<tr>
<td>M6</td>
<td>0.45</td>
<td>12.56⁺</td>
<td>33.52⁺</td>
<td>4.91</td>
<td>0.60</td>
<td>0.82</td>
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<td>Place of research</td>
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<td></td>
</tr>
<tr>
<td>Poznań</td>
<td>0.42</td>
<td>10.76⁺</td>
<td>35.47⁺</td>
<td>4.31⁺</td>
<td>0.54</td>
<td>0.75⁺</td>
</tr>
<tr>
<td>Elbląg</td>
<td>0.52</td>
<td>20.55⁺</td>
<td>26.08⁺</td>
<td>6.28⁺</td>
<td>0.60</td>
<td>1.16⁺</td>
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<tr>
<td>p</td>
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<tr>
<td>Genotypes x place of research</td>
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<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
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</tr>
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</table>

a, b – values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05, n.s. - non significant

### TABLE 3

Factors significantly affect the yield and content indicated elements *Miscanthus* grass

<table>
<thead>
<tr>
<th>Components</th>
<th>Investigated factors</th>
<th>years of research</th>
<th>interaction genotypes x years</th>
<th>interaction place of research x years</th>
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<td>Yield</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<tr>
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<td>*</td>
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<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>N</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>H</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>SiO₂</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<tr>
<td>P</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>K</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>*</td>
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<tr>
<td>Ca</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Mg</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>S</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Na</td>
<td>*</td>
<td>n.s.</td>
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* - the level of significance for p ≤ 0.05, n.s. - non significant
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<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
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<tbody>
<tr>
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<td>109.5b</td>
<td>7.6c</td>
<td>346.4c</td>
<td>531.2b</td>
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<td>18.2b</td>
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<td>129.7b</td>
<td>9.5c</td>
<td>324.8c</td>
<td>566.5b</td>
<td>112.9b</td>
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<td>5.1a</td>
<td>7.8a</td>
</tr>
<tr>
<td>M5</td>
<td>54.0a</td>
<td>3.9a</td>
<td>123.8a</td>
<td>244.1b</td>
<td>38.4a</td>
<td>4.2a</td>
<td>10.0a</td>
</tr>
<tr>
<td>M6</td>
<td>61.1a</td>
<td>4.1a</td>
<td>115.4a</td>
<td>308.0b</td>
<td>45.2a</td>
<td>5.5a</td>
<td>7.5a</td>
</tr>
</tbody>
</table>

Genotypes

<table>
<thead>
<tr>
<th>Place of research</th>
<th>Poznań</th>
<th>Elbląg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>52.7a</td>
<td>108.7b</td>
</tr>
<tr>
<td>P</td>
<td>8.68b</td>
<td>9.38b</td>
</tr>
<tr>
<td>K</td>
<td>36.00c</td>
<td>23.82b</td>
</tr>
<tr>
<td>Ca</td>
<td>7.07a</td>
<td>6.77a</td>
</tr>
<tr>
<td>Mg</td>
<td>10.34</td>
<td>11.46</td>
</tr>
<tr>
<td>S</td>
<td>5.49b</td>
<td>11.13b</td>
</tr>
</tbody>
</table>

p

a, b, c - values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05

### TABLE 5

#### Bioaccumulation factors (BF) of macronutrients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>8.68b</td>
<td>0.90b</td>
<td>36.00c</td>
<td>7.07b</td>
<td>10.34</td>
<td>1.74</td>
<td>9.17b</td>
</tr>
<tr>
<td>M2</td>
<td>9.38b</td>
<td>0.81b</td>
<td>23.82b</td>
<td>6.77b</td>
<td>11.46</td>
<td>1.50</td>
<td>9.90b</td>
</tr>
<tr>
<td>M3</td>
<td>6.25a</td>
<td>0.56a</td>
<td>15.73a</td>
<td>7.72a</td>
<td>10.08</td>
<td>1.30</td>
<td>9.55b</td>
</tr>
<tr>
<td>M4</td>
<td>6.71a</td>
<td>0.66a</td>
<td>17.43a</td>
<td>11.01b</td>
<td>9.81b</td>
<td>1.26</td>
<td>6.64b</td>
</tr>
<tr>
<td>M5</td>
<td>8.51b</td>
<td>0.70b</td>
<td>19.27b</td>
<td>9.75b</td>
<td>10.13</td>
<td>1.13</td>
<td>12.20b</td>
</tr>
<tr>
<td>M6</td>
<td>8.11b</td>
<td>0.62b</td>
<td>15.32b</td>
<td>10.34b</td>
<td>10.45</td>
<td>1.50</td>
<td>6.31b</td>
</tr>
</tbody>
</table>

Genotypes

<table>
<thead>
<tr>
<th>Place of research</th>
<th>Poznań</th>
<th>Elbląg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5.49b</td>
<td>11.13b</td>
</tr>
<tr>
<td>P</td>
<td>0.58a</td>
<td>1.15b</td>
</tr>
<tr>
<td>K</td>
<td>28.31b</td>
<td>22.58a</td>
</tr>
<tr>
<td>Ca</td>
<td>6.43b</td>
<td>13.95b</td>
</tr>
<tr>
<td>Mg</td>
<td>6.43b</td>
<td>13.95b</td>
</tr>
<tr>
<td>S</td>
<td>1.20</td>
<td>1.43</td>
</tr>
</tbody>
</table>

p

a, b, c - values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05, n.s. - non significant

### TABLE 6

#### Chemical composition of crude ash from biomass genotypes Miscanthus

<p>| (in percent of ash = 100%, the mean 55 kg ash from 1 t burned biomass) |
|-------------------------|----|----|----|----|----|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.84</td>
<td>37.96</td>
<td>58.22</td>
<td>11.47</td>
<td>1.20</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>0.91</td>
<td>31.18</td>
<td>54.38</td>
<td>10.84</td>
<td>1.14</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>0.76</td>
<td>24.89</td>
<td>46.07</td>
<td>8.80</td>
<td>0.94</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>0.96</td>
<td>26.62</td>
<td>62.25</td>
<td>9.27</td>
<td>0.87</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>0.87</td>
<td>27.33</td>
<td>53.87</td>
<td>8.47</td>
<td>0.93</td>
<td>2.22</td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>0.82</td>
<td>22.84</td>
<td>60.94</td>
<td>8.93</td>
<td>1.09</td>
<td>1.49</td>
<td></td>
</tr>
</tbody>
</table>

Genotypes

<table>
<thead>
<tr>
<th>Place of research</th>
<th>Poznań</th>
<th>Elbląg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.76</td>
<td>0.94</td>
</tr>
<tr>
<td>P</td>
<td>37.36</td>
<td>47.42</td>
</tr>
<tr>
<td>K</td>
<td>19.56</td>
<td>14.42</td>
</tr>
<tr>
<td>Ca</td>
<td>64.49</td>
<td>7.84</td>
</tr>
<tr>
<td>Mg</td>
<td>6.49</td>
<td>0.98</td>
</tr>
<tr>
<td>S</td>
<td>1.36</td>
<td>1.09</td>
</tr>
<tr>
<td>Na</td>
<td>2.11</td>
<td></td>
</tr>
</tbody>
</table>

All the experimental factors under study significantly affected the uptake of individual macronutrients under study (Table 4). Significantly more nitrogen, phosphorus, calcium, magnesium, sulphur and sodium was uptake in biomass of Miscanthus genotype M2. A much higher uptake of the analysed elements was recorded in biomass Miscanthus grass grown in Elbląg. A similar uptake nitrogen, potassium and magnesium was observed by [27, 28].

In the Table 5 show factors of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and sodium bioaccumulation. The genotypes, experiment sites as well as interaction between them significantly differentiated the bioaccumulation factor N, P, K, Ca and Na. The largest bioaccumulation coefficients calculated for potassium. Bioaccumulation factors for calcium, magnesium, nitrogen and sodium on remained average a similar level. The variation in the richness of grasses in macronutrients and other organic components (hemicellulose, cellulose, et.) can be caused by the plants varied capacity for absorbing mineral nutrients from soil and their biological and genotype properties [29].

In the Table 6 shows the percentage share macronutrients in ashes obtained after the combustion of Miscanthus biomass. The genetic features, soil, rainfall, fertilization, date of harvest have a significant influence on the chemical composition of ash [19, 28, 30, 31]. Analysed ashes were characterized by
high percentage of alkaline elements (mean Ca (53.09), K (27.30), Mg (9.63), Na (1.73), S (1.03) and P (0.86). In agricultural practice for fertilization ash from *Miscanthus* seems to be the most useful [19].

The results of individual analysis of GE interaction of genotypes are given in Table 1 for the yield and in Table 2-5 for K, Ca, Mg, and Na. The individual analysis for genotypes that follows, for each genotype: the main effect estimate, the F-statistic for it and the F-statistic for the interaction. Additionally, coefficients of regression and determination and F-statistic for regression are also given in this table. By comparing the F-values with the corresponding critical values given at the lower of the table, for levels 0.05 and 0.01, it is possible to evaluate the significance of the genotypes with regard to their main effects and interactions with environments. Finally, in order to investigate the relationship between the yield level of the *Miscanthus* genotypes and the chemical elements in *Miscanthus* biomass, multiple regression analyzes were performed separately for Poznań and Elbląg, and for both cities combined. Below, the multiple regression equations for yield (Y) for Poznań Y = 11.015 - 0.539K + 0.552Mg + 5.490Na (Ca - non-significant influence on yield), correlation coefficient R = 0.576, coefficient of determination (R²) = 33.13%. For Elbląg Y = 33.729 - 0.486K - 1.108Ca + 3.021Mg (Na - non-significant influence on yield), correlation coefficient R = 0.844, coefficient of determination R² = 71.24%. For Poznań and Elbląg together correlation coefficient R = 0.523, coefficient of determination R² = 27.32%, Y = 25.310 - 0.409Ca (K, Mg and Na - non-significant).

In studies of nutrient uptake by plants, it is not only their absolute content that is important, but also their weight and molar ratios. Such ratios are derivatives of their content in biomass. They provide grounds for conclusions concerning the availability of determined elements to plants, their antagonism or synergism. It must be stressed that the data only partially explain the relations between the soil and a plant, because the content of nutrients in a plant is to a certain extent determined by its genetic features. The weight components of K, Na, Ca and Mg in *Miscanthus* biomass are presented in Table 7. The monovalent-to-divalent cation ratio (K+Na) : (Ca+Mg) in biomass of the clones harvested in Poznań was highly diverse. It was much lower in biomass of M1 and M6 than in the others, which indicates a higher content of divalent cations. This indicates that M1 and M6 took up more divalent cations than monovalent ones. This may result from genetic factors and from larger amounts of these elements in soil. It increases the resistance to lodging, which facilitates the biomass harvest considerably. The K:Ca ratio in the genotypes under study was similar. This indicates that contrasting the ratio of monovalent-to-divalent cations was affected mainly by potassium and calcium cations. Considerable changes in the cation ratios were found in *Miscanthus* biomass harvested in Elbląg. The ratios were twice higher (0.68) than in Poznań (0.29). They are indicative of greater accumulation of potassium and sodium in *Miscanthus* biomass harvested in Elbląg. The total content of potassium and sodium in biomass harvested in Poznań was 11.51 g kg⁻¹ D.M. whereas in Elbląg it was 21.71 g kg⁻¹, with a much smaller difference in the total content of Ca+Mg of 39.77 and 32.37 g kg⁻¹ D.M. respectively. The differences may have been caused by the properties of soil in Poznań and Elbląg, mainly its pH of 6.82 and 5.12, respectively. A varying (K+Na) : (Ca+Mg) ratio was also observed in the *Miscanthus* biomass harvested in Elbląg, where the highest value was calculated for the biomass of M1 and the lowest was for M4. In studies with eastern galega in which the seeds had been inoculated with the blue-green *Nostoc* algae calculated ratios (K+Na) : (Ca+Mg) and K : Ca were level 0.33 and 0.35 [32]. The (K+Na) : (Ca+Mg) ratio in *Miscanthus* in the consecutive study years in Poznań decreased, whereas in Elbląg it was the greatest in the second year, and the same in the first and the third year (Table 8). The mean value of the ratio was more than twice as high in the biomass harvested in Elbląg as in Poznań. The values for the consecutive years made the following sequence: 2nd year > 1st year > 3rd year. The study years significantly differentiated K : Mg ratios in seeds, straw and pea pods [33].

TABLE 7

Value of the (K + Na):(Ca + Mg) and K:Ca ratio in *Miscanthus* grass, depending on the place of research and genotypes (mean for years of cultivation)

<table>
<thead>
<tr>
<th>Element</th>
<th>Genotypes</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poznań</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K+Na): (Ca+Mg)</td>
<td></td>
<td>0.24</td>
<td>0.33</td>
<td>0.31</td>
<td>0.32</td>
<td>0.35</td>
<td>0.20</td>
<td>0.29</td>
</tr>
<tr>
<td>K:Ca</td>
<td></td>
<td>0.25</td>
<td>0.35</td>
<td>0.33</td>
<td>0.34</td>
<td>0.37</td>
<td>0.21</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Elbląg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K+Na): (Ca+Mg)</td>
<td></td>
<td>1.08</td>
<td>0.73</td>
<td>0.68</td>
<td>0.47</td>
<td>0.60</td>
<td>0.53</td>
<td>0.68</td>
</tr>
<tr>
<td>K:Ca</td>
<td></td>
<td>1.39</td>
<td>0.92</td>
<td>0.81</td>
<td>0.53</td>
<td>0.66</td>
<td>0.59</td>
<td>0.82</td>
</tr>
</tbody>
</table>
The ratios for monovalent (K:Na) and divalent (Ca:Mg) elements also made an interesting pattern (Table 9). An analysis of the K:Na ratio revealed its different values for different clones and experiment sites. The greatest value of the ratio was calculated for biomass of M4 clone grown at both sites, which indicates an increased capability for taking up potassium, and the lowest was for the M3 clone in Poznań and M5 in Elbląg. Moreover, higher levels of sodium were found in biomass harvested in Poznań than in Elbląg. The pattern for the Ca:Mg ratio was the opposite to that of K:Na. The values were nearly twice as high for Miscanthus grass harvested in Poznań than that harvested in Elbląg (8.23 and 4.27, respectively). This indicates that more calcium than magnesium was taken up by the plants grown in Poznań. An analysis of this ratio within the clones revealed that it was the highest for M6 and the lowest for M3 in the biomass harvested in Poznań; the respective values for Elbląg: M4- 5.23 and M1-2.94. These ratios for Miscanthus grass are important in regard to the chemical composition of the ash produced. Biomass with a high content of potassium has a negative effect on ash, which tends to form slag in the combustion process [19, 34] and is a deciding factor in using ash in the soil-plant circulation. Determination of the demand for nutrients in potential years of biomass growth and harvest is an important element of studies on the content of minerals in biomass of perennial plants and its changes. Miscanthus grass is of particular importance in this regard because studies [35, 36] have shown that while producing large amounts of biomass, these plants respond poorly to fertilisation, whose effectiveness was low. The low effectiveness of fertilisation may be a consequence of high photosynthetic capability of Miscanthus, which follows the C4 photosynthesis pathway, the function of rhizomes which accumulate large amounts of nutrients and effective use of light and water [37]. An important role in growth and development is played by rhizomes, which accumulate nutrients; report that the accumulated nutrients are transferred from rhizomes to the over ground parts during the growth period and then back to rhizomes at the end of the growing season [13, 38]. This may suggest that nutrients must be replenished to meet the Miscanthus needs in terms of fertilisation.

The relationships between the amount of biomass and the total content of Ca, K, Mg and Na in biomass are also interesting (Table 10) and negatively significant. A year-by-year increase in the yield of Miscanthus results in an increase in its biomass with a simultaneous decrease in the total content of the elements under study in the biomass during the phase of technological usability as a source of energy. Similar relationships have also been reported by [39] for Miscanthus as a perennial crop.

### Table 8
Value of the (K + Na):(Ca + Mg) ratio in Miscanthus grass, depending on the place of research and the years of cultivation (mean for genotypes)

<table>
<thead>
<tr>
<th>Place of research</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poznań</td>
<td>0.42</td>
<td>0.23</td>
<td>0.19</td>
<td>0.28</td>
</tr>
<tr>
<td>Elbląg</td>
<td>0.58</td>
<td>0.86</td>
<td>0.59</td>
<td>0.68</td>
</tr>
<tr>
<td>Mean</td>
<td>0.50</td>
<td>0.55</td>
<td>0.39</td>
<td>0.48</td>
</tr>
</tbody>
</table>

### Table 9
Value of the K:Na and Ca:Mg ratio in Miscanthus grass depending on the genotypes and place of research

<table>
<thead>
<tr>
<th>Research of elements</th>
<th>Genotypes</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>Poznań</td>
<td>13.43</td>
<td>15.44</td>
</tr>
<tr>
<td>Elbląg</td>
<td>22.09</td>
<td>18.62</td>
</tr>
<tr>
<td>Ca:Mg</td>
<td>8.32</td>
<td>8.10</td>
</tr>
<tr>
<td>Elbląg</td>
<td>2.94</td>
<td>3.18</td>
</tr>
</tbody>
</table>

### Table 10
Relationship between the yield of Miscanthus grass (t ha⁻¹ D.M.) and the sum of the content elements in years of cultivation

<table>
<thead>
<tr>
<th>Year of cultivation</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (t ha⁻¹ D.M.)</td>
<td>7.84</td>
<td>12.39</td>
<td>17.93</td>
</tr>
<tr>
<td>Sum of the K, Ca, Mg and Na content (g kg⁻¹ D.M.)</td>
<td>63.02</td>
<td>53.03</td>
<td>42.02</td>
</tr>
</tbody>
</table>

r = -0.99 y = 7.94 - 2.09x (for year cultivation)
CONCLUSIONS

1. The average yield of Miscanthus biomass amounted 12.72 t ha⁻¹.
2. Biomass of genotypes of Miscanthus grass grown in diverse soil and climatic conditions in Poland during three years contained the largest amount of carbon, hydrogen, silica, calcium, followed - in a decreasing order - by K, Mg, N, Na, S and P.
3. Uptake macronutrients by the biomass yields of Miscanthus was higher for calcium, potassium, magnesium and nitrogen.
4. The Miscanthus biomass yield has been found to be significantly negatively correlated with the total concentration of Ca, K, Mg and Na.
5. The content of the elements under study in biomass differentiated the weight ratios of monovalent and divalent elements, which indicated the nutritional demands of the genotypes under study and the chemical composition of the ash produced.
6. Ashes from burned biomass of Miscanthus can be suitable for use in agriculture as a liming agent to be applied on medium and heavy soils.

ACKNOWLEDGEMENTS

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Dorota Kalembasa, Stanisław Jeżowski, Barbara Symanowicz have contributed equally to this work.

REFERENCES


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LANDSCAPE PATTERN AND URBAN COOLING ISLANDS

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ABSTRACT

Urbanisation is an important factor related to microclimate and one of the main reasons for the formation of an Urban Heat Island (UHI). In the general sense, vegetated areas (e.g. forests, urban green spaces) form Urban Cooling Islands (UCIs), mitigate the adverse effects of UHI and deliver benefits for human health and urban sustainability. However, it is still unclear how the land use/land cover (LULC) pattern characteristics and different types of green spaces affect the UCI benefits. This paper uses landscape metrics and quantitative statistical analysis to assess the influence of the spatial pattern of the landscape and different types of green spaces on delivering the UCI benefits in the case of Samsun, Turkey. In this study, land surface temperature (LST) and LULC types in Samsun, Turkey were obtained from Landsat 8 imagery, Urban Atlas 2012 and ESM 2012 datasets. I found that forests, urban green spaces, and water features were the most important components of the landscape in terms of delivering UCI benefits. The larger the patch area, edge perimeter and the more complex the shape of forests and urban green spaces with a more physically connected configuration had the stronger the UCI benefits. The paper concluded with a short review of how the findings of this study can be useful for future landscape planning regarding the UCI mitigation in urban areas.

KEYWORDS:
Urban cooling islands, land surface temperature, spatial pattern, landscape metrics

INTRODUCTION

Urbanisation is a process where the land is mainly converted into urban areas (e.g. settlements, industrial areas, and transportation networks), and the built-up areas cover a large proportion of the land surface [1, 2]. Urbanisation is an important factor related to microclimate since the temperature in urban areas is higher than the surrounding areas. This impact is called as the Urban Heat Island (UHI) effect [3, 4]. The UHI effect leads to increased energy consumption [5], adversely affects vegetation growth [6, 7], air and water quality [8, 9], and poses an important threat to the health of citizens [10, 11]. Therefore, the mitigation of the UHI has received considerable attention as an urgent subject in landscape planning [7, 12, 13]. In the meantime, several studies have proved that, in urban environments, the vegetated areas create the Urban Cool Island (UCI) effect. The UCI effect means a lower temperature in green areas than its surrounding areas and impervious surfaces [14, 15]. So, in urban areas, the UCI effect is considered as one of the major factors affecting urban climate [16, 17], mitigating the adverse effects of the UHI effect and also improving the thermal comfort for citizens [18, 19]. Considering all of these, it is clear that a better understanding of the UCI effect is obviously of critical importance for improving the urban living conditions.

Landscape pattern is one of the most important factors relevant to UCIE [5, 20]. The relationship between the UCI effect and land use/land cover (LULC) pattern is well documented in various studies for the cooling effect of the vegetated areas and water features [21, 22, 23] as well as certain type of urban green spaces (e.g. parks [24], gardens [14], and green roofs [25]). Further to that, several studies have reported that the size, shape, type, and pattern of green spaces have an influence on the UCI effect [14, 26, 27]. For example, large urban forests and urban green spaces have been found to create the UCI effect [28, 29]. The purpose of this study was thus to investigate the UCI effect and its relationship to the patterns of the urban landscape. This study thus used part of Samsun district as a case study and analysed the influence of spatial pattern of the urban landscape and LULC types on delivering UCI benefits. The objectives were (1) to determine the effect of LULC pattern on the UCI, and (2) to compare the efficacy of different types of green spaces in terms of delivering UCIE benefits.

MATERIALS AND METHODS

Study Area. Samsun is located in the north of Turkey, the Black Sea Region, at 41.28°N, 36.34°E, and composed of 17 districts. With a population of 1261810 in 2013, Samsun is the most populous city in the Black Sea region and sixteenth most populous city in Turkey [30]. Samsun has the Black Sea climate which is characterized by rainy, warm winters, and hot summers in all seasons. The highest average temperatures in the last century in Samsun province
were observed in September and August (39.0°C and 38.3°C, respectively) [31]. In this study, the case study area is formed from the coastal parts of Central, Ondokuzmayis and Tekkekoy districts of Samsun province (Figure 1).

Samsun has witnessed a rapid urbanisation process over the past few decades, and this trend is expected to continue in the near future because of the recent population growth [32]. Rapid urbanisation and increase in the population have profoundly changed Samsun’s urban landscape and the pattern of LULC [33].

**Land Use/Land Cover (LULC) Identification and Landscape Metrics.** For the identification of LULC types, I used the Urban Atlas 2012 (UA2012) and the European Settlement Map (ESM 2012) – Release 2017 datasets [34, 35]. Whilst the Urban Atlas dataset provided general information on different LULC types, ESM helped me to identify LULC types at a finer resolution.
### TABLE 1
Selected landscape metrics

<table>
<thead>
<tr>
<th>Metric and unit</th>
<th>Level</th>
<th>Acronym and Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of Landscape (%)</td>
<td>Class</td>
<td>0 &lt; PLAND ≤</td>
<td>The proportion of landscape occupied by a particular LULC type</td>
</tr>
<tr>
<td>Area Weighted Patch Area (ha)</td>
<td>Class</td>
<td>AREA_AM &gt; 0, without limit</td>
<td>The area-weighted mean size of the given LULC type</td>
</tr>
<tr>
<td>Number of Patches</td>
<td>Class</td>
<td>NP ≥ 1, without limit</td>
<td>The number of patches of the given LULC type</td>
</tr>
<tr>
<td>Patch Area (ha)</td>
<td>Patch</td>
<td>PA &gt; 0, without limit</td>
<td>The size of each LULC patch</td>
</tr>
<tr>
<td>Shape index</td>
<td>Patch</td>
<td>SI ≥ 1</td>
<td>SI approaches 1 for a patch with simple circle shape. Higher SI indicates higher shape complexity</td>
</tr>
<tr>
<td>Patch Perimeter (m)</td>
<td>Patch</td>
<td>PERIM &gt; 0, without limit</td>
<td>The perimeter of each LULC patch</td>
</tr>
<tr>
<td>Proximity Index</td>
<td>Patch</td>
<td>PROX_AM ≥ 0</td>
<td>The degree of isolation and fragmentation within a specified search radius for the given LULC type</td>
</tr>
</tbody>
</table>

I combined 22 detailed LULC categories into 9 broad LULC categories to prevent bias in the analysis process and the results: agricultural areas, built-up areas, forests, natural/semi-natural areas, open spaces with little/no vegetation, railways, roads/streets, urban green spaces, and water features. The final vector formatted LULC map was converted into raster format with a resolution of 30m (Figure 2).

For this study, 7 landscape metrics at patch and class levels were used, because of their widespread use and straightforward interpretation [36, 37, 38, 39, 40, 41]. At the class level, the percentage of landscape (PLAND, %), number of patches (NP) and area weighted mean area (AREA_AM) were selected. In addition to this, I used 4 metrics at the patch level to explore the relationship between the pattern of LULC categories and the UCI effects (Table 1).

#### Land Surface Temperature (LST) Retrieval and Urban Cooling Island (UCI)

The UHI and UCI effects are generally derived from meteorological observations [42, 43], and thermal remote sensing retrieval [20, 44]. Even though the traditional method of meteorological observations yield more accurate data, this method is restricted to a small portion of a landscape and requires a long term study for its observations. On the other hand, thermal remote sensing retrieves the land surface temperature (LST) and provides better synchronicity and higher spatially continuous coverage for the mean radiative temperature of a land surface [45-47]. The effect of UCI is generally stronger during summer [48]. Since the LULC map was dated to 2012, three cloud-free Landsat-8 images were acquired to represent average temperature patterns at the hottest period between 2013 and 2015 in Samsun (for the dates of 13 September 2013, 31 August 2014 and 3 September 2015). I calculated UCI based on LST (°C). I calculated the UCI based on the LST (°C). The LST was retrieved from the thermal infrared sensor (TIRS) on Landsat-8 imagery dated, 13 September 2013. I used the LST retrieval process used in previous research [e.g. 15, 20, 47]. The UCI effect was represented by the minimum, mean, maximum, standard deviation, majority and minority value of LST for each of the LULC type.

#### Statistical Analysis
In order to evaluate the relationship between the patch level landscape metrics and the mean LST values, I calculated cross-correlation coefficients (e.g. patch area value versus LST mean value, proximity index value vs. LST mean value, etc.). The variation of the mean LST values did not include high-frequency components (ripples). So, each of the series of the other 6 parameters were pre-processed to suppress high-frequency characteristics to have more reliable cross-correlation coefficients. The pre-processing was composed of two stages. At the first stage, the values which were enormously away from the related mean values were neglected, and at the second stage, a moving average filter was applied in which the window size was adjusted to the number of data points. Spearman’s rank correlation coefficient was used to quantify the effect of the LULC patch pattern on UCI. Statistical analyses were performed in the MatlabTM [49].

#### RESULTS

**Landscape Pattern and UCI Effects.** Built-up areas of Samsun is mainly located on the coast of Black Sea region and scattered into the patches of forests, semi-natural lands, and agricultural lands. Within the total study area of 109513.17ha, built-up areas occupy 2.13% of the total study area with a large number of patches (64823) and an area-weighted mean area of 0.88ha (Table 2).

Railways and roads/streets only occupy a small portion of the whole study area (0.08% and 1.15%, respectively). The dominant LULC type, forests (55.33%), reported a smaller number of patches.
(2315) with the largest area-weighted mean area of 25079.44ha) as an indication of least fragmented pattern compared to the other LULC types. The other two widespread LULC types were agricultural lands (19.77%) and open spaces with little/no vegetation (15.39%). With the lower number of patches and large area weighted mean area, agricultural lands are structurally more connected than open spaces with little/no vegetation. Urban green spaces and natural / semi-natural lands occupy only a small portion of the study area (3.33% and 2.79%, respectively). However, urban green spaces are mainly distributed around built-up areas and have a much more scattered pattern compared to natural / semi-natural lands. As the rarest LULC type, water features occupy only 0.03% of the whole study area with the second lowest area-weighted mean area (2.65ha) and largest NP (206).

**UCI Effects of the LULC Types.** For each year, the spatial distribution of LST and accordingly the UCI effects corresponded to the pattern of the landscape is shown in Figure 3.

The highest maximum LST and the biggest temperature differences for the four dates occurred in September 2013. All other dates had lower maximum LSTs and lower temperature differences, indicating that in September 2013 had the most heterogeneous thermal environment in the study area (Table 3).

| TABLE 2 |

<p>| Land use/land cover types in the study area |</p>
<table>
<thead>
<tr>
<th>LULC TYPE</th>
<th>PLAND</th>
<th>NP</th>
<th>AREA_AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forests</td>
<td>55.33</td>
<td>4749</td>
<td>25079.44</td>
</tr>
<tr>
<td>Agricultural lands</td>
<td>19.77</td>
<td>19118</td>
<td>212.87</td>
</tr>
<tr>
<td>Open spaces with little/no vegetation</td>
<td>15.39</td>
<td>88402</td>
<td>273.40</td>
</tr>
<tr>
<td>Urban green spaces</td>
<td>3.33</td>
<td>18768</td>
<td>6.57</td>
</tr>
<tr>
<td>Natural/semi-natural lands</td>
<td>2.79</td>
<td>8267</td>
<td>13.03</td>
</tr>
<tr>
<td>Built-up areas</td>
<td>2.13</td>
<td>64823</td>
<td>0.87</td>
</tr>
<tr>
<td>Roads/streets</td>
<td>1.15</td>
<td>6268</td>
<td>189.01</td>
</tr>
<tr>
<td>Railways</td>
<td>0.08</td>
<td>40</td>
<td>10.31</td>
</tr>
<tr>
<td>Water features</td>
<td>0.03</td>
<td>206</td>
<td>2.65</td>
</tr>
</tbody>
</table>

**FIGURE 3**

LST (°C) of three selected dates and the total average LST
Forests formed the largest UCIIEs for the selected three dates. Even though water features occupied only a small amount of the study area, they had cooling effects compared to the other LULC types. On the other hand, built-up areas, railways, and roads/streets formed the UHIs in all three dates. Surprisingly, the mean UCI effects formed agricultural lands, urban green spaces, natural and semi-natural lands and open spaces with little/no vegetation were hotter than the LST of the whole study area in all three dates. Part of the reason for this result was that agricultural areas had less vegetation cover (e.g. crops had been harvested and pastures were withered) in late August and early September. Similarly, areas with open spaces with little/no vegetation had less vegetation cover compared to forests, natural and semi-natural lands, and urban green spaces. This interpretation was confirmed by the lowest NDVI values in agricultural lands for the three dates. On the other hand, urban green spaces were surrounded by built-up areas, whereas the natural and semi-natural lands were surrounded by agricultural lands and they were both affected by the UHI effect created by surrounding LULC types.

Whilst the value of the total average LST was 29.18°C, the max and minimum LST were 40.39°C and 20.97°C for the whole study area (Table 4). The max LST of forests, agricultural areas and open spaces with little/no vegetation were quite high with 40.39°C. Also, with the highest difference between their minimum and maximum LST values, forests and open spaces with little/no vegetation reported the most heterogeneous thermal environment amongst the all other LULC types. However, the value of total average LST inside the forests (27.64°C) was obviously lower than the average LST of the whole study area (29.18°C) and built-up areas (30.41°C). Also, the majority of its LST values were between 26.79°C - 27.44°C. So, I could safely claim that among the all LULC types, forests formed the largest UCI area with the lowest mean and minimum LST.

As expected, the minimum and mean LST of agricultural areas and open spaces with little/no vegetation were higher than all the LST values for the whole study area and forests. The minimum LST of urban green spaces was the same with built-up areas, its mean and maximum LST values were slightly lower than built-up areas. This inconsistency attributed to the presence of intense built-up areas around small sized urban green spaces. However, the second lowest standard deviation value together with the low values of majority LST for this LULC type confirmed that urban green areas among built-up areas also formed small UCIs in the study area (see Figure 2 and 3).

### Table 3

<table>
<thead>
<tr>
<th>Study Area LULC types</th>
<th>Temperature (°C)</th>
<th>Min</th>
<th>Mean</th>
<th>Max</th>
<th>Standard Deviation</th>
<th>Majority</th>
<th>Minority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural lands</td>
<td>25.02</td>
<td>35.22</td>
<td>45.84</td>
<td>18.89</td>
<td>25.8</td>
<td>34.96</td>
<td>24.97</td>
</tr>
<tr>
<td>Built-up areas</td>
<td>24.01</td>
<td>32.84</td>
<td>44.51</td>
<td>17.57</td>
<td>26.99</td>
<td>34.07</td>
<td>23.97</td>
</tr>
<tr>
<td>Forests</td>
<td>22.8</td>
<td>30.62</td>
<td>44.8</td>
<td>16.77</td>
<td>22.6</td>
<td>33.18</td>
<td>22.93</td>
</tr>
<tr>
<td>Natural/semi-natural lands</td>
<td>24.71</td>
<td>34.48</td>
<td>45.98</td>
<td>18.28</td>
<td>24.91</td>
<td>32.19</td>
<td>24.84</td>
</tr>
<tr>
<td>Open spaces with little/no vegetation</td>
<td>23.92</td>
<td>34.91</td>
<td>47.35</td>
<td>17.55</td>
<td>26.15</td>
<td>32.83</td>
<td>24.06</td>
</tr>
<tr>
<td>Railways</td>
<td>27.29</td>
<td>32.97</td>
<td>45.8</td>
<td>22.88</td>
<td>26.66</td>
<td>30.27</td>
<td>26.61</td>
</tr>
<tr>
<td>Roads/streets</td>
<td>25.87</td>
<td>32.62</td>
<td>43.31</td>
<td>20.16</td>
<td>25.53</td>
<td>31.34</td>
<td>39.2</td>
</tr>
<tr>
<td>Urban green spaces</td>
<td>24.05</td>
<td>33.04</td>
<td>43.39</td>
<td>17.62</td>
<td>26.15</td>
<td>32.77</td>
<td>24.13</td>
</tr>
<tr>
<td>Water features</td>
<td>25.45</td>
<td>29.75</td>
<td>37.19</td>
<td>22.97</td>
<td>25.9</td>
<td>30.5</td>
<td>24.34</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Study Area</th>
<th>Temperature (°C)</th>
<th>Min</th>
<th>Mean</th>
<th>Max</th>
<th>Standard Deviation</th>
<th>Majority</th>
<th>Minority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural lands</td>
<td>20.97</td>
<td>29.18</td>
<td>40.39</td>
<td>2.95</td>
<td>28.09-28.73</td>
<td>39.74-40.39</td>
<td></td>
</tr>
<tr>
<td>Built-up areas</td>
<td>23.11</td>
<td>31.1</td>
<td>40.39</td>
<td>2.4</td>
<td>30.68-31.32</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Forests</td>
<td>21.83</td>
<td>30.41</td>
<td>38.94</td>
<td>1.64</td>
<td>30.03-30.68</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Natural/semi-natural lands</td>
<td>20.97</td>
<td>27.64</td>
<td>40.39</td>
<td>2.45</td>
<td>26.79-27.44</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Open spaces with little/no vegetation</td>
<td>21.42</td>
<td>31.57</td>
<td>38.69</td>
<td>2.55</td>
<td>29.38-30.03</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Railways</td>
<td>25.79</td>
<td>30.17</td>
<td>34.08</td>
<td>1.42</td>
<td>30.03-30.68</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Roads/streets</td>
<td>24.5</td>
<td>30.39</td>
<td>37.7</td>
<td>1.34</td>
<td>30.03-30.68</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Urban green spaces</td>
<td>21.83</td>
<td>30.13</td>
<td>37.69</td>
<td>1.8</td>
<td>29.38-30.03</td>
<td>22.91-23.56</td>
<td></td>
</tr>
<tr>
<td>Water features</td>
<td>24.26</td>
<td>28.51</td>
<td>34.05</td>
<td>2.07</td>
<td>27.44-28.09</td>
<td>32.62-33.26</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION AND CONCLUSION

I used landscape metrics and quantitative statistical analysis to assess the influence of the spatial pattern of the landscape and forests on delivering UCI benefits. This study revealed that patch size, shape, edge perimeter and physical connectivity of LULC types had both positive and negative effects on LST and UCI benefits. According to the results presented in Table 3 and 4, there are significant differences in the min, mean and max LST values of different LULC types. For example, forests and open spaces with little/no vegetation had the most heterogeneous thermal environment amongst the all other LULC types (also see Figure 3). Whilst the inner parts of forests had the lowest LST and UCI effect for the selected three years and their average LST and UCI effect, the mean LST was higher at the edges of forests. This was the exact opposite for the areas of open spaces with little/no vegetation.

In relation to the average values of LST, the LULC types with lower temperatures have a direct relationship with the presence of dense vegetation, such as forests and urban green spaces. Furthermore, the LST and UCI benefits depend on vegetation cover of green spaces itself but also their surrounding characteristics [15, 47, 50]. As emphasised above, the inner parts of forests with dense vegetation reported the lowest LST and UCI effect for the selected three years and their average LST and UCI effect. Also, a significant negative correlation was found between forest and urban green space UCI effect and their composition and configuration [51]. As the size and shape complexity of these two green spaces increase and the distance between their patches decreases, their UCI effects get stronger. On the other hand, larger and complex patches of built-up areas and open spaces with little/no vegetation within a short distance to each other lead stronger UHI effects.

The UHI effect leads to increased energy consumption, air, and water quality and threatens the physical and mental health of citizens [5, 7, 11]. Therefore, efficient methods to mitigate the UHI effects and to deliver UCI benefits in urban environments require simple but important actions, such as increasing the amount and diversity of vegetated areas and water features, decreasing the amount of impervious surfaces [52, 53]. For example, enlarging urban green spaces with dense vegetation, adding complexity to the layout and shape of them and linking individual urban green spaces to each other with vegetated alley would help landscape planners to decrease the UHI effects of built-up areas and impervious surfaces. Such an approach can simply increase the amount of photosynthesis and transpiration, decreases LST and creates larger UCI in urban areas [15, 54].

<table>
<thead>
<tr>
<th>TABLE 5</th>
<th>Spearman correlation coefficients among landscape metrics and UCIs of total average LST</th>
</tr>
</thead>
<tbody>
<tr>
<td>AREA</td>
<td>PERIM</td>
</tr>
<tr>
<td>Roads/streets</td>
<td>0.04</td>
</tr>
<tr>
<td>Railways</td>
<td>0.33</td>
</tr>
<tr>
<td>Open spaces with little/no vegetation</td>
<td>0.53</td>
</tr>
<tr>
<td>Natural/semi-natural areas</td>
<td>0.24</td>
</tr>
<tr>
<td>Forests</td>
<td>-0.32</td>
</tr>
<tr>
<td>Built-up areas</td>
<td>0.47</td>
</tr>
<tr>
<td>Agricultural lands</td>
<td>-0.13</td>
</tr>
<tr>
<td>Urban green spaces</td>
<td>-0.26</td>
</tr>
<tr>
<td>Water features</td>
<td>Nan</td>
</tr>
</tbody>
</table>

Positive bold values: Significantly dependent (> 0.4)
Negative bold values: Significantly inversely dependent (< (-0.4))
Nan: Not a number (cannot be computed)
REFERENCES


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**ABSTRACT**

Fire blight caused by *Erwinia amylovora* is a difficult disease to control and has a high destructive effect. There is no certain management practice against this disease. Prohibition of antibiotics, harmful effects of the proposed chemicals to the ecosystem and health, emergence of the residues in customs controls, and increasing organic farming tendency, make the use of resistant varieties, rootstocks, and interstocks increasingly more important in the control of the disease. For this purpose, 32 hybrid F1 pear genotypes were evaluated for fire blight resistance and fruit quality characteristics and they were compared with their parents. How the maternal and pollenator parents transferring their features to the next generations also investigated. As a result of the study, among cultivars, ‘Magness’ found better for the transmission of fire blight resistance as maternal parent. Similar result obtained from ‘Moonglow’ as pollenator. ‘Santa Maria’ and ‘Magness’ transferring good fruit attractiveness, eating quality and shape while ‘Williams’ transferring irregular and rusty shaped genes as female parent. ‘Akca’ and ‘Moonglow’ hybrids’ fruit is smooth and in the form of a pear-shaped bulb, cheeky on a yellow-green background. So they have good attractiveness with low stone cell amount. Hybrids of ‘Conference’ and ‘Kaiser Alexandre’ have smoothly shaped and finely textured fruits that are good in terms of eating quality but their surface are usually green coloured and rusty. Coloration of ‘Guz’, ‘Tas’ and ‘Ankara’ hybrids’ fruit are also inadequate and they have high stone cell amount with irregular shape. As a conclusion of the study, registration of the genotypes which are found fire blight resistant and have superior fruit characteristics (29CD-4, 22CD-28, 29CD (7/6) and 29CD-14) is thought to contribute to fire blight disease management and pear cultivation.

**INTRODUCTION**

Cultivation of pear is being made approximately in 15 849 560 acre and 27 345 930 tons of pear has been harvested from these areas in the world [1]. Pear is mainly grown in temperate climatic zones of both two hemispheres, and 2 748 285 tons of pear is merchandized between countries [2]. However, in order to optimize the production of pears and increase the amount of trade, struggling with the most devastating disease, fire blight caused by *Erwinia amylovora*, is very important [3].

Fire blight is a disease that can cause the plant to die by systematically infecting the entire underground and aerial parts of the plant and leading to substantial crop losses [4]. Due to the fact that chemical management is not a definite solution, it causes adverse effects on human and environmental health and chemical residue problems; the use of resistant cultivars, rootstocks and interstocks are prominent ways in the control of the disease. In this context, breeding programs are being carried out to develop resistant genotypes [5, 6].

Hybridization is generally used in breeding studies, due to the polygenic nature of fire blight resistance [7] and complexity of its mechanism [8]. However, varieties with high resistance to disease also have poor characteristics such as grittiness, spininess, small fruit and malformations, so hybridization programs have been carried out with different combinations such as susceptible x susceptible, susceptible x resistant, resistant x resistant and resistant x susceptible in order to obtain genotypes that have superior fruit quality and disease resistance [9, 10]. Because the right choice of parents appears to be the most significant criterion for obtaining genotypes that are have desired characteristics. Therefore, determination the abilities of parents (maternal and pollenator) for the transmission of fire blight resistance and fruit characteristics to the progenies is crucial for the later stages of breeding programs.

For these reasons, 32 F1 pear hybrid genotypes were evaluated which were obtained from different hybridization combinations in terms of fire blight resistance and fruit quality aspects. Investigated char-
characteristics were compared with the parental cultivars. Each cultivar analysed for how they transferring their characteristics to the next generation.

**MATERIALS AND METHODS**

**F₁ hybrid plants.** The study was carried out using 32 F₁ hybrid pear plants which were obtained from different hybridization combinations that were formed with the moderate fire blight resistant 'Kaiser Alexandre', 'Magness', 'Moonglow', 'Conference', 'Ankara' and the susceptible 'Williams', 'Santa Maria' 'Tas' and 'Akca' varieties/cultigens. Hybrid plants were obtained from projects (TOVAG 106O719 and TOVAG 110O938) initiated in order to develop new pear varieties which are resistant to fire blight and have superior fruit characteristics. 32 Hybrids that were started to bear fruits in successive years of 2016 and 2017 were evaluated.

Pathogenic bacteria. Highly virulent seven *Erwinia amylovora* strains, that were chosen according to their pathogenicity levels among 75 *E. amylovora* strains, isolated by [11, 12, 13] from different locations in Turkey (Adana, Amasya, Bursa, Eskisehir, Karaman and Konya), were used in the study.

Evaluation for fire blight susceptibility. The susceptibility levels of hybrid plants were defined by artificial inoculations. The hybrids were tested twice with a suspension of 10⁶ cell/millilitre density prepared from the 48-hour bacterial culture developed in King B medium of these isolates when their shoots were reached approximately 15-25 cm height. After eight weeks the average of the two values was taken and the Genotype susceptibility (GS) value was calculated for each hybrid. Susceptibility of the shoots to fire blight was calculated according to the formula shown below (1). Then susceptibility characteristics were determined according to [6].

---

### TABLE 1
Parameters, relative scores, class values and scores of the characteristics of hybrid pear genotypes based on modified weighted ranking method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relative Scores</th>
<th>Class Values and Scores of the Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire Blight Resistance</td>
<td>20</td>
<td>Slightly susceptible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less susceptible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid-susceptible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very susceptible</td>
</tr>
<tr>
<td>Eating Quality</td>
<td>20</td>
<td>Very good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bad</td>
</tr>
<tr>
<td>Fruit Attractiveness</td>
<td>20</td>
<td>Very good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bad</td>
</tr>
<tr>
<td>Harvest Time</td>
<td>10</td>
<td>Late &gt; From Williams</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle – Between Akca and Williams</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early &lt; From Akca</td>
</tr>
<tr>
<td>Fruit Size</td>
<td>5</td>
<td>Very big (&gt; 220g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Big (175-220g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle (130-175g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Small (75-130g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very small (&lt; 75g)</td>
</tr>
<tr>
<td>Length/Diameter</td>
<td>5</td>
<td>Very long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very short</td>
</tr>
<tr>
<td>Soluble Solids Content</td>
<td>5</td>
<td>High (&gt; % 13.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle (% 10 - 13.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low (&lt; % 10)</td>
</tr>
<tr>
<td>Stone Cell Status of Fruit</td>
<td>5</td>
<td>Few</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lot</td>
</tr>
<tr>
<td>Fruit Firmness</td>
<td>5</td>
<td>Very firm (&gt;11 kg/cm²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Firm (8-11 kg/cm²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle (6-8 kg/cm²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soft (&lt;6 kg/cm²)</td>
</tr>
<tr>
<td>Rustiness</td>
<td>5</td>
<td>Very few</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Few</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lot</td>
</tr>
</tbody>
</table>
Determine superior hybrid genotypes. Weighted ranking method was used in order to determine the superior genotypes. For this purpose, the breeder defines the selection criteria and gives those criteria the relative points based on the significance level. The total score belonging to each genotype is determined with this revealed scoring system. [5].

While determining the score, in addition to the properties obtained with the measurement (susceptibility level of hybrids to fire blight, fruit size, length/diameter ratio, soluble solids content, harvest time and fruit firmness), sensorial properties (eating quality, fruit attractiveness, stone cell status of fruit and rustiness) which were detected by 5 panelists, were added to weighted ranking method (Table 1).

International pear identification documents were used in selection of the criteria used in the weighted ranking table and in setting the reference values of the criteria [5, 6, 14]. All these parameters were evaluated with the 2-year data.

RESULTS AND DISCUSSION

Breeding new pear genotypes that will establish a market presence in the World, requires to supply the requests of the consumers in every sense. For this purpose, the sensorial characteristics have been added to the measured properties and it has been tried to determine the superior genotypes by subjecting all the genotypes to the weighted ranking method and the result are given in Table 2.

### TABLE 2
Scores of hybrid pear genotypes in terms of properties according to Modified Weighted Ranking Method

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fire Blight Resistance</th>
<th>Harvest Time</th>
<th>Eating Quality</th>
<th>Fruit Attractiveness</th>
<th>Fruit Size</th>
<th>Length/Diameter Ratio</th>
<th>Soluble Solids Content</th>
<th>Stone Cell Status of Fruit</th>
<th>Fruit Firmness</th>
<th>Rustiness</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>22CD-28 (*S. Maria x Akca)</td>
<td>200</td>
<td>40</td>
<td>200</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>905</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29CD-4 (S. Maria x Moonglow)</td>
<td>200</td>
<td>100</td>
<td>200</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>35</td>
<td>35</td>
<td>895</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Magness</strong></td>
<td>100</td>
<td>40</td>
<td>200</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29CD(7/6) (S. Maria x Moonglow)</td>
<td>200</td>
<td>40</td>
<td>140</td>
<td>40</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>50</td>
<td>780</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29CD(14) (S. Maria x Moonglow)</td>
<td>200</td>
<td>100</td>
<td>140</td>
<td>80</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>775</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Santa Maria</strong></td>
<td>60</td>
<td>40</td>
<td>200</td>
<td>50</td>
<td>40</td>
<td>35</td>
<td>50</td>
<td>50</td>
<td>760</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-104 (S. Maria x K. Alex)</td>
<td>60</td>
<td>100</td>
<td>200</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>20</td>
<td>745</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34-292 (Williams x Conference)</td>
<td>100</td>
<td>100</td>
<td>140</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>715</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-525 (S. Maria x K. Alex)</td>
<td>20</td>
<td>100</td>
<td>200</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>20</td>
<td>705</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-113 (S. Maria x Conference)</td>
<td>200</td>
<td>100</td>
<td>140</td>
<td>40</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>50</td>
<td>700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-187 (S. Maria x Tas)</td>
<td>200</td>
<td>100</td>
<td>140</td>
<td>50</td>
<td>25</td>
<td>35</td>
<td>50</td>
<td>50</td>
<td>685</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29-33 (S. Maria x Moonglow)</td>
<td>60</td>
<td>100</td>
<td>140</td>
<td>50</td>
<td>40</td>
<td>35</td>
<td>25</td>
<td>50</td>
<td>675</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29-188 (S. Maria x Moonglow)</td>
<td>60</td>
<td>100</td>
<td>140</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>675</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-427 (S. Maria x Tas)</td>
<td>60</td>
<td>100</td>
<td>140</td>
<td>40</td>
<td>50</td>
<td>35</td>
<td>25</td>
<td>50</td>
<td>650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20CD-1 (Magness x Tas)</td>
<td>200</td>
<td>100</td>
<td>80</td>
<td>40</td>
<td>25</td>
<td>50</td>
<td>5</td>
<td>35</td>
<td>650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32CD(8/19) (Williams x Ankara)</td>
<td>200</td>
<td>100</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>20</td>
<td>645</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20CD(4-17) (Magness x Tas)</td>
<td>200</td>
<td>40</td>
<td>140</td>
<td>80</td>
<td>15</td>
<td>15</td>
<td>25</td>
<td>50</td>
<td>635</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-384 (S. Maria x Akca)</td>
<td>60</td>
<td>40</td>
<td>200</td>
<td>140</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>635</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Williams</strong></td>
<td>60</td>
<td>40</td>
<td>140</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>20</td>
<td>630</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-504 (S. Maria x Akca)</td>
<td>200</td>
<td>100</td>
<td>140</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>20</td>
<td>615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-101 (S. Maria x K. Alex)</td>
<td>60</td>
<td>100</td>
<td>140</td>
<td>80</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34-466 (Williams x Conference)</td>
<td>60</td>
<td>100</td>
<td>140</td>
<td>80</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>20</td>
<td>600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32CD(8-20) (Williams x Ankara)</td>
<td>200</td>
<td>100</td>
<td>80</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>595</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29-301 (S. Maria x Moonglow)</td>
<td>20</td>
<td>100</td>
<td>80</td>
<td>40</td>
<td>40</td>
<td>25</td>
<td>25</td>
<td>35</td>
<td>575</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-611 (S. Maria x **K. Alex)</td>
<td>60</td>
<td>40</td>
<td>80</td>
<td>140</td>
<td>40</td>
<td>40</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-2 (S. Maria x Tas)</td>
<td>60</td>
<td>40</td>
<td>140</td>
<td>80</td>
<td>50</td>
<td>35</td>
<td>25</td>
<td>50</td>
<td>540</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-348 (S. Maria x Akca)</td>
<td>60</td>
<td>40</td>
<td>80</td>
<td>140</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>515</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-249 (Williams x K. Alex)</td>
<td>60</td>
<td>40</td>
<td>140</td>
<td>80</td>
<td>15</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>515</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-535 (S. Maria x Akca)</td>
<td>60</td>
<td>40</td>
<td>140</td>
<td>80</td>
<td>15</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>515</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29-176 (S. Maria x Moonglow)</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>50</td>
<td>25</td>
<td>20</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-64 (S. Maria x Bursa)</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>40</td>
<td>25</td>
<td>20</td>
<td>455</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29-176 (S. Maria x Moonglow)</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>455</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Santa Maria, **: Kaiser Alexandre

\[
(GS) = \frac{\text{Length of the Infected Part (cm)}}{\text{Total Length of Shoot (cm)}} \times 100 \quad (1)
\]
As a result of the modified weighted ranking method, the total point varied between 340-905 (Table 2). ‘Magness’ (800 points) got the highest point among the commercial varieties included into the weighted ranking, used as a reference. This study was carried out within the breeding program so hybrids which got higher point than ‘Santa Maria’ are considered as a candidate to become a new variety.

In terms of total points, evaluations of the fruits of the hybrids remaining between ‘Williams’ and ‘Santa Maria’ varieties (760-630) will be continued one more year and those with lower points than ‘Williams’ variety (630) will be eliminated. In consequence of the method formed by the parameters which bring the commercial value of the genotypes to the forefront, 4 genotypes (29CD-4, 22CD-28, 29CD (7/6) and 29CD-14) have been found to have the potential of being registrable. As the new candidate of cultivar, the fruits which belong to some hybrids having the potential are seen in Figure 1.

Although the fire blight has been known for about 250 years, no way has been found effective to struggle with the disease. It is impossible to prevent the disease factor reaching to the plant. So, laying out the gardens with disease resistant rootstocks and cultivars is very significant for economic and sustainable pear cultivation. For this reason, studies have been carried out in many countries primarily on determining the susceptibility level of genetic resources [15, 16, 17, 18, 19]. Then, breeding programs are being carried out to develop resistant genotypes and studies are still going on [5, 20, 21]. Some genotypes were registered as a result of these programs such as ‘Harrow Queen’, ‘Harrow Delight’, ‘Harrow Sweet’ [22], ‘Shenandoah’ [23], ‘Aida’ and ‘Boheme’ [24] and they were introduced to the European market. Totally 16 fire blight resistant F1 genotypes [5, 6] have not been registered but they were reported being registerable thanks to their superior fruit characteristics. In addition to resistant scions ‘Geneva series’ QR517-9’ and ‘QR193-2’ were improved against to fire blight as resistant rootstocks [25, 26]. Because disease effects all upper and under organs such as flowers, shoots, roots and branches of host plants and kills whole plant [4, 27].

In the combinations in which the ‘Magness’ cultivar is used as the maternal parent, the hybrids are generally tolerant to the disease, the fruits are superior in terms of eating quality and soluble solids content, while the size of the fruit and attractiveness are moderate. The hybrids of the combinations in which the ‘Williams’ cultivar is used as the maternal parent are generally susceptible to disease and the fruit is irregularly shaped and rusty. Although the fruits are good in terms of eating quality, they fall behind in other parameters. When the ‘Santa Maria’ variety is used as the maternal parent, the hybrids are generally susceptible to disease while length/diameter ratio, the size, eating quality and attractiveness are good, and it has soluble solid content and fruit firmness in the moderate level. ‘Santa Maria’ and ‘Magness’ have good attractiveness, eating quality and shape while ‘Williams’ has irregularly shaped and rusty. ‘Magness’ and ‘Williams’ have higher soluble solid content than ‘Santa Maria’. The sort of varieties in terms of red cheeks on fruit surface is ‘Magness’> ‘Santa Maria’> ‘Williams’.

In combinations where the ‘Moonglow’ and ‘Akca’ variety is used as pollinator, generally the fruit is smooth and in the form of a pear-shaped bulb, cheeky on a yellow-green background. For this reason, in ‘Moonglow’ and ‘Akca’ hybrids are having good attractiveness, the amount of stone cells is low and the eating quality is high. In addition, hybrids of these cultivars have apparent resistance to the disease while ‘Akca’ is highly susceptible to fire blight and the entire fruit surface is yellow and green.

When the ‘Conference’ and ‘Kaiser Alexandre’ varieties are used as pollinators, the hybrids are resistant to the disease and the fruits are smoothly shaped and finely textured. Fruits that are good in terms of eating quality are usually green coloured and rusty. All these features are resemble both ‘Conference’ and ‘Kaiser Alexandre’.

In combinations where the ‘Guz’, ‘Tas’ and ‘Ankara’ varieties are pollinators, the fruits are generally irregularly shaped, have a large amount of stone cells and rusty with bad attractiveness. Also coloration of these hybrids are inadequate. All these features are acceptable for ‘Guz’ and ‘Tas’. Ankara is a bit better than the others in terms of all these features.

In the previous studies, among the cultivars that were used as a parent; ‘Ankara’, ‘Conference’, ‘Kaiser Alexandre’, ‘Moonglow’ and ‘Magness’ cultivars were relatively resistant to fire blight disease, whereas the ‘Akca’, ‘Santa Maria’, and ‘Williams’ were determined to be susceptible. The results of the study agree with previous findings. Generally, the disease-tolerant cultivars’ hybrids were found less susceptible against to fire blight.

One of the most remarkable result of the study is effect of the parental interaction in terms of transferring the fire blight resistance and fruit characteristics. For example, generally ‘Santa Maria’ gives disease resistant hybrids with ‘Moonglow’ but susceptible hybrids with ‘Bursa’. Similarly, ‘Williams’ hybrids give lighter fruits with ‘Kaiser Alexandre’ and ‘Ankara’, while it is heavier with ‘Conference’. The fact that one cultivar in a combination showed a poor performance in disease resistance or fruit characteristic does not mean that the result will be poor in every combination. Because, recombinations of the genes vary according to cultivars and these features are show quantitative and polygenic inheritance [6, 28].
Development of disease-resistant genotypes is the current priority of the breeders in terms of integrated management against fire blight. In this study, 32 hybrid pear genotypes were analyzed in terms of disease resistance and fruit quality aspects. Superior hybrids were identified via weighted ranking method which was consisted of parameters measuring the commercial preference of the fruits. As a conclusion of the study, registration of the genotypes which are found fire blight resistant and have superior fruit characteristics (29CD-4, 22CD-28, 29CD (7/6) and 29CD-14) is thought to contribute to fire blight disease management and pear cultivation.

Among cultivars, ‘Magness’ found better for the transmission of fire blight resistance as maternal parent. Similar result obtained from ‘Moonglow’ as pollinator. ‘Santa Maria’ and ‘Magness’ transferring good fruit attractiveness, eating quality and shape while ‘Williams’ transferring irregular and rusty shaped genes as female parent. ‘Akca’ and ‘Moonglow’ hybrids’ fruit is smooth and in the form of a pear-shaped bulb, cheeky on a yellow-green background. So they have good attractiveness with low stone cell amount. Hybrids of ‘Conference’ and ‘Kaiser Alexandre’ have smoothly shaped and finely textured fruits that are good in terms of eating quality but their surface are usually green coloured and rusty. Coloration of ‘Guz’, ‘Tas’ and ‘Ankara’ hybrids’ fruit are also inadequate and they have high stone cell amount with irregular shape.

FIGURE 1
Fruits of some hybrids with registration potential (A: 29CD-4, B: 29CD-14, C: 29CD (7/6), D: 22CD-28)
REFERENCES


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CORRELATION ANALYSIS BETWEEN INTERFERENCE INDEX AND TREE GROWTH FACTORS OF *POPULUS SIMONII*

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ABSTRACT

This study was aimed to explore the correlations between wood competition and tree growth factors in *Populus simonii* artificial forests. Grey relational analysis was used to analyze the correlations of wood interference index with diameter at breast height (DBH), canopy size, height-diameter ratio, and habitat utilization rate. It was found that the interference index was negatively correlated with DBH, canopy size, and habitat utilization rate, but was positively correlated with height-diameter ratio. The main inhibitory factor on forest growth was stand density. Grey relational analysis showed that the correlation degrees of tree growth factors with interference index ranked as follows: height-diameter ratio > DBH > canopy size > habitat utilization rate. This study is a basis for stand structure optimization of artificial forests and eco-construction in middle east Inner Mongolia, China.

KEYWORDS: *Populus simonii*, Interference index, Tree growth factors, Correlation analysis

INTRODUCTION

Stand structure factors basically include tree species, forest age, plant height, diameter and individual distribution. Stand structures cover all information of forests, which can be acquired through investigation. Meanwhile, the good or bad stand structure reflects the strong or weak functions of forest.

Space structure affects the health and stability of artificial forests. Spatial structure and allocation decide the overall ecological functions of artificial forests [1-3]. The spatial distribution of tree species significantly affects the growth and survival of tree populations [4] and thereby leads the differences in forest health and stability. So far, neighbor interference models are used to guide research on forest culture and management [5-6], but have been rarely used to analyze the stand structures of protection forests in middle east Inner Mongolia, China.

*Populus simonii* is major rural tree species and more importantly, a protection forest species in semiarid regions of North China. Because of strong adaptability, *P. simonii* was selected as the major afforestation species in Chifeng City in middle east Inner Mongolia during 1980s. However, due to excessively high planting density and lack of necessary fostering measures, the forests grew into massive “small old-trees”, which severely inhibited the functions of local forestry. Thus, in this study, forest interference theory was used to analyze the space growth competition rules of *P. simonii* forests, aiming to underlie the stand structure optimization of artificial forests in middle east Inner Mongolia. This study would significantly guide us to strengthen the ecological protection functions of *P. simonii* artificial forests in middle east Inner Mongolia.

EXPERIMENTAL

Study area and methods. Study area. The study area was Huanghuadianzi in Aohan Banner, a typical small watershed in Chifeng, Inner Mongolia. The geographical position was 42°17′-42°33′ N and 119°36′-119°53′ E, and the altitude was 440-906 m. The annual precipitation there was 400-470 mm and the annual evaporation was 2290-2400 mm. The soils there were basically chestnut soils with alkalinity or weak alkalinity. Most vegetation in the small watershed were artificial and included natural grassland, shrub forests, tree-shrub mixed forests, coniferous-broadleaf mixed forests, and broadleaf forests. The major artificial tree species include *P. simonii*, *Pinus tableulaeformis*, *Pramus sibirica* and *Caragana microphylla*.

Methods. Investigation and sampling. We selected *P. simonii* stands with basically the same habitat conditions, forest ages, and different densities in the study area. Then study plots were set up, and the diameter at breast height (DBH), tree height and canopy size of both base plants and neighbors were measured. Also the interference index of base plants of *P. simonii* was computed.
Data analysis. (1) Definition of base plant and neighbour. The study trees were defined as the base plants, while the nearby individual plants that affected the growth of a base plant were called the neighbors [7].

(2) Neighbor interference index computed. Neighbor interference index was computed using a reported neighbor interference model [8]:

$$I_j = \frac{1}{n} \sum_{i=1}^{n} \left[ \frac{D_{ij}^2}{H_i} \cdot \frac{H_j}{D_{ij}^2} \cdot H_{ij} \cdot L_{ij}^{-1} \right] \cdot A_i$$

where, $I_i$ is the interference index of base plant $i$, or namely the interference degree of base plant $i$; $D_i$ is the DBH of base plant $i$; $H_i$ is the tree height of base plant $i$; $D_j$ is the DBH of neighbor $j$ around base plant $i$; $H_j$ is the tree height of neighbor $j$ around base plant $i$; $A_i$ is the canopy size of base plant $i$; $L_i$ is the distance between neighbor $j$ and base plant $i$; $n$ is the number of quadrants of the selected neighbors (here $n=6$). The interference index of a base plant is the average of neighbor interfering effects from all quadrants, and indicates the relative position of a tree in the forest stand [8].

(3) Grey relational analysis. The basic clues of grey relational analysis are as follows: geometric similarity is built between the curves plotted separately from the test sequences and from the reference sequences; the geometric shapes of test sequences and reference sequences are compared; if the curves are closer, the degree of relation is larger; vice versa [9].

The reference sequence is set as $Y_0(k)$, and the test sequence is $Y(k)$, $k=1, 2, 3, \ldots, m$, with the sequence length of $N$; the aim of relational analysis is to determine the relation degree between $Y_0(k)$ and $Y_0(k)$. The computational method can be found in Ref. [9].

(4) Data processing. Statistical analyses were conducted on Excel2003 and DPS 8.0.

RESULTS AND DISCUSSION

Correlation between tree interference index and tree DBH. Analyses show DBH is negatively correlated with interference index among the base plants in both study plots (Fig. 1), or namely the DBH of base plants declines with the increase of their interference index.

The trees with different DBHs are interfered to different degrees, as the dominant trees are less interfered by the neighbors, while small-DBH trees are more severely interfered by neighbors and are under the compressed state in the forests, which indicate the competition of “survival of the fittest” in the forests. For instance, at DBH = 15.7, 28.1, 14.7 and 25.1 cm, the interference index in study plot #1 is 3.51, 2.01, 3.95 and 2.45, respectively. As for different study plots, the interference degree of base plants with the same DBH may be different. For instance, at DBH of 20 cm, the interference indices of base plants in study plots #1 and #2 are 2.77 and 3.05, respectively. The average DBH of base plants is smaller but the average interference index is larger in study plot #2 than study plot #1. Specifically, the average DBHs of base plants in study plots #1 and #2 are 20.9 and 19.4 cm, respectively, while the average interference indices are 2.75 and 3.19, respectively, indicating the competition among trees is more severe in study plot #2 than in study plot #1. Except different densities, other conditions are basically the same between these two study plots, indicating the density effect largely affects the growth of DBH. At large stand density, the between-tree interference is evident, as the small-DBH trees are more severely interfered, which indicates the strong self-thinning ability of forests. As reported, when other conditions are the same or similar, the stand density is the major influence factor on DBH following certain general rules, which can be explained by classic theories [6].

Correlation between wood interference index and wood canopy size. Canopy size is a concrete manifestation of nutrition space and growth space. Generally, a larger canopy size indicates more extensive growing space, or namely, the competitive ability for nutrition is stronger. The between-neighbor competition leads to significant variation and response of individual trees in size and morphology. To uncover the changing rules, we analyzed the correlations between canopy size and neighbor interference index, and found the canopy sizes of base plants were negatively correlated with interference index (Fig. 2), or namely the canopy sizes of base plants declined with the increase of interference index.

<table>
<thead>
<tr>
<th>No. of standard plot</th>
<th>Closure</th>
<th>Average canopy size of dominant trees (m)</th>
<th>Average DBH (cm)</th>
<th>Average tree height (m)</th>
<th>Existing density (plants-m$^{-2}$)</th>
<th>Soil thickness (cm)</th>
<th>Altitude (m)</th>
<th>Slope (°)/aspect</th>
<th>Age (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>0.8</td>
<td>4.4x4</td>
<td>20.9</td>
<td>5.7</td>
<td>0.04</td>
<td>&gt;100</td>
<td>662</td>
<td>4°/SW</td>
<td>22</td>
</tr>
<tr>
<td>#2</td>
<td>0.7</td>
<td>4.1x3.7</td>
<td>19.4</td>
<td>6.4</td>
<td>0.05</td>
<td>&gt;100</td>
<td>669</td>
<td>5°/W</td>
<td>22</td>
</tr>
</tbody>
</table>

TABLE 1:

Basic information in the study plots of Populus simonii stands
Trees with different canopy sizes are interfered to different degrees, as the dominant trees are less interfered by their neighbors and grow with larger canopy, while the suppressed trees are more interfered by their neighbors and grow with smaller canopy. For instance, at canopy size = 2.3, 4.4, 2.7 and 3.8 m, the interference index of base plants in study plot #1 is 3.51, 2.01, 3.95 and 2.86, respectively. As for different study plots, the interference degree of base plants with the same canopy size may be different. For instance, at canopy size of 3.6 m, the interference indices of base plants in study plots #1 and #2 are 2.52 and 3.05, respectively. The average canopy size of base plants is smaller and the average interference index is larger in study plot #2 than study plot #1. Specifically, the average DBHs of base plants in study plots #1 and #2 are 2.52 and 3.05, respectively. The average canopy size of base plants is smaller and the average interference index is larger in study plot #2 than study plot #1. Similarly, the density effect largely affects the growth of canopy size. When the stand density is large, the individual trees occupy less resource and space and the interference between individuals is severe. Moreover, the trees are smaller with more severe interference from their neighbors, become less competitive for resources and space, and are more likely to be washed out [10].

Correlation between wood interference index and wood height-diameter ratio. Tree height-diameter ratio of a tree reflects its size and the trunk shape and roundness, and indirectly reveals the size of its occupied space. Height-diameter ratio is an important indicator whether the trunk shape and texture are good or not. A larger height-diameter ratio indicates the growing rate of tree height surpasses that of DBH, and vice versa [8]. The interference index is somehow correlated to the height-diameter ratio among base plants (Fig. 3). Clearly, the height-diameter ratio of base plants is positively correlated with their interference index, or namely the height-diameter ratio increases with the rise of interference index. The trees with different height-diameter ratios are interfered to different degrees. For instance, at height-diameter ratio = 28.46, 15.57, 41 and 24.79, the interference index is 3.51, 2.01, 3.95 and 2.45, respectively. The average height-diameter ratios of base plants in study plots #1 and #2 are 22 and 36, respectively, while their average interference indices are 2.77 and 3.05, respectively, indicating the tree competition in study plot #2 is more severe, and the
growing rate of height surpasses that of DBH for the base plants. For trees with large planting density, due to resource competition, density restricts the transversal growth of DBH, so the trees have to complete for light resources only through height growth. The above analyses reflect the importance of density on tree growth. For trees with large planting density, trees complete severely for resources, and usually become straight and round with small and fewer nodes and excellent texture.

Correlation between interference index and habitat utilization rate. The habitat use production efficiency, or habitat utilization rate (namely the habitat resources), can be evaluated by many indices. Generally, habitat utilization rate is measured by wood volume, which is a comprehensive indicator of DBH, tree height and canopy size. A larger wood volume indicates a higher habitat utilization rate. In this study, habitat utilization rate is defined as the wood volume per unit nutritive area of canopy size. In a forest community, each tree individual occupies certain space and area, so the tree canopy size reflects the size of its occupied space and nutritive area, while the wood volume per unit nutritive area is called the habitat utilization rate. Habitat utilization rate reflects the environment utilization ability of trees as well as the space utilization degree of resources.

The habitat utilization rates of base plants are negatively correlated with the interference index in both stand plots (Fig. 4). The trees with different habitat utilization rates are located at different positions in the forests, and are interfered to different degrees. For instance, the habitat utilization rates of base plants in study plots #1 and #2 are 0.0104-0.0343 (mean 0.0166) m$^3$·m$^{-2}$ and 0.0051-0.0241 (mean 0.0133) m$^3$·m$^{-2}$, respectively, while their average interference indices are 2.77 and 3.05, respectively. The larger stand density of study plot #2 led to more severe resource competition, so the tree individuals were less competitive for resources and space. When the tree individuals were smaller, this inferior position was more evident, so the trees were more likely to be washed out. Thus, forests with large planting density could not well fulfill the production potential.

The above analyses suggest the interference index well reflects the interfered degree of base plants by the neighbors, and well indicates the quality of stand structures. The interference index of base plants is closely correlated with their diameters, crown development, and trunk height-diameter ratio, and reflects the tree growing condition and its position in the forest [10]. The interference index of forest indirectly reflects the quality of its spatial structure, as the forest with smaller interference index grows with better spatial structure [11]. To explore the degree of intimacy between forest growing index and interference index and thus to screen out the guiding growth indicators for stand structure adjustment, we further analyzed the correlations between different growth indicators and the interference index.

\[ y = -7,4936x^2 + 51,606x - 60,608 \]

\[ R^2 = 0.8175 \]

**FIGURE 3**
Correlations between interference index and height-diameter ratio of base plants in the study plots

\[ y = -9,2394x^2 + 71,121x - 96,189 \]

\[ R^2 = 0.8877 \]

**FIGURE 4**
Correlations between interference index and habitat utilization rate in base plants of study plots
Grey relational analysis between interference index and tree growth factors. Since growing indicators were complexly correlated with the interference index, we used a comprehensive index in the analyses. To compare the correlations between different growth indicators and the interference index, we used grey relational analysis here and computed the correlation coefficients between different growth indicators and the interference index. According to the correlation degree rule in grey relational theories, a larger correlation degree means it is closer to the ideal value and the corresponding processing is better. Therefore, the processing effects of different methods rank as follows: height-diameter ratio (0.71) > DBH (0.7) > canopy size (0.46) > habitat utilization rate (0.28). Comprehensive analysis suggests the interference index is most correlated with the height-diameter ratio of base plants, followed by DBH.

Theoretical analysis and production experiences both suggest trees with large height-diameter ratio are more susceptible to harms of winds or snow, so during wood thinning, trees with too large height-diameter ratio should not be reserved\[10\]. The above analyses also suggest interference index is most correlated with height-diameter ratio, but height-diameter ratio is more unmeasurable in practice, so we adopted DBH instead, which is more measurable\[6\]. Thus, DBH was used to analyze the interference degrees of basal plants.

Smaller-DBH trees are more stressed and less competitive in a forest and would be easily washed out. On the contrary, larger-DBH trees tend to form a severe resource competition relationship with other trees, which is unfavorable for other trees or for the stability of stand structures and the healthy development of forests. Thus, trees with too large or too small DBH should be given special fostering and management measures.

Stand density is an efficient control factor in forest culture and management, underlies the creation of appropriate spatial structure, and decides the sizes of growth and development spaces of individual trees. Stand density largely decides the internal structure of a forest and thereby determines the health and stability of stand structure. The density effect is extremely important for tree growth. When the stand density is large, the weak trees should be cut down, so as to create a stand structure with appropriate density, control the number of neighbors and restrict the neighbor interfering effect. Such measures could facilitate the individual growth and natural pruning, and also create forests with strong overall defending ability. Moreover, mixed forests compared with pure forests are less interfered and grow with larger biomass and stronger defending ability. Mixed forests compared with pure forests could more comprehensively utilize forest land resources. As the resource utilization rates of forests differ, the mutual competition is less severe, and the carbon sequestration ability is stronger. Thus, one important way to improve forest carbon sink function is to transform pure forests to mixed forests. As reported, mixed forests grow at rates 20% - 45% faster than pure forests, as mixed forests could fully utilize optical energy, nutrient and moisture, which are reflected by the faster growing rate and larger unit area yield\[12\].

CONCLUSIONS

When the forest ages are the same and other site conditions are similar, the interference index of basal plants is negatively correlated with tree diameter at breast height, canopy size, and habitat utilization rate, but is positively correlated with tree height-diameter ratio. The interference is more severe at larger stand density. Grey relational analysis shows the association degrees of tree growth factors with interference index rank as follows: height-diameter ratio > diameter at breast height > canopy size > habitat utilization rate. Based on theory and experiences, we think diameter at breast height is a more feasible indicator for us to analyze the interference degrees of base plants.

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THE PHYSICAL AND CHEMICAL PROPERTIES OF CATERING WASTE OIL AND THE PARTICLE SIZE CHARACTERISTICS OF COMBUSTION PARTICULATE EMISSIONS

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ABSTRACT

With the development of China’s economic construction and the improvement of people's living standards, the catering industry has been greatly developed. However, the complaint of pollution in the catering industry has become a hot spot of pollution complaints. The environmental pollution of catering industry becomes a prominent problem, which has been one of the main problems hindering the further development of catering industry. Based on the analysis on the characteristics of catering exhaust pollution sources and the efficiency of purifier treatment, the author took a catering enterprise as the research object and used the screening model SCREEN3 introduced by the United States Environmental Protection Agency (EPA) to analyze the influence of catering enterprises' exhaust, in order to provide a reference for the environmental assessment of the similar projects.

KEYWORDS:
Catering, Waste oil, Physical and chemical properties, Combustion particulates, Particle size of particulate emissions

INTRODUCTION

Biodiesel is a methyl or ethyl ester fuel made from the transesterification process of alcohols with oils including plant oil, animal fats and catering waste oil, etc. With the tight supply of energy resources and more serious environmental pollution, biodiesel has become a hot spot in the field of energy substitution. Currently, there are few species of oil plants which can be collected on a large scale in our country, and the yield is very limited. The waste oil in catering industry is the main source of raw material for biodiesel production at present in our country. The life cycle assessment is the entire process analysis and evaluation on the consumption of natural resources and the environmental impact for a product or service system in its production process and activities. The life cycle analysis method is used to systematically analyze and evaluate the biodiesel production, the energy efficiency of using biodiesel and the emission characteristics of key pollutants such as CO₂, which are the basis for the large-scale production and use of biodiesel. BERNESSON, etc. conducted the analysis on the life cycle energy consumption and emissions for the biodiesel made from rapeseed oil and soybean oil and other raw materials in Britain and Switzerland. Currently, in China there are few researches on this aspect [1].

The catering industry in our country produces a lot of oil fumes in the cooking process because of the particularity of the cooking methods. The exhaust pollutants of the catering enterprises are mainly composed of sedimentable particles, fine particles and hydrocarbons. Tan Desheng, etc. did the sampling analysis on the cooking oil fumes in the catering unit’s kitchen in Beijing. It was found that the particle size of the main sedimentable particles was 10~400 um, and the peak size of the quantity concentration was concentrated at 10~100 um. The size of the inhalable particles in the oil fume was mainly below 1.0 um, and the peak particle size of the quantity concentration is concentrated between 0.063 and 0.109 um, the peak size of the mass concentration is 6.56 ~ 9.99 um. Wang Xiuyan and so on, monitored the VOCs in a medium-sized restaurant in Tianjin at the peak period of the meal. In the kitchen oil fume, 66 kinds of VOCs were detected, including 23 alkanes, 8 olefins, 14 aromatic hydrocarbons, 8 halogenated hydrocarbons, 6 oxygenated organic compounds, 6 sulfur compounds and 1 limonene. The oxygenated organic compounds in the VOCs of kitchen and exhaust were the main pollutants, accounting for more than 55% of the total, in which the most was alcohol, followed by propane [2]. Feng Yanli et al. [3] analyzed the compounds of the exhaust fumes from the catering enterprises, and found that the fumes contained 18 compounds, in which the content of acetaldehyde, formaldehyde and acetone were relatively high.
The life cycle analysis method was used to analyze the energy consumption and CO₂ emission of biodiesel produced by chemically catalyzed reaction using catering waste oil as raw material [4-8]. According to the energy consumption and CO₂ emission characteristics of biodiesel in all stages of life cycle [9], the corresponding improvement suggestions were provided, which was a reference for promoting the clean production of biodiesel in China.

**Experimental Research objectives and scope.** Using the waste edible rapeseed oil as the raw material, the biodiesel produced from chemical catalyzed method was used as the research object. The energy consumption characteristics, the fossil energy efficiency (energy efficiency ratio) and the CO₂ emission characteristics were analyzed. The energy consumption and CO₂ emission in different stages were compared and the corresponding improvement suggestions were provided. The biodiesel products with 1 MJ energy were used as the functional unit. The research included the entire process including raw material production and transportation, product production and transportation, and the use of product on vehicle diesel engines (see Fig. 1). The production of raw materials has experienced the process of rape planting and transportation, the production and transportation of rapeseed oil, consumption of catering industry, the collection of kitchen waste and the waste oil refining and so on. The energy consumption of various transportation, processing equipment and building construction and manpower consumption were not considered.

**Evaluation indices.** The calculation of the biodiesel life cycle energy consumption and CO₂ emission are based on the ISO 14040 “the principle and framework of environmental management life cycle assessment (2006)”. The evaluation indices of biodiesel life cycle energy consumption include total energy consumption, fossil energy consumption, fossil energy efficiency ratio and net energy output. Total energy consumption (TE) refers to all the energy invested in the life cycle; Fossil energy consumption (FE) refers to the fossil energy consumed in the life cycle; The fossil energy efficiency ratio (η) refers to the output product energy per unit fossil energy. Net energy output (NE) is the difference between life cycle energy output (BE) and fossil energy consumption. The calculation formula of total energy consumption:

\[
TE = RME + FE
\]

\[
RME = G \times E_{\text{material}}
\]

\[
FE = \sum_i FE_i = \sum_i \left( FE_{\text{Explicit}} + FE_{\text{invisible}} \right)
\]

\[
FE_{\text{Explicit}} = X_j \times E_j
\]

\[
FE_{\text{invisible}} = Y_k \times P_{nk} \times E_j
\]

In the formula, RME is the energy contained in the raw materials for biodiesel production, MJ; G is the raw material consumption in the process of biodiesel production, kg; E_raw material refers to the raw material calorific value, MJ / kg; FE is the fossil energy consumption at the life cycle i stage, MJ; FE_{Explicit} is the fossil energy consumption in the form of coal, electricity, oil and so on at life cycle i stage, MJ; FE_{invisible} is the fossil energy consumed by the raw materials in the production life cycle.
laboratory for analysis.

tray with cover and brought the samples back to the dishes. After the sampling completed, closed the edge of the wok. When the chef started cooking, the samples were 15, 30, and 45 cm away from the edge of the wok, so that the three groups of parallel stainless steel tray was placed 15 cm away from the edge of the tray. Then the tray was covered and waited for sampling. When sampling, the sampling places of the other four restaurants were all at the exhaust outlet of the fume purifier. The sampling tube was placed at the end of the exhaust outlet, close to the edge of the sampling port. According to the actual situations of each restaurant and online analysis data, sampling time was about 10 – 30 min.

**Sample analysis. (1) Sedimentable particles.** The blood cell counting plates after sampling were brought back to the laboratory. The metallurgical microscope (L3230, Shenzhen SangNond Technology Co., Ltd.) was used to observe the sedimentable particles in the square grids of the blood counting plates by zoom in 5 times. The size of the sedimentable particles was measured and photos were taken by the real-time image measurement software and a digital camera attached to a metallurgical microscope. Under the microscope, the number of sedimentable particles was manually counted according to the grid subarea. The grid area was 3 mm × 3 mm = 9 mm². The total number of sedimentable particles in the square grid was divided by the square grid area to obtain the number of sedimentable particles per mm². The sedimentable particles on the blood cell counting plate were basically with size below 300 μm, the diameter of the settleable particles could reach 1 mm at most, and the number of sedimentable particles >400 μm was very small. And after this part of the oil dropping to the blood cell counting plate, the sedimentable particles were distributed with an irregular shape. For this reason, this experiment did not count > 400 μm diameter of sedimentable able particles.

**Inhalable particles.** From 2010-04-14 to 2010-04-15, sampling was conducted on inhalable particles produced in cooking canteens, barbecues, hotpots, fast food and western restaurants in a university in Haidian District, Beijing. The sampling instrument was an Electrical Low Pressure Impactor (ELPI), from a Finnish Dekati company, with a flow of 30 L·min⁻¹. During sampling, the sampling was taken directly on the side of the oven and at the same height as the chef's face. And the sampling places of the other four restaurants were all at the exhaust outlet of the fume purifier. The sampling tube was placed at the end of the exhaust outlet, close to the edge of the sampling port. According to the actual situations of each restaurant and online analysis data, sampling time was about 10 – 30 min.

(2) Inhalable particles. The particle size distribution and concentration of inhalable particles in cooking fumes of catering industry were measured using ELPI. The working principle of ELPI [10] is to charge the measured particles, and then measure the charge of the particles that enter the low-voltage cascade real sampler to obtain particle concentrations with different particle sizes. ELPI divides inhalable particles according to the dividing particle size at each level. The inhalable particles generate a certain amount of current at each level and it is recorded in real time by the electrometer channel. The result is recorded by computer. ELPI has 12 levels with current identifiers, plus a level with an upper dividing diameter of 10 μm.

**MATERIALS AND METHODS**

The main production stages of the sedimentable particles in the cooking oil fume are the three processes including adding the food materials, frying and the seasoning. Most of the produced particles are freely subsided by gravity and a few of them are extracted into the fume pipe by fans. Therefore, the sampling place is chosen inside the kitchen. The production stages of the inhalable particles in the oil fume of catering industry are the processes of heating oil, adding food materials, frying, seasoning and taking foods to the dish. Among them, the processes of adding food materials, frying and taking foods to the dish produce the most quantity of the inhalable particles. Because of the low gravity, most of the inhalable particles in the oil fume are extracted away by the exhaust fan, therefore, the sampling place is chosen at the exhaust port.

**Sedimentable particles.** The blood cell counting plates (XB-K-25 type, Qiujing medical instrument factory) were used to sample the sedimentable particles with more than 10 μm diameter which were produced during the cooking of Hunan cuisine, fast food, and Cantonese cuisine, respectively. The blood cell counting plates were cleaned with carbon tetrachloride before sampling, and then were fixed in a self-made stainless steel tray (480 mm × 230 mm × 35 mm). The counting plates were placed in 3 parallels at 0 cm, 15 cm, and 30 cm from the edge of the tray. Then the tray was covered and waited for sampling. When sampling, the stainless steel tray was placed 15 cm away from the edge of the wok, so that the three groups of parallel samples were 15, 30, and 45 cm away from the edge of the wok. When the chef started cooking, the cover of the stainless steel tray was removed. Due to the pause during the cooking process, the sampling time was set as the time of chief to cook 3 dishes. After the sampling completed, closed the tray with cover and brought the samples back to the laboratory for analysis.
RESULTS AND DISCUSSION

As shown in Table 1, the closer to the edge of the wok, the higher the concentration of sedimentable particles; At the same distance of the sampling position, the highest concentration of sedimentable particles was in Hunan cuisine, followed by fast food, and Guangdong cuisine was least. Since Hunan cuisine uses a lot of oil during cooking, and the fire is fierce, the intensity and frequency of stir-fry are high, the number of sedimentable particles produced is higher than that of fast food and Cantonese cuisine. Fast food is focused at fast. The main food materials are generally well-prepared before cooking. The garnish is added to stir fry quickly during cooking, and finally after adding spices it can be served on the dish. Overall, the cooking time of one dish of fast food is shorter than that of a dish of Hunan cuisine. Cantonese cuisine is more complex than Hunan cuisine, but because of its emphasis on freshness, light and palatable taste, it does not add too much oil when cooking, and the fire and the intensity of stir-frying are moderate, making the fume produced less than Hunan cuisine and fast food.

<table>
<thead>
<tr>
<th>Cuisine Type</th>
<th>Place</th>
<th>15cm</th>
<th>30cm</th>
<th>45cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunan cuisine</td>
<td></td>
<td>21.02</td>
<td>12.48</td>
<td>7.26</td>
</tr>
<tr>
<td>Fast Food</td>
<td></td>
<td>14.24</td>
<td>7.00</td>
<td>3.44</td>
</tr>
<tr>
<td>Cantonese cuisine</td>
<td></td>
<td>7.11</td>
<td>4.44</td>
<td>2.55</td>
</tr>
</tbody>
</table>

TABLE 1
The quantity concentration of sedimentable particles in the grid of blood cell counting plates/num:mm²

After studying the morphology of the sedimentable particles in the oil fumes of the three restaurants, the particles showed various morphologies, but there was almost no difference in the morphology between different restaurants. As shown in Figure 1, there were mainly regular round shape (A), oil containing food residue (B), oil-water mixture (C), oil-in-water (D), the small oil droplets (E), oil-in-water (F) and so on, that appear around the large oil droplets after the sediment of relatively large oil water mixture and the water film rupture. In addition, there was food residue (G) that was broken down by high-temperature carbonization during the cooking process. It can be seen that the particles of oil fume contain oils, organic matter, and their thermal decomposition or cracking products that are volatile during food cooking and processing. In the literature [11], the analytical method for oil fumes was infrared spectrophotometry. This method excluded the oil fume particles other than grease. Zhou Yamei et al, heated oils at a constant temperature and concluded that the particle concentration of inhalable particulates measured by the autosampler was with nearly 2 orders of magnitude difference from that measured by the UV-visible spectrophotometer. For this reason, the use of infrared spectrophotometry to measure the concentration of oil fumes did not consider the pollution of other particles in the fumes to the environment.

The area of the blood cell counting plate grid was 3 mm × 3 mm = 9 mm². The total number of oil droplets in the square grid was divided by the area of the square grid to obtain the number of oil droplets per mm². As shown in Fig. 2 to 4, the closer to the edge of the wok, the larger the number of sedimentable particles, the peak number was at 10 to 100 μm. Because of gravity, there were a large quantity of the sedimentable particles near the burner, and the particles tended to be round shape when they settled. When the particle size or the initial velocity of the particles was too large, the
particles would settle down and be irregularly distributed. When the number of sedimentable particles was large, some oil droplets would have oil droplets overlapping. Due to the relatively moderate cooking method and less oil consumption, for the Cantonese cuisine the settled particles on the counting plate were mainly with a particle size of less than 100 μm, and the quantity of particles >100 μm was very small. However, Hunan cuisine and fast foods have faster cooking speeds, more intense fires and more oil, resulting in more sedimentable particles than Cantonese cuisine. This shows that the concentration and diameter of sedimentable particles in oil fumes have a great relationship with the amount of cooking oil and cooking methods.

CONCLUSION

(1) The quantity of oil fumes produced in the catering industry has a direct correlation with the traditional cooking habits. The greater the amount of oil used, the fiercer the fire, and the more frequent stir-frying, the more oil fumes will be produced.

(2) Although there is large quantity of sedimentable particles in cooking fumes of catering industry, due to the effect of the particles own gravity, their pollution to the environment is limited to the interior of the kitchen and near the exhaust outlet, and will not form long-lasting pollution to the atmosphere.

(3) Inhalable particles in the fumes of catering industry can exist in the environment for a long time, and the quantity concentration is much greater than that of sedimentable particles. Usually, 0.1 μm of inhalable particles are generated instantaneously in the restaurant's burner with the concentration as high as 10^6 to 10^8 num·cm^-3. With the rapid increase in the number of urban restaurants, from the point of view of airborne suspended particulates only, the inhalable particles may cause more serious environmental pollution in some areas than vehicle exhaust pollution. Therefore, the oil fume treatment and monitoring can be focused on the inhalable particles.

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ECOLOGICAL STUDY OF PISTACHIO BARK BEETLE, 
CHAETOPTELIUS VESTITUS (MULS & REY, 1861) 
(COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The pistachio bark beetle, Chaetoptelius vestitus (Muls & Rey) (Coleoptera, Curculionidae, Sco-lytinae) is a serious pest of pistachio trees in Tunisia. Despite its important damage, its seasonal life cycle is still unclear. In this study, we investigated the seasonal life cycle and distribution of C. vestitus between November 2013 and October 2016 in the center and south of Tunisia. We recorded the direct damage caused by beetles on pistachio branches and buds. The highest density of C. vestitus attack was detected in Elguettar 2 site (Gafsa governorate). The insect overwinters both in adults and larval stage on pistachio trees. The adults emerge to attack new suitable trees at mid-May. Reproduction started in autumn. Also, females searched suitable hosts to attack. Copulation took place either on the bark in the initial phase of egg tunnel construction or inside the tunnel system. Mean fecundity was 50.95 (±9.68 SD) eggs/ female. The colonization process occurred in two phases. During the first phase, feeding galleries were drilled through the buds. In the second phase, reproduction galleries were constructed in the thick branches of the tree. The results obtained in this work are considered essential for developing optimal control of the pest. Information on the seasonal life cycle allows to anticipate the time when plant damage may take place and to design the proper management of the pest.

KEYWORDS:
Pistacia vera L., Chaetoptelius vestitus, seasonal life cycle, fecundity, insect colonization, Tunisia

INTRODUCTION

Pistachio cultivation is a major fruit production activity in Tunisian, especially in semi-arid and arid zones [1-2]. In the central and southern regions of Tunisia, planted areas have dramatically increased since the 1970s, reaching 27,060 ha producing 1200 tons in 2013 [3]. However, in addition to drought, the pistachio culture is facing insect attack. Pistachio trees can be attacked by many insect species including the bark beetle Chaetoptelius vestitus (Muls & Rey, 1861) (Coleoptera, Curculionidae) [4-5-6-7-8-9]. This insect attacks all species of pistachio (Pistacia), which are considered as its primary hosts. It causes significant losses in yield if not suppressed, and it proved to be critical to the economics of the pistachio industry. It is found in almost all the main pistachio-producing countries in the world [10].

C. vestitus affects both buds and bark parts of the pistachio tree [11-12-13-14]. Currently, C. vestitus in Tunisia is managed mainly by contact insecticides (Fenitrothion 50% EC: organophosphate family) and by the elimination and incineration of infested branches [15]. However, the misuse of insecticides causes environmental pollution and can be harmful to human health as well. In addition, insecticides must be applied to all infested plants and orchards at the same time to prevent further infestation of neighboring farms. For that reason, alternative strategies are needed. The management of the insect requires the understanding of environmental effects on the dynamics of the beetle populations. Environmental effects usually influence insect activities, such as feeding, development, egg laying and dispersal. Environmental attributes, including temperature, humidity and light intensity are density-independent factors that affect the dynamics of populations. However, despite the importance of pistachio trees, no detailed study has been conducted. In addition, little data is available about its biology and damages [16-17-18-19-20-21-22-12-13]. In Tunisia, only one study was carried out on the biology of C. vestitus [15]. Thus, knowledge of the life history and biology of the pistachio bark beetle is important for the development of an integrated pest management program. Therefore, for effective management of the pest, it is pertinent to investigate the developmental biology of this pest in local environmental conditions. Environmental factors, including temperature and precipitation during the growing season and winter temperature, certainly have a great impact on the seasonal life cycle of the beetle. Therefore, the
objectives of this study were to describe the seasonal life cycle of pistachio bark beetle, and determine the role of climatic factors (Temperature and Precipitation) in the population fluctuations of the pest.

MATERIALS AND METHODS

Seasonal life cycle of *C. vestitus*. Field collection. The *C. vestitus* seasonal life cycle was investigated in the field in Elguettar (Gafsa governorate) between November 2013 and October 2016. The study site is located at 34°23’37.42”N and 9°06’46.96”E at an elevation of 241 m. As can be seen in Figure 1 July is the hottest and January is the coldest month in the year [23]. To study the population fluctuations of *C. vestitus*, we analyzed the total number of specimens collected by direct sampling of the whole infested trees. Every month, 10 pistachio trees (about 4 meters in height) with insect entry holes (perforations), which are typical symptoms of pistachio bark beetle infestation, were selected [10]. Thick branches, buds and twigs were cut. For each plant, the number of larvae, pupae and adults was recorded and adults were placed in plastic containers then transported to the laboratory to be sexed according to Ghrissi et al. [24]. The meteorological factors evaluated were maximum and minimum temperatures and precipitation.

An observation of pistachio phenology was carried out in parallel with the monitoring of the seasonal life cycle of *C. vestitus* at nine study sites. Five pistachio trees were selected in each study site for phenological monitoring. For each individual, four branches of each tree exposure (North, South, East and West) were selected and examined throughout the study period. The observations started on 10/11/2013 and were completed on 20/11/2016. As can be seen in Figure 2, the differentiation of buds is the first stage that announces the beginning of the vegetative cycle in mid-February. After the dormancy is lifted, flowering takes place from the first two weeks of April to the end of June. The fruiting of pistachio occurs from mid-April to the end of July. In late July, maturation lasts about a month and a half. All along this period, the nut ripens. In fact, the total fresh weight increases due to the magnification of the nut. During this stage, the fruit becomes ripe and edible. In October, most leaves drop until the beginning of December, and the tree remains dormant until the following February.

Laboratory Rearing. *Chaetoptelius vestitus* adults had been reared since 15 November 2013 in rearing boxes (35 x 25 x 15 cm) at 28°C, 75% RH and a 16L: 8D us photoperiod. The plastic boxes was filled with sand on which we put 20 branches of pistachio (about 30 cm long and 2 cm in diameter). Ten infested branches with *C. vestitus* were put with ten healthy branches. One week after the infestation, the ten newly infested branches were examined every two days. We observed the appearance of infestation holes, the duration of each developmental stages of the insect until the emergence of the first adults and therefore the total life cycle duration. At that time, the teneral (newly emerging) adult stage was observed. Larval instars were determined by head capsule width measurements. The collected larvae were placed in 70% ethanol and head capsule maximum width was measured using a stereomicroscope equipped with an ocular micrometer.

![FIGURE 1](image)

Average monthly temperature (line) and rainfall (bars) in the investigated region during the years of the study. (From November 2013 to October 2016)

(Data obtained from the Regional Directorate of Agriculture of Elguettar Gafsa Tunisia).
FIGURE 2

Seasonal changes in pistachio bark beetle population in relation to developmental stages of the pistachio trees at Elguettar, Gafsa, Tunisia.

Mating behavior, females’ fecundity, maturation period were recorded. Fifty females were used for these tests. Thus, only newly emerged adults were used for these studies. Each female was held with a male with freshly cut pistachio branch in a plastic box. The couples were maintained in the laboratory at 28°C with a 75% as photoperiod and checked daily. The numbers of oviposition holes and eggs per branch were recorded daily. Gallery systems were also studied. Twenty females were used for this test. The length and width of the maternal galleries and the larval ones were measured.

Damage distribution of *C. vestitus* in Tunisia. To determine the degree of damage, a survey of the beetle was conducted at 9 localities in the major pistachio growing areas of southern and central Tunisia from October 2015 to October 2016. The first five plantations are located in Gafsa governorate "Elguettar 1", (34°19’53.13’’N; 8°56’20.12’’E), "Elguettar 2", (34°23’37.42’’N; 9°06’46.96’’E), "Sidi Aich", (34°37’49.15’’N;

8°57′39.45″E), "Sened" (34°27′32.65″N; 9°15′44.60″E) and "Gafsa Nord" (34°24′53.50″N; 8°46′34.55″E). The sixth plantation; "Mazouna" is located at sidi bouzid governorate (34°36′30.47″N; 9°49′27.51″E). Two plantations sites are located in Kasserine Governorate; "Sheitla" (35°20′39.17″N; 9°13′12.91″E) and "Majel Bel Abbes" (34°43′56.80″N; 8°31′25.40″E). The last plantation site is located in Sfax Governorate (34°17′13.78″N; 10°00′46.67″E). At each site, thirty pistachio branches were sampled. All samples were examined for the presence of entry holes.

Statistical Analysis. The data of the head capsule widths were analyzed using the Hcap program developed by Logan et al. [25]. The program allows an accurate separation between each larval instar. In addition, this program estimates the mean and standard deviation of head capsule widths for each instar.

The length and width of maternal galleries were compared to those of larval galleries using a 6WXGHQW¶V t-test (P <0.05). We used GLM test to analyse the difference between percentages of damages at different localities. All of the analyses were performed using R 3.3.2 software [26].

RESULTS

Seasonal life cycle. The population fluctuation analyses of C. vestitus demonstrated that adults were present throughout the study period. High peak was observed in May and June, when the maximum temperature (29.8 °C) was recorded (Figure 2).

The greatest number of adult C. vestitus occurred during the reproductive phase when the plants produced pods. The number of each developmental stage generally varied during the study period (Nov2013-Oct2016). In fact, Pistachio bark beetles were found to occur almost throughout the year both on the buds and on the thickened branches. The number of adults on branches was stable between November and April of the following year. During this period (from November to April), we noticed fewer adults on the buds. The number of adults on buds showed a steady increase until May, reaching a peak in May and June (Figure 2).

As shown in Figures 2, the number of pupae increased steadily in March and April, but decreased in May and June. However, neither the eggs nor immature stages were noticed in May, June, July, August and September on thick branches or on the flower buds and twigs. Chaetoptelius vestitus overwintered in all developmental stages at the ends of their galleries from the end of November. The larvae remained inactive during winter and resumed their activities from the last week of January the following year. Pupation started at the end of April and the first emerging adults appeared swarming from mid-May. The species produced one generation per year in the center and south of Tunisia. The life cycle of C. vestitus, from oviposition to the emergence of the new generation of adults, was extended from the third week of October to the middle of February of the following year with an entire life cycle that lasted between 80-140 days.

The dissection of the maternal gallery showed that no eggs were collected on each of the first three days. From the 4th day, we noticed the appearance of entries with eggs inside. This indicated that, in the field, newly emerged females mostly fed and oviposited 4 to 8 days after emergence. The successful maturation feeding occurred mostly on weakened trees or trees having some dead branches. The maternal gallery was longitudinal (Figure 3). It was drilled by the female. From the maternal gallery, 36-68 radiated larval galleries were derived perpendicularly. Measurements of the length and width of the gallery system are detailed in Table 1. The mean length and width of the maternal galleries were significantly higher (Student test, P< 0.0001) than those of the larval galleries.

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After the entire development of the beetle, the infested branches had thousands of adult exit holes or shot holes on the entire bark surface. A bark dissection showed that eggs were placed on either side of the mother gallery at regular distances along
its entire length. On average, 50.95 ($\pm$9.68 SD) eggs were laid per female per system (Table 1). The incubation period (i.e. the period between egg laying and egg hatching), lasted 5 to 9 days. We determined the number of larval instars of *C. vestitus* according to head capsules width measured for each instar. Therefore, larval development was completed through five distinct stages separated by four moulting stages (Figure 4). It was observed that the size differed according to the age of the larva. After hatching, the larva appeared unmoving, yellowish-white in color, arc-shaped and without eyes. It measured 1.057 mm ($\pm$0.136 SD) in length and 0.58 mm ($\pm$0.091 SD) in width (Table 1). As soon as feeding began, the larvae became curved, legless and cream-colored. Larvae of the last instar stage was 3.36 mm ($\pm$0.316 SD) long and 1.63 mm ($\pm$0.125 SD) wide (Table 1). The color of the head capsule remained the same. The equation of a straight line for linear regression between the average of head capsule measurements and larval instars was $y = 0.16 + 0.32x$, and $r^2 = 0.95$. A linear measure of size increased by a constant factor from one instar to the next. The duration of larval stage was 36 to 60 days.

The pupae took place at the end of larval gallery in pupal chamber. The pupae was whitish and its average size was 3.19 mm ($\pm$0.207 SD) in length and 1.79 mm ($\pm$0.166 SD) in width (Table 1). The pupal stage lasted 9 to 16 days. The emergence of *C. vestitus* was in mid-February. After emergence, adults flew to colonize the buds of other trees appropriate to spend their feeding phase.

**Damage distribution of *C. vestitus* in Tunisia.** As shown in Figure 5, the percent damages caused by *C. vestitus* adults varied significantly from one locality to another (GLM, $F=9.64$, $p=0.007$). Relatively high infestation rates, higher than 50%, were recorded on the sites of Elguettar 2, Sidi Aich, Sened in Gafsa governorate and Majel Bel Abbes in Kasserine governorate where only pistachio trees is cultivated (Figure 5). On the other hand, in small-scale farmers' holdings such as Gafsa Nord (Gafsa governorate), Sbeita (Kasserine governorate) and Mazouna (Sidi Bouzid governorate), the infestation was moderate (25-50%). Nevertheless, the buds obtained from Elguettar 1 and Sfax (mixed farming) showed very low damage (< 25 % damage).

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean (mm)</th>
<th>STDEV (mm)</th>
<th>Maximum</th>
<th>Minimum</th>
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<tbody>
<tr>
<td>Length Maternal gallery</td>
<td>15</td>
<td>32.658</td>
<td>8.53</td>
<td>45.53</td>
<td>20.33</td>
</tr>
<tr>
<td>Width Maternal gallery</td>
<td>15</td>
<td>3.266</td>
<td>0.45</td>
<td>3.83</td>
<td>2.52</td>
</tr>
<tr>
<td>Length Larval gallery</td>
<td>15</td>
<td>9.02</td>
<td>2.79</td>
<td>12.96</td>
<td>5.09</td>
</tr>
<tr>
<td>Width Larval gallery</td>
<td>15</td>
<td>0.871</td>
<td>0.27</td>
<td>1.24</td>
<td>0.52</td>
</tr>
<tr>
<td>Egg length</td>
<td>10</td>
<td>0.82</td>
<td>0.1549</td>
<td>0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Egg width</td>
<td>10</td>
<td>0.5</td>
<td>0.0816</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Early larval length</td>
<td>10</td>
<td>1.057</td>
<td>0.1362</td>
<td>1.18</td>
<td>0.8</td>
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<tr>
<td>Early larval width</td>
<td>10</td>
<td>0.58</td>
<td>0.0918</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Late larval length</td>
<td>10</td>
<td>3.36</td>
<td>0.3169</td>
<td>3.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Late larval width</td>
<td>10</td>
<td>1.63</td>
<td>0.1251</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Pupal length</td>
<td>10</td>
<td>3.19</td>
<td>0.2078</td>
<td>3.5</td>
<td>3</td>
</tr>
<tr>
<td>Pupal width</td>
<td>10</td>
<td>1.79</td>
<td>0.1663</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Eggs/Female</td>
<td>20</td>
<td>50.95</td>
<td>9.68</td>
<td>68.00</td>
<td>36.00</td>
</tr>
</tbody>
</table>

**FIGURE 5**

Total percent damage buds by *Chaetoptelius vestitus* in different sites of pistachio production in Tunisia (Percentages of damages at different localities followed by the same letter (a, b, c, d, e or f) are not significantly different according to the GLM test (at $p <0.05$)).

Error bars= standard deviation
DISCUSSION

An understanding of the effects of temperature and rainfall on insect lives and population dynamics would help in developing improved pest control strategies. Although Chaetoptelius vestitus adults were observed throughout the year, the average number of each developmental stage of the insect as well as the level of damage showed a significant seasonal variation in the thickened branches. Fragoulis [20] found a significantly high population of beetles (including all developmental stages) on the thickened branches. This implied that the beetle seemed to prefer thickened branches for its reproduction phase in comparison with buds and twigs. In this study, the immature stages of this insect increased steadily after October, began to decline from April, and disappeared from May to September. This may be due to the emergence of new adults. In addition, this period is associated with low rainfall and high daily temperature resulting in dryness of branches, which probably causes the insect to stop reproduction. There are seasonal differences in the relative abundance of adult C. vestitus and preimaginal stages. This finding is consistent with results reported by Rizk et al. [16–17–18] and Ziaaddini et al. [19]. C. vestitus infestation coincides with the phenology of pistachio plants. In addition, changes in cropping practices associated with newly planted Pistacia cultivars may create a favorable environment and thus a high level of infestation. The current study clearly demonstrates the importance of buds and thickened branches for population abundance and the incidence of the pistachio bark beetle throughout the year. As soon as the harvest season of pistachio is over, all the infested, dry and fallen branches accumulated under the trees must be eliminated and incinerated in order to kill the beetle inside.

In the species examined in this study, some biological traits were recorded in both the central and southern populations of C. vestitus. In fact, the total life cycle duration of C. vestitus was similar to that reported by Brahman and Jardak [15] (85 to 191 days). According to Balachowsky [10], females lay an average of 60 to 80 eggs per gallery system. Female fertility varies according to the environmental conditions, the condition of the plant, and the availability of mature males. Brahman and Jardak [15] and Balachowsky [10] also studied the morphological characteristics of larvae, pupae and adults. Nevertheless, no data on morphometric measurements or duration of development of each larval stage were available.

The level of damage on older branches (thickened branches) varied according to the localities studied. This variation may be due to the age or condition of the orchard and to the variety of pistachio [27–28]. It is possible that the level of damage to pistachio branches and buds affects the quality and production of pistachio. Further surveys on the effect of beetle infestation on the quantity and quality of pistachios are needed. This study was done only in the southern and central parts of Tunisia. Future studies should include other pistachio growing areas in different seasons. Moreover, further surveys on the quantity and quality of pistachios are needed.

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FATTY ACID COMPOSITION AND HEAVY METAL ACCUMULATION CHARACTERISTICS OF FRUCTUS TRICHOSTHANTHIS SEED

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ABSTRACT

The safety of agricultural products is receiving increasing attention. Although seed of Fructus Trichosanthis (the fruit of Trichosanthes species) is an important Chinese traditional medicine resource and a popular snack food in China, few reports have appeared on its nutritional value, medically effective components, and safety. In this study, we analyzed the unsaturated fatty acid content and heavy metal accumulation characteristics of Fructus Trichosanthis seed. Fructus Trichosanthis seed oil was found to have many more monounsaturated and polyunsaturated fatty acids than other plant seed and animal oils, thus demonstrating its healthfulness and edibility. According to the heavy metal data, copper is the heavy metal that accumulates most readily in Fructus Trichosanthis seed.

KEYWORDS:
Fatty acid, heavy metal, Fructus Trichosanthis seed, food safety

INTRODUCTION

Seed of Fructus Trichosanthis (mature fruits of the wild cucurbits Trichosanthes kirilowii Maxim and T. rosthornii Harms.), is both an important traditional medicine resource in China and a popular snack food in many places. Despite the importance of the seed, however, few reports have appeared on its nutritional value, medically effective components, and safety [1, 2].

With the continuous development of Chinese industry and urbanization, heavy metal pollution in soil is becoming increasingly serious. Heavy metal pollution alters soil composition, structure, and function and also inhibits crop root growth and photosynthesis, thereby reducing crop yield. In addition, heavy metals can be transferred to animals and humans through the food chain, thus seriously endangering human health. Heavy metal pollution, such as cadmium contamination of rice, arsenic poisoning, and high levels of lead in blood, has been frequently reported in recent years [3, 4]. Soil heavy-metal pollution has become one of the most

concerning public issues related to soil pollution. Heavy metal accumulation, which has been mainly studied in field crops, has not been investigated in Fructus Trichosanthis.

In this study, we analyzed the content of unsaturated fatty acids, the main active component of Fructus Trichosanthis, and the accumulation of heavy metals to provide information on the edibility, nutritional and medicinal value, and safety of Fructus Trichosanthis.

MATERIALS AND METHODS

Materials and Instruments. Dry Fructus Trichosanthis seed (8% moisture content) was purchased from a local market. Petroleum ether (analytical reagent [AR] grade), n-hexane (AR), methanol (AR), anhydrous ether (AR), and potassium hydroxide (AR) were purchased from Beijing Chemical Reagent Company. Analyses were performed on an Agilent 7890A gas chromatograph (GC) and an Agilent 5975C mass spectrometer (MS).

Experimental Methods. Extraction of Fructus Trichosanthis Seed Oil. Accurately weighed 3-g portions of ground, shelled Fructus Trichosanthis seed were filtered through an 100-mesh sieve. After addition of a certain volume of n-hexane according to a pre-determined material:liquid ratio, extraction and elution were carried out in a solvent extractor at 130°C for a certain extraction and elution time. Following extraction, the solvent was removed by rotary evaporation, and the residue was air-dried in an oven at 105 ± 1°C.

Fatty Acid Detection. Fatty acid composition was determined on an Agilent 122362 GC equipped with an flame ionization detector (FID) and capillary column (SGE) [5, 6]. Injector and detector temperatures were 240°C, and the injection volume was 1 μL. For best resolution of methyl esters, the GC oven temperature was programmed to increase slowly to 180°C. FID and injector temperatures were maintained at 240°C. Nitrogen, used as the carrier gas, was maintained at a flow rate of 30 mL/min.
**Determination of Heavy Metals.** Heavy metals were determined following previously published methods [7, 8, 9]. Contents of the heavy metals Pb, Cd, As, Cu, Cr, Hg, and Zn in soil and *Fructus Trichosanthis* seed were measured on an inductively coupled plasma MS (Thermo X7, USA). Finely ground samples were soaked in 6 mL concentrated nitric acid (98%) for 24 h and then digested with HNO₃-H₂O₂ (4:1) for 4–5 h. After dilution of the residue with ultrapure water, mineral element contents were determined.

**RESULTS AND DISCUSSION**

Seed used in this study was harvested from alluvium soil in Jingxian County, Hebei Province, China. Heavy metal contents of the soil, as determined by our study, are shown in Table 1. According to our results, the most abundant heavy metal was zinc, followed in order by chromium, copper, lead, arsenic, cadmium, and mercury (the latter below the detection limit). The levels of the detected heavy metals were consistent with Chinese national standards (GB 15618-2018).

**TABLE 1**

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Contents (mg/kg)</th>
</tr>
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<tbody>
<tr>
<td>Pb</td>
<td>28.76±0.68</td>
</tr>
<tr>
<td>Cd</td>
<td>0.082±0.0094</td>
</tr>
<tr>
<td>As</td>
<td>21.69±0.85</td>
</tr>
<tr>
<td>Cu</td>
<td>43.99±1.34</td>
</tr>
<tr>
<td>Cr</td>
<td>52.36±2.11</td>
</tr>
<tr>
<td>Hg</td>
<td>ND</td>
</tr>
<tr>
<td>Zn</td>
<td>77.51±3.0.19</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Fatty acid</th>
<th>Contents (%)</th>
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</thead>
<tbody>
<tr>
<td>16:0</td>
<td>Palmitic acid</td>
<td>5.98±0.13</td>
</tr>
<tr>
<td>18:2</td>
<td>Linoleic acid</td>
<td>51.33±0.75</td>
</tr>
<tr>
<td>18:1</td>
<td>Methyl oleate</td>
<td>31.94±1.03</td>
</tr>
<tr>
<td>18:0</td>
<td>Methyl stearate</td>
<td>4.61±0.37</td>
</tr>
<tr>
<td>18:3</td>
<td>Linolenic acid</td>
<td>5.03±0.21</td>
</tr>
<tr>
<td>20:1</td>
<td>Arachidonic acid</td>
<td>0.66±0.03</td>
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</tbody>
</table>

Compared with former studies, we found much higher amounts of oil in the seed of *Fructus Trichosanthis* (Table 2). As shown in Table 2, which lists the composition and amounts of fatty acids found in the seed of *Fructus Trichosanthis*, we detected six different fatty acids. Linoleic acid and methyl oleate, with relative contents collectively greater than 83%, were the most predominant fatty acids. These results demonstrate that *Fructus Trichosanthis* seed oil has a higher proportion of monounsaturated and polyunsaturated fatty acids than plant seed oils, such as rapeseed, peanut, and soybean oils, and especially animal oils [10]. *Fructus Trichosanthis* seed oil is thus healthful and edible.

Heavy metal pollution is an important soil environmental problem, but the heavy metal content of *Fructus Trichosanthis* seed has not been reported. The contents of seven important heavy metals in *Fructus Trichosanthis* seed are shown in Table 3. Except for mercury, which was not detected, heavy metal contents were relatively low. The most abundant heavy metal was copper, followed by zinc, which differs from determined soil heavy-metal contents (Tables 1 and 3). These data indicate that copper is the heavy metal that most readily accumulates in *Fructus Trichosanthis* seed.

**TABLE 3**

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Contents (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>0.324±0.036</td>
</tr>
<tr>
<td>Cd</td>
<td>0.037±0.008</td>
</tr>
<tr>
<td>As</td>
<td>0.269±0.057</td>
</tr>
<tr>
<td>Cu</td>
<td>18.473±1.713</td>
</tr>
<tr>
<td>Cr</td>
<td>0.120±0.042</td>
</tr>
<tr>
<td>Hg</td>
<td>ND</td>
</tr>
<tr>
<td>Zn</td>
<td>7.625±0.628</td>
</tr>
</tbody>
</table>

Note: ND stands for undetected

**CONCLUSIONS**

1) *Fructus Trichosanthis* seed oil is healthy and edible and contains many more monounsaturated and polyunsaturated fatty acids than other plant seed and animal oils.

2) Although *Fructus Trichosanthis* seed from the soil and location of this study was safe to eat, it had relatively high levels of the heavy metal copper.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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THE LIFE CYCLE AND EFFICACY OF
ANTHRIBUS NEBULOSUS FORSTER IN REDUCING SOFT
SCALE POPULATIONS IN BELGRADE

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ABSTRACT

Anthrhus nebulo sus Forster, 1770 (Coleoptera, Anthribidae) is a significant predator of soft scales from the Coccidae family. This paper presents data on the biology and efficacy of this species in Belgrade (Serbia) on five species of soft scales: Physokermes hemicyr phus (Dalman), Physokermes piceae (Schrank), Eulecanium tiliae (L.), Parthenolecanium corni (Bouche) and Parthenolecanium rufalum (Cockerell) (Hemiptera, Coccidae). The life cycles of A. nebulo sus and soft scales are synchronized. In April, the female A. nebulo sus lays an egg under the body of a female soft scale. The hatched larva feeds on the eggs of the scale insect, passes through three stages of development, and then pupates under the scale exoskeleton. The duration of the embryonic and postembryonic development of the predator on these species of scales is similar. The only difference is in the duration of the development of third-stage larvae, which is reflected in the duration of the total development of the species that ranges from 43 to 49 days. The percentage of efficacy of A. nebulo sus statistically significantly varies (p<0.001) among the studied species of soft scales, except between Ph. hemicyr phus and E. tiliae. The highest percentage of efficacy (58.03%) was found in Ph. piceae, and the lowest (10.65%) in P. rufalum.

KEYWORDS:
Anthribidae, Coccidae, predator, life cycle, efficacy, Serbia

INTRODUCTION

Anthrhus nebulo sus Forster belongs to the Anthribidae family, in which 3900 species of 372 genera are described [1]. Most of the representatives are mycophagous or saprophagous species, while the genus Anthribus includes predators of soft scale insects, among which the most significant are Anthribus nebulo sus Forster and Anthribus fasciatus Forster [2].

The life cycle of A. nebulo sus is synchronized with that of its host as the entire development, from egg to imago, takes place under the insect’s cover. In the course of a year it develops one generation and it overwinters in the imago stage. To date, it has been recorded as a predator of 15 species from the Coccidae family and one species from the Ker mesidae family [3, 4]. During the 1980s, it was introduced from Hungary to Virginia (USA) for the biological control of Physokermes hemicyr phus, with good localized results [3, 5]. Twenty-nine years after its introduction, it was determined that the species had spread up to 32 km in several directions from the initial location, but that further expansion was hampered by geographical barriers [6]. The predatory role of this species was studied on Physokermes piceae and Physokermes hemicyr phus in Germany [7], and on Parthenolecanium rufalum in Italy [8].

In Serbia, A. nebulo sus was first registered 35 years ago on Ph. piceae [9], and later as a significant predator on Ph. piceae [10]. Data on the biology of the species and its efficacy are related to individual species of scales, but at the same time, there are no such data for multiple species. Herein we present findings on the life cycle and efficacy of the predator on 5 species of soft scales from the Coccidae family in Belgrade.

MATERIALS AND METHODS

Material collection and laboratory work. With the aim of monitoring the life cycle of A. nebulo sus, on March 15 in 2015 and 2016 adults of the predator were collected by the shaking method from Picea abies infested with Physokermes piceae. Adults were grown in a 10% sucrose solution in a laboratory at the Faculty of Agriculture in Belgrade, at 22-29°C and 50-70% relative humidity. During April of 2015 and 2016, in the period of formation of female soft scales, twigs with five species of soft scales from five localities and five host plants were brought to the laboratory (Table 1).
**TABLE 1**
Locality and host plants from which scale-infested twigs were collected

<table>
<thead>
<tr>
<th>Locality</th>
<th>Host plants</th>
<th>Species of soft scales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zemun Polje</td>
<td>Picea abies (L.)</td>
<td>Physokermes hemicyclus</td>
</tr>
<tr>
<td>Zemun</td>
<td>Picea abies (L.)</td>
<td>Physokermes picea</td>
</tr>
<tr>
<td>Novi Beograd</td>
<td>Tilia tomentosa Moench</td>
<td>Eulecanium tiliae</td>
</tr>
<tr>
<td>Radmilovac</td>
<td>Ulmus glabra Huds.</td>
<td>Parthenoecanion corni</td>
</tr>
<tr>
<td>Ada Ciganlija</td>
<td>Quercus robur L.</td>
<td>Parthenoecanion rufulum</td>
</tr>
</tbody>
</table>

**TABLE 2**
The developmental cycle of *A. nebulosus* by species of soft scales and year

<table>
<thead>
<tr>
<th>Stage of <em>A. nebulosus</em> development</th>
<th>Species of soft scales</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ph. hemicyclus</em></td>
</tr>
<tr>
<td>Egg</td>
<td>01.04. 06.04. 03.04. 08.04. 09.04. 12.04. 13.04. 18.04. 21.04. 25.04.</td>
</tr>
<tr>
<td>L1</td>
<td>15.04. 20.04. 17.04. 22.04. 23.04. 26.04. 27.04. 02.05. 05.05. 09.05.</td>
</tr>
<tr>
<td>L2</td>
<td>22.04. 27.04. 24.04. 29.04. 01.05. 03.05. 04.05. 09.05. 12.05. 16.05.</td>
</tr>
<tr>
<td>L3</td>
<td>29.04. 04.05. 01.05. 06.05. 08.05. 10.05. 11.05. 16.05. 19.05. 23.05.</td>
</tr>
<tr>
<td>Pupa</td>
<td>06.05. 11.05. 10.05. 15.05. 20.05. 23.05. 18.05. 23.05. 27.05. 31.05.</td>
</tr>
<tr>
<td>Adult</td>
<td>14.05. 19.05. 18.05. 23.05. 28.05. 31.05. 26.05. 31.05. 04.06. 08.06.</td>
</tr>
<tr>
<td>Total development</td>
<td>44 43 45 45 49 49 43 43 44 44</td>
</tr>
</tbody>
</table>

L1, L2, L3 – first, second and third instar larvae

Sample twigs with 100 females of each species were placed in Petri dishes (20 cm wide x 5 cm deep), each containing a pair of copulating *A. nebulosus*. The oviposition of female *A. nebulosus* was monitored and the female soft scales in which the predator laid its egg were marked. Regular inspection under a binocular microscope determined the length of the embryonic and postembryonic development of the predator. The average number of eggs laid per female soft scale was determined based on the number of eggs laid by 10 females. For species determination the keys of Schmutterer [11], Kosztarab and Kozár [2] and Gill [12] were used.

**Sampling of infested twigs.** To determine the predatory role of *A. nebulosus*, research was carried out between 2015 and 2017 at the given localities on the plants and soft scale species listed (Table 1). Five trees from each locality were chosen. Sampling was carried out 5 times in the period from April to June, i.e. during development of the predator in the scales of the insects. From each tree, on opposite sides of the crown at a height of 2 m, four branches 50 cm in length were taken. In the laboratory, the soft scales were examined under a binocular microscope, and the percentage of efficacy of the predator was determined according to the formula $P = B \times 100 / a$, where $P$ is the percentage of efficacy, $B$ is the number of soft scales with different stages of *A. nebulosus* and $a$ is the total number of soft scales examined in the sample.

**Statistical analysis.** The obtained data on the efficacy of *A. nebulosus* were processed using analysis of variance (ANOVA), and for subsequent comparisons, the Tukey test was used [13].

**RESULTS**

During the research, *Anthrhus nebulosus* was raised from the covers of five species of soft scales – *Physokermes hemicyclus*, *Physokermes picea*, *Eulecanium tiliae*, *Parthenoecanion corni* and *Parthenoecanion rufulum*.

The developmental cycle of *Anthrhus nebulosus*. In spring, overwintered adults of the predator feed and then find female soft scales in which they lay their eggs. Under our conditions, the female scale insects formed during April. In the first ten days, female *Ph. hemicyclus* and *Ph. picea* were registered, in the second ten days, *E. tiliae* and *P. corni*, and in the third ten days, *P. rufulum*. After the formation of female soft scales, the oviposition of *A. nebulosus* was observed (Table 2).

During oviposition, the females are very focused, undisturbed by the strong light of the lamps, so it is possible to monitor the laying of eggs under a binocular microscope. After copulation, which lasts between 5 and 17 minutes, the female passes over the scales of the insects, examining them with antennae, in search of soft scales in which predator eggs have not already been laid. Then, after choosing a suitable host, it usually makes a small opening on the side of the insect’s cover, turns around, inserts the ovipositor and lays its egg under the scale’s body. It takes 1-2 minutes for the female to
lay its egg, after which it continues its search for the next soft scale in which to lay another egg. The egg of the predator is white and several times larger than that of the soft scale, so it is easily spotted. A female lays between 24 and 28 eggs during its lifetime, usually a single egg per host. During this period, female soft scales also begin to lay eggs, the number of which depends on the species. A female scale, on average, lays between 447.3±21.1 and 1564.1±10.9 eggs, with the lowest number recorded in and the highest in (Table 3).

The development cycle of in all species of soft scales is very similar. Embryonic development lasts 2 weeks, and the hatched larvae feed on the eggs of the hosts. During its development, the larva passes through three stages (L1, L2, L3) and most often eats all the scale’s eggs. The duration of the development of L1 and L2 predators is 7 days in all soft scales, while the length of L3 development varies, depending on the species of soft scale, or the number of eggs as a food source. Thus, the development of L3 lasts 7-9 days in and and the highest in . In four species of soft scales (<i>E. tiliae</i> and <i>P. corni</i>) development in <i>P. rufulum</i>, which lays an average of 1564.1 eggs, lasts 12-13 days. After it develops, the larva pupates in the scale exoskeleton. The pupal stage lasts 8 days in all soft scales. The different duration of L3 development resulted in the different duration of total development of the predator, which is 43-49 days. The eclosion of adults has been recorded from mid-May and lasts until the second ten days of June, with the earliest imago eclosion from <i>P. hemiphyrus</i> and the latest from <i>P. rufulum</i> (Table 2). Immediately after eclosion, the imago is light in color and in the next 5-6 hours, the color darkens. The individuals with the longest development of 49 days that eclosed from <i>E. tiliae</i> are also the largest. In the following period, the imago feeds for a longer or shorter time on the exoskeleton of the soft scale, which usually breaks up due to its brittleness, resulting in its release. After this, the imago enters diapause until the next spring.

The efficacy of <i>Anthribus nebulosus</i>. The role of <i>Anthribus nebulosus</i> in reducing the numbers of populations of the studied soft scales varies. Pairwise comparisons of the predator’s efficacy were performed using Tukey’s test (Table 4).

<table>
<thead>
<tr>
<th>Species of soft scales</th>
<th>Average number of eggs per female (SD – standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physokermes hemicryphus</td>
<td>447.3±21.1</td>
</tr>
<tr>
<td>Physokermes piceae</td>
<td>845.5±9.5</td>
</tr>
<tr>
<td>Eulecanium tiliae</td>
<td>1564.1±10.9</td>
</tr>
<tr>
<td>Parthenolecanium corni</td>
<td>924.3±14.0</td>
</tr>
<tr>
<td>Parthenolecanium rufulum</td>
<td>694.6±19.8</td>
</tr>
</tbody>
</table>

A – significance of difference between the years
a, b, c, d – significance of difference among soft scale species within a year

In studying the developmental cycle of <i>A. nebulosus</i> in laboratory conditions on <i>Physokermes hemicryphus</i>, <i>Physokermes piceae</i>, <i>Eulecanium tiliae</i>, <i>Parthenolecanium corni</i> and <i>Parthenolecanium rufulum</i>, it was found that there is no difference between the length of embryonic development and the length of development of the first two larval stages. Differences were manifested in the length of L3 development, depending on the amount of food available. In four species of species of soft scales (<i>P. hemiphyrus</i>, <i>P. piceae</i>, <i>P. corni</i> and <i>P. rufu-
that lay an average of between 447.3 and 924.3 eggs, the development of L3 is 7-9 days because after eating all the eggs the larvae pass into the pupal stage. In E. tiliae, which lays an average of 1564.1 eggs, the larva continues to grow and after 12-13 days, pupates. Similar data on variations in the length of L3 development depend on the amount of available food were obtained when studying the biology of Anthribus niveovariegatus Roei-lofs on Eriocerus pela Chavannes in Japan [14].

Data on the A. nebulosus development cycle are very similar to the data obtained by other authors. Differences were observed in the period of oviposition, which was recorded on the territory of Belgrade during April, while on the territory of Moldova [15], Germany [16], Hungary [3] and Turkey [17] it was registered during May, and in Kazakhstan in June [18]. Due to the different time of laying eggs, the eclosion of adults in Belgrade was recorded from mid-May to the second ten days of June. Statistically significant differences (p<0.001) in the efficacy of A. nebulosus were found on the studied scale species, except between Ph. hemicryphus and E. tiliae. The percentage efficacy on P. rufulum was 10.65-12.18%, on P. corni 21.82-23.47%, Ph. hemicyprys 40.7-44.48%, E. tiliae 46.33-47.86% and on Ph. piceae 55.28-58.03%. No statistically significant differences were found between the species of soft scales and year (p=0.950), or between the 3 years studied (p=0.096).

CONCLUSIONS

In the area of Belgrade, A. nebulosus is a predator of Physokermes hemicryphus, Physokermes piceae, Eudecanium tiliae, Parthenolecanium corni and Parthenolecanium rufulum (Hemiptera, Coccidae). The life cycles of the predator and of soft scales are synchronized. In April, the female A. nebulosus lays an egg under the body of the female soft scale. The lengths of the embryonic and first two larval developmental stages of the predator do not differ, while the length of L3 development depends on the species of scale. In four species of soft scales (Ph. hemicryphus, Ph. piceae, P. corni and P. rufulum) that lay an average of between 447.3 and 924.3 eggs, the development of L3 is 7-9 days, and the total length of development is 43-45 days. In E. tiliae, which lays an average of 1564.1 eggs, the length of L3 development is 12-13 days, and the total length of development is 49 days. Under our conditions, the imago eclosion occurs from mid-May to the second ten days of June. Statistically significant differences (p<0.001) in the efficacy of A. nebulosus were found on the studied scale species, except between Ph. hemicryphus and E. tiliae. The percentage efficacy on P. rufulum was 10.65-12.18%, on P. corni 21.82-23.47%, Ph. hemicyprys 40.7-44.48%, E. tiliae 46.33-47.86% and on Ph. piceae 55.28-58.03%. No statistically significant differences were found between the species of soft scales and year (p=0.950), or between the 3 years studied (p=0.096).

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ANALYSIS AND FORECAST OF AGRICULTURAL AND FORESTRY INDUSTRY STRUCTURE BASED ON GREY SYSTEM THEORY: A CASE STUDY OF LIAONING PROVINCE, CHINA

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ABSTRACT

The present study aims to clarify the developments in agricultural and forestry industrial structures, and to forecast the tendency of agricultural and forestry output values and the output of various agricultural and forestry products in Liaoning Province, P.R. China. The grey correlation analysis method was applied based on the statistical yearbook in Liaoning province from 2008 to 2017. This study analyzed the agricultural and forestry output values, such as grain, meat, fruit, and aquatic product output and the afforestation area of Liaoning Province in the last decade. The GM(1,1) model was used to forecast the agricultural and forestry output values and productions of kinds of agricultural products. Results showed that the relationship of planting and animal husbandry on the gross output value of agriculture were relatively closer in the total agricultural output values. The relationship among corn, rice, potato yield, and total crop yield; apple yields and total yields of fruit; poultry meat, pork output, and total meat output, marine product and output of total aquatic products were relatively close. Moreover, the relationship of the protection forest on the afforestation area and total afforestation area were the closest. Furthermore, the total output of agricultural products and the output of agricultural products in 2023 are predicted to increase by 2018. Therefore, the growth rate of meat production should be given considerable attention. This way, the output value of the agricultural service industry and the outputs of pear, poultry, and aquatic products in terms of growth rate will be expedited.

KEYWORDS:
Agricultural and forestry industrial structure, Liaoning province, Grey theory, Agricultural and forestry output

INTRODUCTION

Agriculture and forestry are the foundations of the national economy. Moreover, revitalizing northeast China’s industry and agriculture development are pressing matters. The economic recovery in Liaoning Province, which has the largest economic volume in the northeast region, is related to the success or failure of the revitalization of the northeast region.

Agricultural and forestry (Hereinafter referred to agricultural) industry structure is also called the agricultural production structure. It refers to the internal composition and the relationship of the various agricultural industry sectors and the components within each sector is in per region (Country, area, agricultural enterprise). It is divided into three levels: production structure, product structure, and variety structure [1]. At present, Liaoning Province has made great progress in modern agriculture. However, the development of agricultural modernization remains slow compared with rapid developments in industrialization, urbanization, and information technology. The output of agriculture has increased annually; however, farmers’ incomes have remained low. The agricultural development is restricted by their resources and rising demand. Thus, the contradiction of agricultural economy has increased. The fundamental cause of these conditions is the inefficiency of the agricultural industry. Therefore, optimizing the agricultural industrial structure is needed for the development of agriculture and rural economy in the new period. Furthermore, this will help achieve agricultural efficiency, increased farmers’ income, and harmonious development of the rural economy [2].

Scholars have studied the agricultural industry by using the grey correlation analysis. Examples of these scholars are Li et al., who studied grain production in Henan province [2], Bai et al., who studied agricultural production goods [3], and Duan, who studied various grain yield influencing factors [4]. In addition, several scholars, such as Xie, who pre-
dicted China’s gross domestic product [5], has studied how to forecast data by using grey prediction. Grey correlation analysis has solved numerous practical applications problems, such as economic forecasting, management decision making, scientific evaluation, energy management, industrial process control, water resource analysis, agricultural breeding, research and development of complex equipment, and post-evaluation of major projects.

During our research, we took the current situation of agriculture in Liaoning Province as the starting point. This paper is directed to problems, such as an insufficiently comprehensive grey correlation analysis and grey prediction of agricultural products in this area, small reference value on account of a relatively long study period, and lack of research about afforestation area and aquatic product. Furthermore, this paper forecasts the agricultural output value and the outputs of various agricultural products by selecting appropriate data and applying the GM(1,1) model. Finally, this paper provides combined references and recommendations for the optimization and adjustment of agricultural industry in Liaoning Province.

**MATERIALS AND METHODS**

**Research index and data sources.** In this research, we selected total agriculture output value and output value of each agriculture industry, total output of grain crops, meat, fruit, aquatic products, the output of various agricultural products in these agricultural products, total afforestation area, and various types of forest land area as research indexes. The data were acquired from the statistical yearbook in Liaoning province (written by Bureau of Statistics in Liaoning Province and published by China Statistics Press).

**Research method.** The system with accurate information is called the White System; the system where information is completely unknown is called the Black System; and the system where one part of the information is known and the other part is unknown is labeled the Grey System. Grey System is the research object of the Grey System Theory. By generating and developing the known information, extracting valuable information, the system operation behavior and evolution law are correctly described and effectively monitored [6].

Grey correlation analysis is a method for analyzing the Grey System. By comparing the time series between different factors and measuring the degree of association between the various factors, the dominant factor and a dynamic research process of the system development trend analysis were developed [7]. The similarity degree or dissimilarity degree among the various factors is described as the grey relational degree. It provides a quantitative measure for the development and change of the system. The steps of grey relational analysis are shown in (1-6).

Grey prediction establishes a grey model to determine the future development trend of the system and to provide basis for the planning and decision-making based on past and present known or uncertain information [8]. The most commonly used prediction method is the GM(1,1) model. The steps of grey prediction (7-18).

The steps of grey relational analysis: Determine reference sequence and compared sequence by taking total agricultural output value or total output of agricultural products of Liaoning province in the recent ten years as reference sequence. Gather the output value of each industry in agriculture or the output of all kinds of agricultural products of Liaoning province in the recent ten years as the compared sequence.

**FIGURE 1**

General introduction of research region
Reference sequence, sign as $X_0$:

$$X_0(t) = \{x_0(1), x_0(2), \ldots, x_0(n)\}, t = 1, 2, \ldots, n.$$  

(1)

Compared sequence, sign as $X$:

$$X(t) = \{x_1(i), x_2(i), \ldots, x_m(i)\}, i = 1, 2, \ldots, m, t = 1, 2, \ldots, n.$$  

(2)

Initialize reference sequence:

$$X'_0(t) = \frac{X_0(t)}{X_0(1)}, t = 1, 2, \ldots, n.$$  

(3)

Initialize compared sequence:

$$X'_i(t) = \frac{X(t)}{X_0(1)}, t = 1, 2, \ldots, n.$$  

(4)

When $t = k, \; k \in (1, 2, \ldots, n)$, the grey relational coefficient is:

$$\xi_{i(k)} = \min \left\{ \frac{|X'_0(k) - X'_i(k)| + \rho \max X'_0(k) - X'_i(k)|}{|X'_0(k) - X'_i(k)| + \rho \max X'_0(k) - X'_i(k)|} \right\}$$

(5)

where

- $|X'_0(k) - X'_i(k)|$ is the absolute difference between reference sequence and comparison sequence
- $\min |X'_0(k) - X'_i(k)|$ is the minimum absolute difference value
- $\max |X'_0(k) - X'_i(k)|$ is the maximum absolute difference value
- $\rho$ is the resolution coefficient. $0 < \rho < 1$, usually $\rho = 0.5$

The grey correlation degree between time sequence $X_0$ and time sequence $X_i$:

$$\gamma_{0i} = \frac{1}{n} \sum_{i=1}^{n} \xi_{0i}(k).$$

(6)

Analyze the obtained correlation value. In general, the influence factor of the grey relational degree value that is greater than or equal to 0.70 is the strong influence factor. The influence factor of the grey relational degree value that is between 0.60 and 0.69 is the general influence factor. The influence factor of the grey relational degree value that is less than or equal to 0.60 is the weak influence factor [9].

The steps of grey prediction:

1. Calculate a cumulative sequence by original time sequence: $X_0(t) = \{x_0(1), x_0(2), \ldots, x_0(n)\}$

$$X'_0(t) = \frac{X_0(t)}{X_0(1)}, t = 1, 2, \ldots, n.$$  

(7)

where

$X_0(k+1) = x_0(k) + x_0(k+1), \; x_0(1) = x_0(1), \; k = 1, 2, \ldots, n.$

2. Calculate a consecutive neighbour sequence by cumulative sequence $X'$:

$$Z_i = \{z_1(2), z_1(3), \ldots, z_i(n)\}.$$  

(8)

where $z_i(k) = x_i(1) + x_i(k-1), \; k = 2, 3, \ldots, n.$

3. Set up upper and lower limit vector $B$:

$$B = \left[ \begin{array}{c} x_0(2) \\ x_0(3) \\ \vdots \\ x_n(n) \end{array} \right].$$

(9)

Solving the parameter column by using the least square method:

$$\hat{\theta} = (\hat{\alpha}, \hat{\mu})^T = \left( B^T B \right)^{-1} B^T Y_n.$$  

(10)

where $\alpha$ is development coefficient and $\mu$ is grey action.

4. Set up GM(1,1) model:

$$\frac{dx}{dt} + \alpha x = \mu.$$  

(11)

5. Set up time response series:

$$\hat{z}(k+1) = \left( x_0(1) - \frac{\mu}{\alpha} \right) e^{-\alpha} + \frac{\mu}{\alpha}.$$  

(12)

6. Reduce to original sequence:

$$\hat{z}(k+1) = \hat{z}_0(k) + \hat{z}_0(k) - \hat{z}_0(k) = (1 - e^{-\alpha}) \left[ x_0(1) + \frac{\mu}{\alpha} \right] e^{-\alpha}. \; k = 1, 2, \ldots, n.$$  

(13)

7. Diagnose the model by testing with posterior error (C) and small error probability (P) for analyzing the reliability of the model.

- Variance:

$$S_1^2 = \frac{1}{n} \sum_{i=1}^{n} \left( x_{0i}(k) - \bar{x}_0 \right)^2.$$  

(14)

- Residual:

$$\varepsilon_0(k) = x_{0i}(k) - \hat{x}_0(k).$$  

(15)

- Residual variance:

$$S_2^2 = \frac{1}{n} \sum_{i=1}^{n} \left( \varepsilon_0(k) - \bar{\varepsilon}_0 \right)^2.$$  

(16)

- Posterior error ratio:

$$C = \frac{S_2^2}{S_1^2}. $$  

(17)

- Small error probability:

$$P = p \left\{ |\varepsilon_0(k) - \bar{\varepsilon}_0| < 0.6745S_1 \right\}. $$  

(18)

The accuracy test grade reference table for $C$ and $P$ is shown in Table 1:

<table>
<thead>
<tr>
<th>Accuracy level</th>
<th>Small error probability/P</th>
<th>Posterior error ratio/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade one (Excellent)</td>
<td>$P \leq 0.95$</td>
<td>$C \leq 0.35$</td>
</tr>
<tr>
<td>Grade two (Good)</td>
<td>$0.80 &lt; P &lt; 0.95$</td>
<td>$0.35 &lt; C \leq 0.50$</td>
</tr>
<tr>
<td>Grade three (Qualified)</td>
<td>$0.70 &lt; P &lt; 0.80$</td>
<td>$0.50 &lt; C \leq 0.65$</td>
</tr>
<tr>
<td>Grade four (Unqualified)</td>
<td>$0.60 &lt; P &lt; 0.70$</td>
<td>$0.65 &lt; C \leq 0.80$</td>
</tr>
</tbody>
</table>
When establishing the GM(1,1) model, we can get different $\alpha$ and $\mu$ in selecting data for different dimensions. Hence, we can get different prediction models. The different predicted values are calculated by different prediction models. Different dimensions are considered in building the model to ensure the accuracy and reliability of the forecast value. According to Table 1, the appropriate model is selected. The grey model data generally require not less than five dimensions [10]. In this paper, we choose 5–10 dimensional data for the prediction.

Statistical analysis. The data processes and plotting are completed with SPSS version 21.0 (IBM Inc. NC, USA), and the plots are performed by using OriginLab. OriginPro 9.0 (OriginLab Inc., Northampton, MA, USA).

RESULTS AND ANALYSIS

Correlation analysis of agricultural industrial structure in Liaoning Province. The correlation degree of each factor in different time periods is calculated by using the grey correlation analysis. In accordance with the time sequence, the grey relational dynamic matrix of each level of agricultural industry is formed (Figure 2–7). The status of agricultural industry structure in Liaoning province in the recent 10 years is analyzed. The results are as follows:

According to Figure 2, the correlation degree between the total value of agriculture and its components is: farming $>$ animal husbandry $>$ forestry $>$ fishery $>$ agricultural services industry. Farming, forestry, animal husbandry, and fishery industry are the four strong influencing factors. The agricultural services industry is the weak influence factor. The correlation degree between gross output value of agriculture and planting industry is the most closely related. Moreover, the average correlation degree is 0.94. Hence, farming is the leading agricultural industry in Liaoning Province, followed by animal husbandry, with an average correlation degree of 0.91. The average correlation of forestry and fishery is close, which are respectively 0.73 and 0.73. However, the average correlation degree between the two industries and the planting or animal husbandry is relatively large. The smallest average correlation degree, which is 0.46, is agricultural services industry.

On the basis of the dynamic development trend, the correlation degree between the total value of agriculture and the output value of farming or animal husbandry shows an initial drop and then rise in the trend. The two industries reach the lowest value, which are respectively 0.92 and 0.88. The correlation degree between the total value of agriculture and the output value of the fishery shows a slow downward trend. The correlation degree between the total value of agriculture and the output value of forestry shows that the agriculture services are gradually reduced.

According to Figure 3, the correlation degree between the total output of agriculture and its components is: corn $>$ potato $>$ rice $>$ soybean $>$ millet $>$ sorghum $>$ wheat. Corn and potato are strong influencing factors. The average correlation degree of corn is 0.92. Corn is most closely related to the total output of grain crops followed by potato with an average correlation degree of 0.73. Rice is the general influence factor with a 0.69 average correlation degree. Soybean, millet, sorghum, and wheat, as the weak influence factors, have the average correlations of 0.60, 0.54, 0.44, and 0.42, respectively.
On the basis of the dynamic development trend, the correlation degree between the total grain yield and corn is overall relatively stable. The correlation degree between the total grain yield and potato shows a slow downward trend except for the increase by 4.62% during 2015–2017 compared with 2014–2017. The correlation degree between the total grain yield and rice shows an initial drop and then rise in the trend. The rice reaches its lowest value, that is, 0.6496, during 2010–2017. The correlation degree between the total grain yield and soybean shows a slow downward trend. The correlation degree between the total grain yield and millet rises 0.31% and 1.44% respectively during 2010–2017 and 2016–2017. The correlation degree between the total grain yield and sorghum shows a gradual downward trend except for a rise of 0.45% during 2016–2017 compared with 2015–2017. The correlation degree between the total grain yield and sorghum shows a gradual downward trend except for an increase by 0.13% during 2016–2017 compared with 2015–2017.

According to Figure 4, the correlation degree between the total output of fruit and its components is: apple > pear > grape. (The fruit type is relatively few because of the slightly cold climate in Liaoning Province) Apple is the strong influence factor with a 0.79 average correlation degree. It is most closely related to the total output of fruit. Pear and grape are the weak influence factors, where their average correlation is 0.57 and 0.42, respectively.
On the basis of the dynamic development trend, the correlation degree between the total output of fruit and apple shows a slow downward trend. However, apple is still the most important fruit product. The correlation degree between the total output of fruit and pear shows a gradual downward trend except for an increase by 1.34% during 2016–2017. The correlation degree between the total output of fruit and grape shows a trend of first drop and then rise, thereby showing a certain development potential of grape.

According to Figure 5, the correlation degree between the total output of meat and its components is: poultry meat > pork > beef > mutton. Poultry meat is the strong influence factor with a 0.87 average correlation degree. It is most closely related to the total output of meat followed by pork with an average correlation degree of 0.85. The correlation degree between the total output of meat and pork is also close. Beef is the general influence factor with an average correlation degree of 0.60. The average correlation degree of mutton, as the weak influence factor, is 0.36.

On the basis of the dynamic development trend, the correlation degree between the total meat and poultry meat or pork is slightly fluctuant. The correlation degree value of the total meat and poultry meat or pork is relatively high and smooth. The correlation degree between the total meat and beef gradually reduces in the rest of the stage except for a slow increase of 0.03% during 2010–2017 compared with upper
stage. The correlation degree between the total meat and mutton shows a downward trend in volatility. It only rises by 0.46% during 2016–2017.

According to Figure 6, marine products are the strong influence factor. The average correlation degree of marine products is 0.77. Marine products also play a lead role in the total output of aquatic products. Freshwater products are the weak influence factor. The average correlation degree of freshwater products, which is 0.38, differs considerably from marine products.

On the basis of the dynamic development trend, the correlation degree between the total output of aquatic product and marine products or freshwater products shows a slow downward trend.

According to Figure 7, the correlation degree between the total afforestation area and various types of forest land area is: protection forest > timber forest > economic forest > fuelwood forest. The planting area of protection forest is the strong influence factor with a 0.94 average correlation degree. Protection forest is the mainly planted tree species. Timber forest and economic forest are the general influence factors with 0.69 and 0.64 average correlation degree, respectively. Fuelwood forest, as the weak influence factor, has an average correlation degree of 0.60.

On the basis of dynamic development trend, the correlation degree between the total afforestation area and protection forest is overall relatively stable. The correlation degree between the total afforestation area and timber forest decreases first and then increases. The correlation degree shows the minimum at 0.62 in 2014–2017. The correlation degree between the total afforestation area and economic forest shows a gradual downward trend. The correlation degree between the total afforestation area and fuelwood forest shows an initial drop and then rise in the trend. Moreover, the correlation degree achieves the lowest value, which is −0.54.

**Grey prediction of the development trend of agricultural industry structure in Liaoning Province.** In accordance with the reference data from 2008 to 2017, the data of grain crop yield and afforestation area are not stable, and the annual change is large. Hence, the two items are not suitable for modelling. This study construct GM(1,1) models for total agricultural output value, internal industrial output value, total output of fruit, meat, aquatic products, and output of their various types of agricultural products. The dimension, the optimal forecasting model, and the model test results are shown in Table 2. The predicted values from 2018 to 2023 are shown in Table 3.

As shown in Table 3 combined with the original data from 2017, this study predicted that the gross output value of agriculture in 2023 will increase by 105.71% compared with that in 2017. Farming, forestry, animal husbandry, fishery, agricultural services industry output will increase by 111.04%, 136.32%, 102.61%, 90.97%, and 139.51%, respectively. Farming and animal husbandry still occupy an important position in agricultural gross output value. The output value of agricultural services industry is the fastest growing, and fishery output value is the slowest. Total fruit production in 2023 will increase by 75.05% compared with that in 2017. Apple, pear, and grape production will increase by 85.37%, 100.36%, and 31.43%, respectively. Apple still accounts for a high proportion of fruit production, which is 44.29% of the output of the fruit yield. Pear production is the fastest growing, and grape production is the slowest. Total meat production in 2023 will increase by 10.07% compared with that in 2017. Pork, beef, mutton, and
poultry meat production will increase by 7.98%, 9.63%, 4.06%, and 20.93%, respectively. Pork and poultry meat have the greater impact. Moreover, pork production will reach 54.93% of meat production. Poultry meat production is the fastest growing. Beef and mutton that account for the proportion of total meat production is still small. The growth of mutton production is the slowest. The total output of aquatic products in 2023 will increase by 27.92% compared with that in 2017. The production of marine products and freshwater products will increase by 27.89% and 27.60%, respectively. Marine products production is still dominant, thereby accounting for 81.37% of the total output of aquatic products.

**DISCUSSION**

On the basis of the present situation of agricultural industry structure in Liaoning Province and combined with the research results of this paper, the structure of agricultural industry can be adjusted as follows:

Grain production plays an important role in agricultural production. It is also directly related to social harmony and stability [11]. The relationship between quantity and quality of grain should be handled to adapt to the new consumer demand, which is a long-term strategic task for the agricultural production department [12-21]. Efficient economic crops, forage, and green manure crops should be developed.
to adjust the farming structure for a stable grain crop production. The main cultivation of grain crops are corn and rice in Liaoning Province. Planting high-quality rice, corn, and other similar crops should be promoted on the basis of the stability of the two major crops to format the regional division of crop planting. Superior resources and the product of economic crops with regional characteristics in Liaoning Province should be taken advantage of. Modern agricultural technology should be utilized to form a scale production of economic crops, such as sunlight greenhouses.

The economy of forestry industry based on the market should be developed. Tree species and forest age structure should be adjusted in accordance with the structure of forestry industry. The timber forest area should be maintained. The protection forest, special forest, fast-growing plantation, and industrial raw material forest area should be propagated. The forestry ecological protection should be strengthened while adhering to the scientific logging. The farmland should be appropriately restored as a forest to increase the forest wood stocking of Liaoning Province. Forest by-products, such as economic fungus, mushrooms, pine nuts, chestnut, hazelnut, ephedra, and ginseng, should be developed. New forestry industry projects, such as forest tourism, woody grain and oil production, and wild animal domestication and breeding, should also be developed.

Combined with the statistical yearbook in Liaoning province, the forecast data of agricultural output value and agricultural product output showed that, with the improvement of people's living standard, the development of animal husbandry has become increasingly important. The structure of animal husbandry should be adjusted by starting from industrialization. The industrialization can combine the spread of animal husbandry individuals and markets and accelerate commercialization, marketization, and modernization of animal husbandry. The feed sources for livestock farming, such as independent production of feed crops, should also be expanded. At the same time, this expansion can also promote the adjustment of planting structure.

The key point of adjustment of fishery industry structure is the change of traditional to modern fishery and the change of quantity type to quality type fishery. While remaining market oriented, the structure of a single species of freshwater aquaculture should be adjust and the Liaoning marine ranching should be developed. The two offshore fishing grounds, namely, Liaodong Bay and Ocean Island, should be relied on for products such shrimp, crab, and kelp and sea curiosa such as sea cucumber, abalone, and scallop. The development of ocean fishing and aquaculture should be given adequate attention. In the development of the fishery industry, the protection of the ecological environment of fishery should be considered. The development should be hastened, and the scale of the fishery economy should be adapted to the carrying capacity of resources and environment.

In addition, the following measures adjust the agricultural industry structure in Liaoning Province: developing agricultural services industry, farm produce processing industry, and agricultural products transportation industry; promoting the industrialization of agricultural scientific and technological achievements; constructing agricultural infrastructure; establishing agricultural leading enterprises; and increasing the funds for the construction of modern agricultural industry system.

CONCLUSION

We predicted that the total output value of agriculture in 2023 is more than doubled that in 2017. The planting and the animal husbandry are the two largest industries that affect the gross output value of agriculture. Therefore, the overall agricultural development strategy of Liaoning Province in the future is taking the farming and animal husbandry as the leading industry and developing forestry, fishery, and agricultural services industry collaboratively. The agricultural services industry, as the third industry in agriculture, is also the predicted fastest growing industry; the adjustment of agricultural industry structure in Liaoning province cannot be ignored. Under normal circumstances, from 2018 to 2023, the overall agricultural output will increase annually and the development trend will show good momentum.

We predicted total fruit production in 2023 will increase by roughly 75% compared with that in 2017. Apple and pear are the largest and fastest growing fruits, respectively. Pork production will account for more than 50% of meat production. In addition to poultry in 2023 will increase by more than 20% than that in 2017, the grown rate other meat production will increase slowly. So that the total output growth of meat is only about 10%. Meat production is an important part of solving the problem of agricultural production in Liaoning Province. The output of marine products and freshwater products will increase by nearly 30% in 2017. The proportion of marine products will account for more than 80% of the total output of aquatic product. In summary, Liaoning province should pay attention to agricultural products closely associated with the total output of agricultural production, such as corn, rice, potato, apple, pear, poultry meat, pork, and marine products. In promoting the advantages of agricultural products, we should also pay attention to the harmonious development of characteristic agricultural products and high-quality agricultural products.

Protection forest plays an irreplaceable role in environmental protection. Protecting and increasing the area of protected forest, that is, increasing timber forest and economic forest area properly, is the strategy of the afforestation area in Liaoning Province.
Moreover, for grey correlation analysis, this paper only shows the correlation degree between various industrial output value and total industrial output value of agriculture, between output of various agricultural products and the total output of agricultural products, and between various types of forest land area and total afforestation area. The impact of the components of agriculture on the total agriculture industrial output value or relation need to be further studied.

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MOLECULAR EPIDEMIOLOGY OF BOVINE RABIES IN EASTERN AND SOUTHEASTERN ANATOLIA REGIONS OF TURKEY

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ABSTRACT

This study was conducted to determine the molecular characterization and filiation of rabies virus isolated from bovine rabies cases in Eastern and Southeastern Anatolia Region of Turkey 2000-15, their genetic proximity to previously isolated viruses in Turkey and in neighboring countries. In the study, 1090 animal brain sample suspected of rabies were studied, using fluorescent antibody test (FAT). The results of the tests indicated 71.83% (786/1090) positivity of total animal rabies, with 41.73% (328/1090) positivity in dogs and 35.49% (279/1090) positivity in bovine. A statistically strong positive correlation was found in the prevalence of dog rabies and cattle rabies according to the years. Partial gene sequencing of rabies virus isolates from naturally infected 7 bovine brains belonging to 2009 and 2013 was performed. These isolates confirmed presence of Rabies virus (RABV) by performing gene screening in the NCBI (The National Center for Biotechnology Information) database. In addition, phylogenetic analyzes of these isolates were performed according to the partial gene sequence of RABV nucleoprotein N gene 0-510 bp. It was determined that the nucleotide sequences of the isolates of the rabies virus obtained during 2009 and 2013 were identical as shown by the partial gene sequences of viruses. In addition, these isolates were determined to have a common ancestor with the nucleotide sequences of previously isolated viruses in Turkey and neighbouring countries bordering Eastern and Southeastern Anatolia Regions. It was concluded that it must be ensured that the bovines are inoculated with the inactivated dog vaccine and the dogs must be protected against rabies disease.

KEYWORDS:
Bovine Rabies, Molecular Epidemiology, Turkey

INTRODUCTION

Rabies is a viral zoonosis that can be prevented by rabies vaccines, it remains a major public health problem in developing countries. It is caused by negative strand RNA-viruses belonging to the genus Lyssavirus. The genus Lyssavirus in the Rhabdoviridae family, cause the highest mortality rate among conventional diseases, is an acute progressive encephalitis caused by viruses. Rabies virus (RABV), the prototype of lyssavirus species, is the causative agent of classical rabies and is responsible for the vast majority of all human rabies cases [1]. Rabies virus is usually transmitted through the saliva of an infected animal during a bite. There are currently twelve recognised members of the genus lyssavirus [2]. RABV is deadly in mammals due to its effect on the nervous system. The agent is single stranded, negative polarized, non-segmented RNA virus [3]. The RABV genome created of N, P and L proteins, forms ribonucleoprotein complex that assists in multiplication of virus in the cytoplasm of host cells. The G protein of the RABV is alone stated on the viral surface, which is responsible for the viral pathogenicity, and stimulate protective immunity against rabies [4].

Rabies is widespread throughout the world. Most countries outside the island are endemic. Most of the countries of Americas and Europe report emergence of disease in restricted areas. Countries like Australia, New Zealand, Japan, Papua New Guinea and Pacific Islands are free of this virus since many years [5]. Rabies is also a serious enzootic disease in Jordan, Syria, Lebanon, Iran and Iraq. Stray dogs around here allow the rabies virus to remain in circulation [6].

Rabies within Turkey is mainly a disease of domestic dogs with sporadic cases appearing in domestic cattle [7]. This tendency has exchanged in recent years and has followed up with the emergence of rabies within the bovine population [8]. Rabies is a notable disease in Turkey and the country has strong links with The World Health Organization (WHO), the World Organization for Animal Health (OIE) and the European Union (EU). Turkish government provide rabies surveillance and diagnosis [6] to Wild and domestic animals found dead or suspected of having rabies were collected. The case density applied in natural conditions is passive surveillance. This program was approved by the European Commission [9].
TABLE 1
Turkey rabies virus samples were grouped according to Genbank Code, species, year, geographical region of isolation.

<table>
<thead>
<tr>
<th>Genbank Code</th>
<th>Animal Species</th>
<th>Sampling Year</th>
<th>Geographical Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM 058759</td>
<td>Cow</td>
<td>2009</td>
<td>Gezin/Elazığ</td>
</tr>
<tr>
<td>KJ 081443</td>
<td>Cow</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081444</td>
<td>Cow</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081445</td>
<td>Cow</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081446</td>
<td>Cow</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081447</td>
<td>Cow</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081448</td>
<td>Cow</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081449</td>
<td>Fox</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
<tr>
<td>KJ 081450</td>
<td>Fox</td>
<td>2013</td>
<td>Karakoçan/Elazığ</td>
</tr>
</tbody>
</table>

A portion of Turkey's territory lies in Asia another part lies in Europe. Turkey is engulfed by water on three sides land borders with countries like Syria, Iraq and Iran that are known to harbor rabies disease.

Molecular epidemiology is acting an enhancement in the comprehension of the rabies virus and its relationship with host species. This access has been performed at different geographic levels, both at private and public levels [10]. The main focus in the molecular characterization of lyssaviruses is the nucleoprotein N gene. The availability of a large number of partial N gene sequence databases facilitates and provides convenience for time, geographical and host distribution of virus [11].

This study was conducted to determine the molecular characterization and filiation of rabies virus isolated from bovine rabies cases in Eastern and Southeastern Anatolia Region of Turkey between the years 2000-2015 and their genetic proximity to previously isolated viruses in Turkey and in neighboring countries.

MATERIALS AND METHODS

Materials, Data. The case density passive surveillance in rabies disease was applied in the eastern and southeastern Anatolia (Elazığ, Malatya, Tunceli, Bingöl, Diyarbakır, Batman, Siirt, Şırnak, Hakkâri, Van, Mardin, Bitlis, Muş) during 2000-2015. The case histories, suspected animal species, and test result information were recorded in the national netork system. Surveillance records were used as material for this study.

Virus isolates. All samples were tested for rabies virus by direct immunofluorescence antibody (dIFA) test (Centocor, Malvern, Pa.) as described previously [12]. Sequence analysis of 7 bovines and 2 foxes isolates was performed in regions with intensive bovine positivity. The species of origin of the samples from Turkey and their GenBank Code, Sampling Year, Geographic origin and are shown in Table 1.

Methods. Genetic analysis was performed on PCR products from the brains of wild and domestic animals. Total RNA was extracted, using RNA easy mini kit (cat no ID/74104, Qiagen), from infected brain tissues for PCR assay according to the manufacturer’s instructions. cDNA synthesis and PCR amplification of the partial nucleoprotein (N) gene was performed as previously described by Haelton et al. [13]. All PCR products were separated on a 2% agarose gel, then purified with the High Pure PCR Product Purification Kit (Roche, Germany). Consensus sequences were obtained by aligning the forward and reverse sequences with the ABI 3130xl Genetic Analyzer (Applied Biosystems, USA). Alignment, translation of the common sequences was performed using the Clustal X package [14]. Neighbor joining (NJ) analysis was performed using MEGA version 2.1 [15] Clustal X and MEGA’s TreeExplorer module NJPLOT program was used to obtain the graphic output. Linear regression model was used to investigate relationships between reported incidence of bovine rabies and dog-mediated rabies. These analyses were performed using the R statistical programming language [16].

RESULTS

A total of 786 rabies infection were confirmed in Eastern and Southeastern Anatolia from 2000 to 2015 including 328 cases from dogs (41.73%) and 279 cases from bovines (35.49%). Positivity rates were detected 5.72% for cat, 4.32 % for fox, 2.92 % for donkey, 2.79 % for wolf, 2.54 % for sheep, 2.16 % for caprine, 1.39 % for horse, 0.25 % for mule, 0.25% for marten and 0.25 % for badger. The highest annual incidence of rabies was recorded with 436 cases in 2012-2015 (Figure 2).

The passive surveillance of animal rabies in the region showed an increased occurrence of rabies in dogs followed by their occurrence in bovine rabies was observed during the period of 2012-2015. There was a strong positive correlation between periodical bovine and dog rabies cases (Figure 2, 3).
FIGURE 1
Rate of annual animal rabies occurrence detected among animals in Eastern and Southeastern Turkey 2000–2015.

FIGURE 2
Some species trends in Rabies cases (2000-2015)

FIGURE 3
The relationship between rabies cases in domestic dogs and bovine reported during 2012-2015 period from the region. The relationship was determined by linear regression test.
Partial nucleotide sequences of 6 isolates cows and 2 isolates fox in 2013 and an isolate cow from 2009, were uploaded to the National Center for Biotechnology Information (NCBI) system by year, animal species and region name. NCBI confirmed that the isolates had Rabies virus (RABV) when compared to the previously installed indexes in the Standard Local Alignment Search Tool (BLAST) program. Rabies Virus Nucleoprotein N gene nucleotide and amino acid sequences of KM 058759, KJ 081443, KJ 081444, KJ 081445, KJ 081446, KJ 081447, KJ 081448, KJ 081449 and KJ 081410 GenBank codes are compared in their own comparisons to be isolated from bovines and foxes at the same time. The 2009 and 2013 bovine isolates were also the same (Figure 4 A, B). Whole, the virus samples from Eastern and Southeastern Anatolia Regions of Turkey shared nucleotide homology.

Mutations in nucleotides 484 (G→A), 544 (C→T) and 574 (A→G) were detected in the nucleotide analysis compared to the partial gene sequence with the virus KM058759, and KJ081443, KJ081447, KJ081450 viruses. it was also found that there was a mutation in the 103 (T→C) nucleotide in addition to the mutations mentioned above between the KM058759 virus and the KJ081443 virus virus (Figure 5).

The N gene sequences of Turkish other RABVs obtained from GenBank were compared with those of the this study’s isolates to analyze molecular epidemic relationships. Phylogenetic analysis of specimens collected in this study’s isolates clearly shows that RABVs have a distinctive geographic correlation.

All Eastern and Southeastern Anatolia Bovine Rabies isolates, Iran, Jordan, Israel, Saudi Arabia, and Lebanon were closely related (Figure 6). Eastern and Southeastern Anatolia Bovine rabies virus variants had 98.8% homology with the Israel-Golan Heights-Human-1996-DQ837383.

The Eastern and Southeastern Anatolia Bovine Rabies isolates were found more closely related to the Middle Eastern isolates than to European isolates.
FIGURE 6
Phylogenetic analysis based on the partial N gene nucleotide sequences of the isolates and other sequences obtained from the GenBank database.
DISCUSSION

Molecular epidemiology is a significant component for the classification of animal virus diseases, including rabies virus, and supply a better understanding of epidemiological relationships [1, 4]. Within the genus Lyssavirus there are different types of virus in terms of antigenic and genetic structure. Rabies virus (RABV) is the main cause of classical rabies in these types. The RABV, which exists worldwide is the potential vector carnivorous [17]. The N gene sequences tree RABVs provided from GenBank were checked with those of the Turkish isolates to analyze molecular epidemiic relationships. The nucleotide sequence analysis demonstrated that all of the Turkish isolates were classified into genotype I of Lyssavirus [18]. It has been determined that the isolates obtained from foxes and cows for which RABV is not specific to the host strain are general.

When analyzed in Eastern and Southeastern Anatolia region in Turkey between the years 2000-2015 it was noted that the total of rabies cases had increased in the 2011-2015 period as the temporal (Figure 1). In the period of temporary increase, the largest number of dogs 41.73% (328) followed by 35.49% (279) bovines were found. According to these data in the regions, rabies is predominant in domesticated dogs. The world's primary reservoir for rabies is the carnivore mammals [19]. The strong positive correlation in the increase of cases of bovine rabies and dog rabies cases during this period indicates that the rabies reservoir of the region is the domestic dogs (Figure 2-3). Domestic dogs are the main reservoir of rabies in Turkey [20]. Although there were 79 cases of bovine rabies in the region between 2000 and 2011, 200 bovines rabies cases were observed in the period 2012-2015 (Figure 1). Bovines in the region are allowed to move freely, especially in the summer and spring. Thus, rabies becomes accessible to animals. This is the reason for the high incidence in bovines [6].

Rabies stays endemic in many regions of Turkey. India is the country where rabies is concentrated. The disease prevalence was 48% in dogs and 61% bovines in India [21]. The prevalence of rabies in 41.73% dogs in bovines 35.49% (279) bovines were found. According to these data in the regions, rabies is predominant in domesticated dogs. The world's primary reservoir for rabies is the carnivore mammals [19]. The strong positive correlation in the increase of cases of bovine rabies and dog rabies cases during this period indicates that the rabies reservoir of the region is the domestic dogs (Figure 2-3). Domestic dogs are the main reservoir of rabies in Turkey [20]. Although there were 79 cases of bovine rabies in the region between 2000 and 2011, 200 bovines rabies cases were observed in the period 2012-2015 (Figure 1). Bovines in the region are allowed to move freely, especially in the summer and spring. Thus, rabies becomes accessible to animals. This is the reason for the high incidence in bovines [6].

Rabies stays endemic in many regions of Turkey. India is the country where rabies is concentrated. The disease prevalence was 48% in dogs and 61% bovines in India [21]. The prevalence of rabies in 41.73% dogs in bovines 35.49% Eastern and Southeastern Anatolia in Turkey. These rates are below from the India. Apparent countries of the Middle East zone are facing promotion problems due to wildlife rabies, including Saudi Arabia, Oman, Yemen, Israel and Iran [22]. Eastern and Southeastern Anatolia have significant ground boundary with countries that are known to port great endemic populations of rabies. According to World Health Organization records, there is no mention of the presence of domestic rabies virus in human and animals that absolutely virus comes from somewhere else to province [23]. The animal movements in the populations in Middle East zone ensure that the virus transits, especially during wartime [24]. Phylogenetic analysis based on the partial N gene nucleotide sequences of the isolates shows the closeness to the isolates of the countries of the Middle East, important in the filiation of the disease (Figure 6). Comparison with a panel of rabies viruses defined a strong geographical connection with endemic viruses in the different regions of Turkey. Molecular epidemiology suggests that the rabies virus isolates identified within Eastern and Southeastern Anatolia are more connected to isolates from the Middle East [10, 20, 24, 25]. This comparison supports anterior investigations.

Eastern and Southeastern Anatolia rabies RABV isolates had homology with host species. Because Phylogenetic analysis based on the partial N gene nucleotide sequences of the isolates shows the closeness to the isolates Fox and Bovines. Rabies virus is not host specific. The virus can infect different animal species. Point mutations were detected in nucleotide sequence analysis of the virus isolates from Eastern and Southeastern Anatolia. Point mutations of the viral genome are rare, by reason of nucleotide differences it is not very effective in the amino acid sequence (Figure 4, B) [10]. Therefore, immunization of domestic cattle with canine inactivated rabies vaccine provides protection against rabies for at least one year is recommended [26].

CONCLUSION

The elimination of canine rabies using 'One Health' strategy is required to generate continuous immunity among dog population, by applying standard immunization approaches at the rural level, along with human population management and community education [27].

REFERENCES


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OPTIMIZATION OF THE INTEGRATION OF Fe/C MICRO-ELECTROLYSIS AND FENTON FOR THE TREATMENT OF COAL CHEMICAL INDUSTRY WASTEWATER BY RESPONSE SURFACE METHODOLOGY

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1Hunan Provincial Key Laboratory of Shale Gas Resource Utilization, School of Civil Engineering, Hunan University of Science and Technology, Xiangtan, 411201, China
2School of Science & Sport, University of the West of Scotland, Paisley PA1 2BE, UK

ABSTRACT

Efficiency of the integration process of Fe/C micro-electrolysis and Fenton for the treatment of coal chemical industry wastewater was studied and the operational parameters were optimized. Response surface methodology was employed to investigate the effects of the main factors (pH, Fe/C ratio and H2O2 dosage) on treatment efficiency and optimize the parameters. The interaction of Fe/C ratio and H2O2 dosage exerted significant effect and the interaction of pH and Fe/C ratio showed moderate effect on COD removal. While the interaction of pH and H2O2 dosage had little effect. The optimal parameters were determined as pH 3.88, 1.04 of Fe/C ratio and 60.25 mg/L of H2O2 dosage, with the predicted optimal COD removal efficiency of 87.27%. The up-flow reactor exhibited high treatment performance and stabilization. The current work could provide scientific and theoretical bases for the treatment of coal chemical industry wastewater and the integration of Fe/C micro-electrolysis and Fenton.

KEYWORDS: Fe/C micro-electrolysis, Fenton, response surface methodology, coal chemical industry wastewater

INTRODUCTION

The coal chemical industry wastewater was discharged from the process of coal gas purification. The wastewater is a typical refractory industrial wastewater with high concentration of contaminants and the composition is complex [1, 2]. Biological process is the generally method adopt for coal chemical industry wastewater treatment on account of its mild treatment condition and lower cost. The performances of traditional biological processes (such as Anaerobic-Anoxic-Oxic process and its modification and SBR et al) in treating coal chemical industry wastewater were unsatisfied due to presence of highly residual toxicity and poor biodegradability [3, 4].

Micro-electrolysis exhibited some advantages in the application to industrial wastewater treatment because of the low cost and maneuverability, which employs nascent Fe2+ and [H] generated in the electrochemical processes to react with pollutants. The nascent Fe2+ and [H] destroy the structure of chromophore and decompose some macromolecular organic matters into small molecular compounds [5, 6], so as to decrease chroma and the concentration of pollutants as well as improve the biodegradability of treated wastewater [7]. The core of Fe/C micro-electrolysis is the formed galvanic cell between Fe anode and C cathode based on the potential difference (Eq. (1) and (2)) [8, 9]. In the galvanic cell, the electrons come from the corrosion of iron, which produces Fe2+ and releases it into the solution. The Fe ions produced by Fe/C micro-electrolysis provide the feasibility for the integration or coupling of Fe/C micro-electrolysis with Fenton process, achieving the synergistic effect of improving the treatment efficiency and Fe ion utilization ratio. In the Fenton system, H2O2 is catalytically decomposed by Fe2+ into •OH which has strong oxidability in terms of degrading or mineralizing the pollutants non-selectively in wastewater [10]. The combination of Fe/C micro-electrolysis and Fenton reaction is one of the development directions for both Fe/C micro-electrolysis and Fenton technology [11-13].

Anode (Fe): \( Fe - 2e^- \rightarrow Fe^{2+} \) (1)
Cathode (C): \( 2H^+ + 2e^- \rightarrow 2[H] \rightarrow H_2 \) (2)

The primary parameters affecting Fe/C micro-electrolysis and Fenton include Fe/C ratio, H2O2 dosage, aeration intensity, temperature and pH etc. Interaction effects exist among these factors. Single factor analysis is incapable to reflect the interaction effect among factors on treatment efficiency and obtain scientific and reasonable optimization. Response surface method (RSM) is a powerful data analysis method based on statistics. The influence degree and significance of both the independent and interaction effects of multivariable on response
value can be evaluated simultaneously with fewer experiments [14]. Variable optimization and response prediction can be achieved based on the impacting analysis [15, 16].

In the current study, RSM coupling with central composite design (CCD) was carried out to investigate the effects of main parameters (Fe/C ratio, H2O2 dosage and pH) on the performance of the integration process of Fe/C micro-electrolysis and Fenton in treating coal chemical industry wastewater, and the parameters were optimized. On this basis, an upflow reactor combining Fe/C micro-electrolysis with Fenton process was designed to estimate the long-term treatment efficiency and stability in the treatment of coal chemical industry wastewater.

MATERIALS AND METHODS

Materials. In the experiments, the coal chemical industry wastewater samples were taken from the outlet of anaerobic treatment process in a real coal chemical industry plant in China. The main quality of the wastewater sample were as following: pH 7.2±0.5, 1332.7±10.3 mg/L of COD, 386.8±3.8 mg/L of BOD5 and 400±20 of chroma. The activated carbon was fabricated by coconut shell and the average diameter was 3.0 mm. It was washed by distilled water assisted with ultrasonic, and subsequently dried in the oven for reserve, prior to use. In order to shield the effect of adsorption on COD removal, the activated carbon was soaked in untreated wastewater for 96 hours to reach the state of adsorption saturation before the experiment. Iron scraps were obtained from a metalworking factory. The samples with an average diameter of 2.0 mm were gained via screening. Iron scraps were soaked and cleaned by NaOH solution (1.0 mol/L) at 50°C, followed by 10% diluted H2SO4 solution to be edulcoration. The pretreated samples were then rinsed with deionized water and dried before use.

Experimental procedures. The parameter optimization experiments were conducted in sequence batch way, which were designed according to the CCD. The activated carbon and iron scraps at a preset Fe/C ratio were blended uniformly and added to the flask reactor (200 mL). Then the real coal chemical industry wastewater (150 mL) after the adjustment of pH with NaOH and H2SO4 solution were poured into the reactor. A certain amount of H2O2 was added into the reactor after 90 min micro-electrolysis reaction. Samples were withdrawn the next 90 min Fenton reaction. Then the pH of the sample was adjusted to 8.5 immediately to quench the reaction and precipitate the ferric ion. Then the samples were centrifuged and the supernatant was filtrated with 0.45 μm filter paper. Finally, the samples were stored at 4°C for further analysis. All the experiments were performed in triplicate, and the results were the average of at least three measurements with an accuracy of ±5%. Schematic diagram of the upflow reactor for the integration of Fe/C micro-electrolysis and Fenton was illustrated in Fig. 1, with the diameter of 3.0 cm and height of 50 cm. The heights of Fe/C micro-electrolysis packing layer and Fenton reaction area were 34.5 and 15.5 cm respectively. The porosity of packing layer was 45.2%. Samples were taken from the effluent at predetermined time and analyzed to evaluate the treatment performance of the continuous operation.

FIGURE 1
Schematic diagram of the up-flow reactor for the integration of Fe/C micro-electrolysis and Fenton

Analytical methods. COD was measured according the standard method. pH was tested with HQ30d multifunction measuring instrument. Total Fe ion concentration was determined with ICP-AAS, of which Fe2+ concentration was obtained from adjacent phenanthroline spectrophotometric method. The RSM analysis and CCD were conducted on software Design Expert. The CCD with three factors consists of eight factorial points, six axial points, and six central points, constituting 20 trials (Table 1).

RESULTS AND DISCUSSION

Validation of the RSM model. The full factorial CCD and the results of each trial were listed in Table 1. COD removal efficiency was selected as the response for RSM analysis and parameter optimization. All the trials was conducted in a random sequence to minimize the systematic error.
According to the experiment results listed in Table 1, a quadratic model was developed based on RSM. The presented data yielded a RSM quadratic model at 95% confidence level (Eq. (3)).

The significance of the regressed RSM quadratic model was evaluated statistically on the basis of the analysis of variance (ANOVA) and the \( F \) test, and the analysis results were listed in Table 2.

### TABLE 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Coded type</th>
<th>( x_1 )</th>
<th>( x_2 )</th>
<th>( x_3 )</th>
<th>Response</th>
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</tr>
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<td>4</td>
<td>1</td>
<td>60</td>
<td>86.65</td>
</tr>
</tbody>
</table>

Annotate: \( x_1 \) pH, \( x_2 \) Fe/C radio, \( x_3 \) \text{H}_2\text{O}_2 \) dosage (mg/L).

### TABLE 2

**ANOVA of the developed RSM model**

<table>
<thead>
<tr>
<th>Source of variations</th>
<th>Degree of freedom</th>
<th>Sum of squares (Adj SS)</th>
<th>Mean squares (Adj MS)</th>
<th>( F ) values</th>
<th>( P )</th>
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<tbody>
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<td>Regression</td>
<td>9</td>
<td>546.003</td>
<td>60.667</td>
<td>96.881</td>
<td>1.549\times10^{-8}</td>
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<tr>
<td>Residual</td>
<td>10</td>
<td>6.262</td>
<td>0.6262</td>
<td>2.935</td>
<td>0.132</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>5</td>
<td>4.671</td>
<td>0.9342</td>
<td>2.935</td>
<td>0.132</td>
</tr>
<tr>
<td>Pure error</td>
<td>5</td>
<td>1.591</td>
<td>0.3182</td>
<td>2.935</td>
<td>0.132</td>
</tr>
</tbody>
</table>

### FIGURE 2

Residual distribution and the comparison of experimental and predicted value

\[
y = -26.376 + 33.8873x_1 + 36.1885x_2 + 0.962x_3 - 4.7077x_1^2 - 40.6189x_2^2 - 0.0133x_3^2 + 2.635x_1x_2 - 0.0031x_1x_3 + 0.6343x_2x_3 \tag{3}
\]

The \( F \) value of RSM regression model was 96.881, considerably larger than theoretical value of 4.94 calculated for 99.9% confidence level, which manifested that the significance of RSM model was at a high level. The \( F \) value calculated for the lack of fit was 2.935, less than 5.05 tabulated on behalf of 95% confidence level, indicating the influence of
lost part was dispensable and the fitting degree was high. The low $F$ value ($<0.00001$) yielded from the $F$ test of the regression model also suggested the high significance of the obtained RSM quadratic model. In addition, the correlation coefficient ($R^2$) of 0.9785 stood for 97.85% of the response variability was examined by this model, confirming the high precision and reliability of the model for explaining the experimental results. The comparison of the experimental data with the predicted data by the obtained RSM model as well as residuals distribution were illustrated in Fig. 2. The random distribution of residuals revealed that the errors of the model got no accumulation in the processes of experiment and data processing, which indicated that the systematic error could be discarded. There was a high consistency between the experimental data and predicted data, which exhibited the RSM quadratic model could well simulate and predict the experimental results of the integration process of Fe/C micro-electrolysis and Fenton in terms of COD removal from coal chemical industry wastewater.

Parameter optimization. According to the ANOVA, the three factors pH, Fe/C ratio and the dosage of $\text{H}_2\text{O}_2$ all exhibited prominent effects on COD removal in the treatment of coal chemical industry wastewater by the integration process. When the solution pH was low, the violent reaction occurred between Fe and $\text{H}^+$ directly and the galvanic cell weakened relatively. While the galvanic cell reactions were constrained due to the decline of potential difference at higher pH [17]. Therefore, either exceeding low or high pH counted against the Fe/C micro-electrolysis. Moreover, exceeding low or high exerted adverse effect on Fenton reactions by affecting the $\text{H}_2\text{O}_2$ decomposition and oxidability of $\cdot\text{OH}$. The number of galvanic cell was directly related to the Fe/C ratio. Fe/C ratio had directly bearing on the number of generated galvanic cell. Thus, an appropriate Fe/C ratio would promote the micro-electrolysis reaction [7, 18]. An appropriate Fe/C ratio also facilitated to generation suitable amount of $\text{Fe}^{2+}$ in micro-electrolysis, which contributed to the process of Fenton to be served as the catalyst and improving the treatment efficiency. $\text{H}_2\text{O}_2$ is the other crucial reagent in Fenton reaction. The COD removal efficiency will turn into a lower level, not only on account of a low yield of $\cdot\text{OH}$ decomposed from a handful $\text{H}_2\text{O}_2$ when $\text{H}_2\text{O}_2$ dosage was low, but also the exorbitant $\text{H}_2\text{O}_2$ is able to capture the $\cdot\text{OH}$ when exceeding $\text{H}_2\text{O}_2$ was added, so as to block the Fenton process (Eq. (4) and (5)) [19]. Thus the low utilization of $\cdot\text{OH}$ and $\text{H}_2\text{O}_2$ mainly accounted for the lower removal efficiency of pollutants.

$$\text{H}_2\text{O}_2 + \cdot\text{OH} \rightarrow \text{HO}_2^- + \text{H}_2\text{O} \quad (4)$$

$$\text{HO}_2^- + \cdot\text{OH} \rightarrow \text{O}_2 + \text{H}_2\text{O} \quad (5)$$

FIGURE 3
Comparative response surfaces generated from RSM and contour plots illustrating the interaction effect
The interaction effects among various factors on the response were illustrated via 3D response surface and 2D contour plots, as shown in Fig 3. The integration effects among factors on response can be obtained from the figures as well as the ANOVA and F test of the developed RSM regression model. The integration effect between Fe/C ratio and H₂O₂ dosage was notable significance since Fe²⁺ generated in Fe/C micro-electrolysis and H₂O₂ were the major reactants in Fenton reactions, which directly depended on Fe/C ratio and H₂O₂ dosage. A proper proportion between H₂O₂ and Fe²⁺ concentrations facilitated the Fenton process as well as improved the utilization of both H₂O₂ and Fe²⁺ ions, leading to a better treatment performance [20, 21]. In the event that Fe/C ratio values was exorbitant, a relatively higher concentration of Fe²⁺ would be subsequently produced in micro-electrolysis, then the excessive quantity of Fe²⁺ consumed •OH (Eq. (6)), which lowered the effectiveness of available •OH and impeded the treatment efficiency. On the contrary, when Fe/C ratio was low, the amount of Fe²⁺ was insufficient to catalyze the decomposition of H₂O₂ to generate adequate •OH for pollutants degradation. On the other hand, relatively superfluous H₂O₂ compared to Fe²⁺ concentration scavenged •OH, resulting in non-oxidizing reaction of •OH (Eq. (6)), which lowered the effectiveness of available •OH and impeded the treatment efficiency. Thus, in order to achieve the best removal efficiency as well as improve the utilization of H₂O₂ and Fe²⁺ simultaneously, a better proportion between Fe/C ratio and H₂O₂ dosage was required. Increasing both Fe/C ratio and H₂O₂ dosage proportionally boosted the Fenton process as well as improved the utilization of both H₂O₂ and Fe²⁺ ions, leading to a better treatment performance [20, 21]. In the event that Fe/C ratio values was exorbitant, a relatively higher concentration of Fe²⁺ would be subsequently produced in micro-electrolysis, then the excessive quantity of Fe²⁺ consumed •OH (Eq. (6)), which lowered the effectiveness of available •OH and impeded the treatment efficiency. On the contrary, when Fe/C ratio was low, the amount of Fe²⁺ was insufficient to catalyze the decomposition of H₂O₂ to generate adequate •OH for pollutants degradation. On the other hand, relatively superfluous H₂O₂ compared to Fe²⁺ concentration scavenged •OH, resulting in non-oxidizing reaction of •OH (Eq. (6)), which lowered the effectiveness of available •OH and impeded the treatment efficiency. Thus, in order to achieve the best removal efficiency as well as improve the utilization of H₂O₂ and Fe²⁺ simultaneously, a better proportion between Fe/C ratio and H₂O₂ dosage was required. Increasing both Fe/C ratio and H₂O₂ dosage proportionally boosted the Fenton process and •OH generation, leading to a promotion of pollutants removals accordingly. The integration effect between pH and Fe/C ratio exhibited moderate significance on COD removal. H⁺ and Fe were the major participants in micro-electrolysis reactions and either excessive H⁺ or Fe was pernicious to the process of micro-electrolysis, thereby reduced the production of nascent Fe²⁺ and [H] and attenuated the efficiency of microelectronics treatment [22]. In comparison, the integration effect between pH and H₂O₂ dosage was not significance mainly owing to the feeble interaction between them in Fenton reactions. pH variation only slightly impacted the stability and decomposition of H₂O₂ when pH was in an appropriate range.

\[ \text{Fe}^{2+} + \cdot \text{OH} \rightarrow \text{Fe}^{3+} + \text{OH}^- \]  

(6)

In the light of the RSM regression model and the response surface, the optimal value of each factor and the predicted value of the response can be obtained by using the response optimizer. The trial at optimal condition obtained by RSM was conducted to be as a validation of the predicted response. The optimal parameters as well as predicted and experimental COD removal efficiency were shown in Table 3.

**Long term performance of up-flow reactor.** Under the conditions of optimal parameters predicted by RSM, treatment efficiency and stability of the integration process of Fe/C micro-electrolysis and Fenton during long term operation were investigated in up-flow reactor. It was observed that COD removal efficiency in the up-flow reactor of the integration of Fe/C micro-electrolysis and Fenton was stable at a high level (average 86.5%e) within 30 days operation. COD removal efficiency decreased only about 4% at the late period, indicating the higher long-term treatment efficiency and running stability. In addition, no evident phenomenon of packing consolidation was found during the treatment process, and only a small amount of metal oxide crystals were attached to the surface of C cathode, which demonstrated that there was no pollutant deposition and sediment covering on the electrodes surface under aerating scouring and shearing cleaning. The iron oxide crystals clung to C cathode was able to form heterogeneous Fenton, which also played an important role in the regeneration of C cathodes [23]. In addition, after the treatment by the integration of Fe/C micro-electrolysis and Fenton, the pollutants in the wastewater were degraded mainly caused in the redox process, then the content of COD was cut down significantly and the biodegradability of the effluent improved obviously with BOD₅/COD increasing from 0.29 to 0.36, which was beneficial to the further treatment in the subsequent processes, especially biological processes.

**CONCLUSIONS**

Response surface method was employed to investigate operational factors and optimize parameters in the treatment of coal chemical industry wastewater by the integration of Fe/C micro-electrolysis and Fenton. A quadratic model with high significance was established by RSM coupled CCD, which could well simulate and predict the COD removal efficiency of coal chemical industry wastewater treated by the integration of Fe/C micro-electrolysis and Fenton. pH, Fe/C ratio and H₂O₂ dosage all exerted remarkable influences on COD removal. The interaction effect between Fe/C ratio and H₂O₂ dosage was notable significance on response. That between pH and Fe/C ratio exhibited impact on

**TABLE 3**

<table>
<thead>
<tr>
<th>pH</th>
<th>Fe/C ratio</th>
<th>H₂O₂ dosage (mg/L)</th>
<th>Predicted COD removal efficiency (%)</th>
<th>Experimental COD removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.88</td>
<td>1.04</td>
<td>60.25</td>
<td>87.27</td>
<td>87.19</td>
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</tbody>
</table>

![Image of the table](image-url)
COD removal at generic level, while inapparent effect was shown between pH and H2O2 dosage. The optimal parameters were determined from the developed RSM quadratic model. The integration process of Fe/C micro-electrolysis and Fenton in an up-flow reactor exhibited high long-term treatment efficiency and operational stability, with COD removal efficiency of around 87%.

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FACILE AND FABRICATION OF SEMICONDUCTOR SILVER TUNGSTATE LOADED ON THE GRAPHENE OXIDE SURFACE AND ITS EFFECTIVE ENHANCED CATALYTIC PERFORMANCE

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ABSTRACT

In recent years, coupling graphene or reduced graphene oxide (RGO) with Ag-based photocatalytic semiconductor materials have received considerable attention owing to their excellent chemical stability, unique electronic structures and their physicochemical properties. In current work, a hierarchical Ag2WO4-reduced graphene oxide (AgGO) visible-light-driven photocatalyst was successfully prepared through a facile and effective hydrothermal method. The as-prepared AgGO semiconductor photocatalysts were characterized by X-ray diffraction pattern (XRD), fourier transform infrared spectra (FT-IR), raman spectroscopy, field emission scanning electronmicroscopy (SEM) and ultraviolet-visible (UV-vis) diffuse reflectance spectroscopy (DRS). It is found that the nano-sized Ag2WO4 particles are deposited on the surfaces of RGO. The AgGO exhibited superior photocatalytic activity and stability to bare Ag2WO4 under visible light. The photocatalytic degradations of methyleneblue (MB) over AgGO photocatalyst were investigated. The significantly enhanced photocatalytic activity and stability of AgGO photocatalyst could be attributed to the excellent electron-accepting and transporting properties of RGO. The possible mechanism of photocatalytic activity enhancement was proposed.

KEYWORDS:
Visible-light photocatalyst, graphene oxide, degradations

INTRODUCTION

In the past decades, semiconductor photocatalysis has attracted much interest because of its potential applications in the fields of environment (degradation of organic pollutants in wastewater) and energy such as such as water splitting for hydrogen production and CO2 photoreduction [1, 2]. Among these photocatalysts, silver-based visible-light-driven photocatalytic semiconductor materials have received considerable attention owing to their excellent chemical stability, physicochemical properties and potential applications. These compounds such as silver halide (X=Cl, Br, I), silver carbonate [3], silver chromate [4] and silver tungstate [5], exhibit excellent photodegradation of organic pollutants under visible light irradiation compared to the traditional photocatalysts. Recently, Ag2WO4 is considered as a promising candidate due to its unique electronic structure and crystal structure [6]; and it is also a semiconductor with a wide bandgap in the range of 2.9 eV to 3.1 eV [7]. Its valence band (VB) is below the O2/H2O potential level (1.23 eV vs. SHE, pH=0) and conduction band (CB) above the H+/H2 potential level (0 V vs. SHE, pH=0), which inducing its high oxidation ability. However, it makes use of visible light because of its wide band gap. Moreover, the low separation rate of the photoexcited electron-hole in Ag2WO4 leads to the limited quantum efficiency. Thus, further study is necessary to enhance its performance for the practical application.

Generally, to improve the photocatalytic activity and stability of silver compounds, it is necessary to rapidly transfer the photogenerated electrons from the surface of silver compounds to an acceptor material before Ag+ is reduced to metallic Ag [8]. To address this problem, different methods, such as ions doping, nanocomposites construction, noble-metal deposition, and heterostructure assembly, have been studied [9]. Among these reported methods, fabricating graphene-based nanocomposite has been regarded as an effective approach to promote the efficiency of photocatalysis. It is believed that graphene or reduced graphene oxide (RGO) is considered to be one of the most promising layer materials for the construction of functional composite materials [10]; it has also received considerable attention recently for photoelectron-chemical and photocatalytic applications due to its extremely high specific surface area, high thermal, chemical stability and fast electron transfer ability, which can effectively inhibit the recombination of the electron-hole pairs [11]. Thence, RGO can be as one of the most ideal
supports for the separation and transfer of the photogenerated charge carriers [12]. In this work, a hierarchical AgGO visible-light-driven photocatalyst was prepared through a facile and effective hydrothermal method. The photocatalytic activity of AgGO was evaluated by the degradation of methylene blue (MB). The results show that the as-prepared hierarchical AgGO photocatalyst exhibit excellent photocatalytic activity under visible light irradiation. A possible mechanism for the transfer of photogenerated carriers was also proposed.

**MATERIALS AND METHODS**

**Materials.** Potassium permanganate (KMnO4), concentrated sulfuric acid (H2SO4), silver nitrate (AgNO3), sodium tungstate (Na2WO4), sodium nitrate (NaNO3), hydrogen peroxide (H2O2), hydrochloric acid (HCl) were purchased from Aladdin Chemical Reagent Co., Ltd. Methylene blue (C16H18ClN3S, Mw=319.86 g mol⁻¹, MB) was purchased from Shanghai Chemical Reagent Co. Ltd.

**Preparation of hierarchical AgGO photocatalyst.** Graphene oxide (GO) nanosheets were prepared according to the modification of Hummers’ method from natural graphite [13]. GO (60 mg) was added to ultrapure water (60 mL), and then was sonicated for 3 h to obtain a GO aqueous suspension. A certain amount of GO aqueous suspension was added to ultrapure water (60 mL), and then was sonicated for 3 h to obtain a GO aqueous suspension. A certain amount of GO aqueous suspension was added to AgNO3 (0.06 mol·L⁻¹, 50 mL) and stirred for 2 h to ensure the adsorption of positively charged Ag⁺ onto the surface of negatively charged GO via electrostatic interaction. Then Na2WO4 solution (0.03 mol·L⁻¹, 50 mL) was dripped into the mixture and kept stirring for 1 h. The suspension was transferred to a 100 mL Teflon-lined stainless steel autoclave. The autoclave was heated to 160 °C and mainstirred for 1 h. The suspension was then separated by centrifugation followed by washing with ethanol and deionized water for several times, respectively. The product (AgGO) was obtained on the Shimadzu LabX-6000. X-ray Diffractometer (40 kV, 30 mA, λ=0.154 nm) with a Cu Kα radiation source at a scanning rate of 4°/min within the range of 5-80°. The photorefractive measurements were made with an electrophysical analyzer (CHI660B, CHI Shanghai, Inc.). The Brunauer-Emmett-Teller (BET) specific surface areas (S BET) of the samples were carried out on the basis of nitrogen adsorption and desorption isotherms (Micromeritics ASAP 2030 nitrogen adsorption apparatus, USA).

**Photocatalytic activity measurement.** The photocatalytic activity of AgGO photocatalyst was evaluated by the degradation of dye (MB) aqueous solution under visible-light irradiation. 50 mg photocatalyst was added to 100 mL 1.0×10⁻⁵ mol·L⁻¹ dye solution under magnetic stirring. The light source was a 250W metal halide lamp equipped with wavelength cut off filters for λ=420 nm. The photocatalytic activity of the prepared samples was performed at ambient temperature [14]. The suspensions were stirred in the dark for 30 min to reach adsorption-desorption equilibrium. After visible light irradiation, the powder was then separated by centrifugation, and the concentration of dye was determined by measuring the absorbance (465 nm) using an UV-2450 spectrophotometer. The removal efficiency of dye was calculated and photocatalytic process was modeled using pseudo first-order kinetics equation according to the following Equations (1) and (2):

\[
D(\%) = \frac{C_0 - C}{C_0} \times 100\% \tag{1} \\
\ln(C_0/C) = kt \tag{2}
\]

where \(D\) is the removal efficiency; \(C_0\) (mg·L⁻¹) is the initial concentration of original dye solution; \(C\) (mg·L⁻¹) is the dye concentration after light irradiation at time \(t\) (min). \(k\) is the apparent rate constant.

**RESULTS AND DISCUSSION**

**Phase and morphology characterization.** XRD patterns of as-prepared Ag2WO4 and AgGO photocatalyst with various RGO contents are shown in Figure 1. From the pattern of Ag2WO4, the diffraction peaks at 2θ= 18.72°, 27.86°, 30.17°, 31.41°, 32.82°, 37.86°, 45.11°, 54.62°, 57.73°, 63.02°, 65.83°, 72.83° and 74.71°, can be assigned to (011), (011), (002), (231), (400), (241), (402), (233), (352), (004), (214), (404) and (633) crystal planes of the
cubic phase of Ag$_2$WO$_4$ (JCPDS NO. 34-0061), which is in agreement with previous reports [15]. In addition, the AgGO photocatalysts exhibit XRD patterns similar to that of pure Ag$_2$WO$_4$ sample. The result indicates that the existence of RGO does not affect the growth of new crystal orientations of Ag$_2$WO$_4$, thus RGO only functions as a platform where the Ag$_2$WO$_4$ microsphere can nucleate and grow as well.

SEM images of RGO (a) and Ag$_2$WO$_4$ (b), FSEM image of AgGO-3 photocatalyst (c) are shown in Figure 2. As can be seen from the Figure 2 (a and b), the RGO nanosheets are curled and corrugated, which is consistent with the previous report [16], while the pristine Ag$_2$WO$_4$ exhibits regular particle morphology with an average size of 150 nm in diameter and 20 μm in length, and the surface is relatively smooth without any secondary nanostructures, indicating a high uniformity of the rod-like morphology. As can be seen from FSEM image of the AgGO-3 photocatalyst (Figure 2c), it seems that large amounts of Ag$_2$WO$_4$ particles are distributed on the surface of RGO sheets, indicating a good loading between Ag$_2$WO$_4$ and RGO. SEM images illustrate the transparent properties of the RGO substrate, where the RGO retains its two-dimensional thin sheet structure with wrinkled regions after loading of the Ag$_2$WO$_4$. This structure might provide enough contact surface area between the RGO sheets and Ag$_2$WO$_4$ particles and also presumably facilitate charge-carrier transport.

EDS spectrum (Figure 2d) shows that all the points in the selected area display the existence of Ag, W, O and C elements. The 2D-projected elemental mapping (Figure 2e) demonstrates the distribution of these elements throughout the AgGO-3 sample.

**Raman spectra and FT-IR characterization.** Raman spectroscopy is a powerful non-destructive tool to characterize the crystalline quality of carbon and can be used to prove the existence of RGO in the AgGO photocatalyst. Raman spectra of RGO (a), pure Ag$_2$WO$_4$ (b) and AgGO-3 photocatalyst (c) are shown in Figure 3. As shown in Figure 3 (a and c), two observably peaks at about 1330 and 1593 cm$^{-1}$ are defined as D band and G bands of RGO in the spectra of pure RGO and photocatalyst [17], corresponding to the E$_{2g}$ phonon of sp$^2$ bonded carbon atoms in a two-dimensional hexagonal lattice, as well as the defects and disorder carbon in the graphite layers, respectively [18]. The sharp peak located at about 881 cm$^{-1}$ are observed in spectrum of pure Ag$_2$WO$_4$ and AgGO photocatalyst (Figure 3 b and c), which is attributed to the WO$_4^{2-}$ symmetric stretching vibration. The obtained Raman spectrum of AgGO photocatalyst shows both the characteristic Raman peaks of RGO and Ag$_2$WO$_4$, implying that the Ag$_2$WO$_4$ particles are successfully anchored onto the RGO support.
FT-IR spectra of GO (a), RGO (b), Ag2WO4 (c) and AgGO photocatalyst (d) are shown in Figure 4. It is clear that the GO (Figure 4 a) shows many strong absorption peaks that correspond to various oxygen functional groups, such as, the OH stretching vibration at 3370 cm\(^{-1}\), the C=O stretching vibration at 1732 cm\(^{-1}\), the stretching vibration of \(sp^2\) hybridized C=C bond at 1616 cm\(^{-1}\). After hydrothermal treatment of the GO, the intensity of all absorption peaks corresponding to oxygen functional groups (C=O, O-H, C-O-C, C-O-H, and C-O) have significant decrease (Figure 4 b), which further demonstrates the effective reduction of GO to RGO by the hydrothermal method, in good agreement with the XRD results (Figure 1). For bare Ag2WO4 (Figure 4 c), the peak at around 825 cm\(^{-1}\) has an absorption maximum, which contains octahedral WO\(_6\) units. In the case of the AgGO photocatalyst (Figure 4 d), the characteristic bands for Ag2WO4 still remain, but two new absorption peaks emerge at 3373 and 1568 cm\(^{-1}\), which are attributed to the stretching vibration of O-H and the skeletal vibration of the graphene sheets. Therefore, the above results further confirmed the reduction of GO and the successful preparation of AgGO photocatalyst. This result suggests the integration of Ag2WO4 and RGO, which is consistent with the Raman spectra. Moreover, the interaction between Ag2WO4 and RGO may benefit the photogenerated electron transfer and then enhance the photocatalytic activity of composites.
Photoluminescence, Transient photocurrent response and UV-vis diffuse reflectance characterization. PL spectra are useful to disclose the migration, transfer, and recombination processes of the photogenerated electron-hole pairs in semiconductors. In general, the lower PL intensity indicates a decrease in the recombination rate of photogenerated charge carriers. The better separation of photogenerated electrons and holes in the AgGO photocatalyst is confirmed by PL emission spectra of single Ag$_2$WO$_4$ and AgGO photocatalyst; and PL emission spectra of bare Ag$_2$WO$_4$ (a) and the AgGO photocatalyst (b) recorded with an excitation wavelength at 363 nm are shown in Figure 5. As can been from Figure 5, the strong emission peak of the bare Ag$_2$WO$_4$ is located at about 496 nm, which could be attributed to a band-to-band emission. Compared with single Ag$_2$WO$_4$, the emission peak intensity of the AgGO photocatalyst decreases moderately, indicating that the recombination of photogenerated charge carriers is suppressed. The result of PL verifies that the AgGO photocatalyst could effectively separate photogenerated electron-hole pairs.
Due to the RGO has excellent electronic conductivity and the high charge carrier, once Ag$_2$WO$_4$ was AgGO photocatalyst with RGO further, AgGO showed the lowest fluorescence emission intensity corresponding to the lowest recombination rate of electron-hole pairs. Photocurrent measurements were also carried out to evaluate the capacity of photogenerated charge separation. To our knowledge, higher photocurrent value means more efficient separation of photogenerated electron-hole pairs. In order to supply evidence to support that the addition of RGO played an important role in the photocatalytic activity of Ag$_2$WO$_4$, transient photocurrent responses for the samples were measured and the results are shown in Figure 6. It can be seen that the AgGO displayed a much enhanced photocurrent density than Ag$_2$WO$_4$. In addition, the transient photocurrent of the AgGO photocatalysts decreased when the RGO content was increased beyond the optimum value. The reason for this phenomenon is that excessive RGO can act as a center for the recombination of electron-hole pairs instead of providing an electron pathway, and it may absorb some visible light, cause a light harvesting competition between Ag$_2$WO$_4$ and RGO. The PL and transient photocurrent response experiment demonstrate that the photocatalytic activity and stability of Ag$_2$WO$_4$ can be improved in a large degree via coupling Ag$_2$WO$_4$ with RGO sheets decorated with Ag nanocrystals. This should be mainly attributed to the fast separation and transfer of photogenerated charge in the heterostructure.
The optical absorption properties of the as-prepared samples are examined by UV-vis diffuse reflectance spectra since the optical absorption behavior of a photocatalyst was essential for its photocatalytic activity, and the results are shown in Figure 7. The pure Ag₂WO₄ showed its sharp fundamental absorption edge around 400 nm, corresponding to the band gap of ~3.07 eV. All the composite samples display similar absorption edge with an intense transition from the visible to UV region. It should be noted that introduction of RGO leads to an obvious enhancement of background absorption in the range of 500-800 nm, and the band-gap energy of AgGO-3 is approximate 2.34 eV. The band gap narrowing might be ascribed to weak interaction between Ag₃WO₄ and RGO, which is in line with the results of the Raman spectra. Moreover, with increasing the RGO content, the composite samples present more intensive absorption over the whole visible-light region, which is in good agreement with the color change of the samples, which turns to darker and darker with increasing the RGO content. Therefore, the formation rate of electron-hole pairs on the photocatalyst surface/interfaces also increases substantially, resulting in the better catalytic performance.

**BET Characterization.** To further obtain the pore size distributions and specific surface area of the as synthesized samples, N₂ adsorption-desorption measurements were performed. Figure 8 shows the N₂ adsorption-desorption isotherms curves for the pure Ag₂WO₄ and AgGO-3 photocatalyst. As can be seen Figure 8, both of them exhibited typical type IV isotherm curves caused by the weak adsorption-adsorbent interaction, indicating the presence of mesopores. Moreover, the nonlimiting adsorption at high P/P₀ is a characteristic of the type H₃ loop, suggesting the formation of slit like pores due to the aggregation of the plate-like particles. The measured BET specific surface area of the AgGO-3 photocatalyst is about 8.12 m² g⁻¹, which shows the relatively high surface area compared to pure Ag₂WO₄ (1.36 m² g⁻¹). The BET specific surface area of AgGO-1, AgGO-2 and AgGO-4 photocatalyst is 2.22, 3.98 and 12.28 m² g⁻¹, respectively. Meanwhile, the corresponding pore volume distributions of both samples were calculated by the BJH method from the adsorption branch of the isotherms. As shown in the inset of Figure 8, both of products contain large mesopores with an average pore size of 17.22 nm and 15.18 nm, respectively.

**FIGURE 7**
(a) UV-vis diffuse reflectance spectra of pure Ag₂WO₄, AgGO;
(b) plots of (hv)² vs. photon energy (hv) of Ag₂WO₄ and AgGO-3

**FIGURE 8**
N₂ adsorption-desorption isotherms (a) and pore size distribution curves (b) of pure Ag₂WO₄ and AgGO-3
The photocatalytic oxidation activity of as-synthesized uniform AgGO-3 photocatalyst has been evaluated using MB as the model pollutant. Figure 9 shows the photocatalytic activity of pure Ag2WO4 and AgGO-3 photocatalyst with different RGO contents. For comparison, the blank test was also conducted under the same reaction conditions. It could be seen that negligible photodegradation of MB was observed when no catalyst is added. About 26% of the MB was respectively removed by pure Ag2WO4 after 180 min of visible light irradiation. Absolutely, it can be seen that the photocatalytic activity of all AgGO photocatalyst is higher than that of the pure Ag2WO4 and about 98% MB was removed by AgGO-3 in 180 min. However, with further enhancement of the RGO amount, the photocatalyst did not possess higher photocatalytic performance. It may be because of the formation of recombination centers of photoinduced carriers in the bulk of the catalyst [5] and shielding effect, which was that the further increase of RGO would wrap the Ag2WO4, and then reduce the contact surface of Ag2WO4 particles. Moreover, a higher mass ratio of RGO in the composites is considered to increase the absorbing and scattering of photons by RGO in the photoreaction system, leading to a decrease in the absorption efficiency of light for Ag2WO4 particles.

The linear relationship between ln(C0/C) and t of the as-prepared AgGO is shown in Figure 9(b), which confirming that the photodegradation reaction of MB dye is fitted pseudo-first-order. According to Eq. (2) and Figure 9(b), the pseudo-first-order rate constant of photodegradation are 0.00189 min⁻¹, 0.00343 min⁻¹, 0.00706 min⁻¹, 0.0246 min⁻¹ and 0.00912 min⁻¹ for pure Ag2WO4, AgGO-1, AgGO-2, AgGO-3 and AgGO-4, respectively. Clearly, the AgGO-3 reveals the highest value with k of 0.0246 min⁻¹, which is found to be about 13 times higher than that of Ag2WO4. The stability of a photocatalyst is important for its assessment and application. Recycling experiments were performed to check the stability of photocatalyst. Comparison of photocatalytic performance within 5 cycles for Ag2WO4 and AgGO photocatalyst under the same experimental condition is shown in Figure 10. As can be seen from Figure 10, the degradation of photocatalyst has no observable change even after 5 recycles. The results indicated that the prepared photocatalyst has an excellent reuse performance.
The possible mechanism. On the basis of the results described above, a proposed mechanism (Figure 11) is discussed to explain enhancement of the photocatalytic activity of the AgGO composite. Under the illumination of artificial solar light irradiation, Ag2WO4 are photoexcited to generate electron-hole pairs. The photogenerated holes (h+) and electrons (e−) transfer to the surface of Ag2WO4 and then drive photocatalytic oxidation reactions. O2•− radicals are produced by the reduction of O2 molecules adsorbed on the catalyst surface by the photogenerated electrons. In addition, the reaction of H2O and active holes generate •OH, and both O2•− and •OH radicals can degrade organic dyes effectively. Due to the introduction of RGO and the close interfacial contact, the photogenerated electrons on the conduction band (CB) of Ag2WO4 could be effectively transferred to the RGO sheets. Thus, the recombination of photoinduced electrons and holes can be sufficiently inhibited, resulting in the improved photocatalytic activity. Efficient electron transfer from Ag2WO4 to RGO sheets also sustains the stability of the AgGO photocatalyst by keeping electrons away from Ag2WO4 and inhibiting photocorrosion. Furthermore, high surface area GR sheets offer more active adsorption sites and photocatalytic reaction sites, which also favor improved photocatalytic activity. According to these results, one important conclusion can be drawn, that is, when discussing the effect of introduction graphene or RGO on the photocatalytic activities of semiconductor particles, the results should be analyzed on the basis of specific reactions.

CONCLUSION

A series of AgGO photocatalysts were successfully and directly produced via a hydrothermal method. The AgGO photocatalyst exhibited superior photocatalytic activity and stability to bare Ag2WO4 under visible light. It is suggested the photogenerated electrons on Ag2WO4 can be quickly transferred to RGO sheets while RGO mainly act as electron acceptors and transfer channels in the heterostructure. In addition, the RGOs can be used as protective coatings that partially inhibit the photocorrosion of Ag2WO4. Among the prepared AgGO photocatalysts, the AgGO-3 shows the best photocatalytic activity despite degradation of MB. The AgGO photocatalyst with excellent photocatalytic performance and enhanced stability could find promising applications in solving environmental protection issues. In conclusion, this work not only promotes the development of Ag2WO4 for practical applications, but also inspires the exploration of similar facile methods to stabilize other easily photocorroded photocatalysts.

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INVESTIGATION OF DROUGHT CHARACTERISTICS OF MURAT RIVER FLOWS, IN EUPHRATES RIVER VALLEY

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ABSTRACT

The scarcity of water and major water demands cause that droughts give rise to important economic, social and environmental results. For this reason, drought periods must be determined beforehand and must be controlled. In this study, stochastic models were developed in order to estimate monthly flow rates of the Karasu River. The best-fitting model was chosen as Autoregressive Movement Average (ARMA(2,1)) Model. By using this model, 100 synthetic series having the same length with the historical series were produced. The drought durations and severities were obtained by subjecting the historical and synthetic series to run analyses for median threshold level (q=0.5). By using these droughts, possible future drought and climate scenarios that might occur in the future may be predicted, precautions may be taken, risks may be foreseen and preparations may be made. In addition, to determine the distribution that best fits the drought characteristics, the Kolmogorov-Smirnov, Anderson-Darling and Chi-Square distribution fitness tests were applied. According to these tests, the majority of the drought durations fit the Gamma distribution, the majority of the drought severities fit the Generalized Extreme Value (GEV) distribution. The determination of the best fit is important in that it facilitates the hydrologic computations and analyses.

KEYWORDS:
Akaike Information Criterion (AIC), Autoregressive Movement Average Model (ARMA), Drought, Run analyses, The best distribution.

INTRODUCTION

The term drought has a relative usage in terms of common and technical use; and may be defined separately for different regions and different resources. Geography, atmosphere sciences and environmental sciences have developed different definitions for drought. Although these definitions do not overlap with each other, because they reflect the viewpoints of different disciplines; in general, they have common points like “lack of precipitation”, “lasting for a long time” and “negative effects emerging”. Based on this, drought may be defined as the lack of water that starts when the average precipitation values decrease in a certain area and lasts for months or years posing a pressure on all components of natural/humane environments that depend on water. The determination of droughts has great importance in hydrological terms. The factors that are required to define drought are the valuation of the occurrence in terms of time, and the magnitude and severity of the drought. The run analyses are one of the most effective methods to determine these factors [1].

Runs theory widely uses probabilistic methodology in drought characterization, which allows the estimation of return periods of extreme events. This was initially proposed by Yevjevich [2], and it models the recurrence of extreme periods based on one selected magnitude (e.g., cumulative deficit, duration, or mean intensity [3, 8]. Drought characteristics like the duration, severity and magnitude vary according to reference level. The purpose in determining the drought characteristics is to determine whether the specific period is dry or wet. Precipitation or flow series with adequate lengths are required to determine the dry and wet periods. If the data are not adequate, synthetic flow series must be produced by establishing the stochastic time series model. In addition, with the help of the produced series, interpretations may be made on floods and drought. These series are also used for the purpose of determining the size of the water bodies.

Mathematical approaches like synthesis and simulation are needed in problems on making decisions experienced in sizing and running the water resource systems. Simulation may be defined as the mathematical expression of the behavior of a water resource system along a certain time period. Although hydrological simulation models are classified in various forms, flow models are collected under two groups: (i) Deterministic or physical simulation of the hydrological system, (ii) Statistical or stochastic simulation of the hydrologic system [4]. Stochastic approach is related with the statistical characteristics of the historical series. Commonly used stochastic models are the ones that are established with time series approaches like Autoregressive (AR), Moving Average (MA) and Autoregressive Moving Average (ARMA).
There are many studies on the stochastic model of stream flows in the literature. In previous studies, generally the Box-Jenkins Methodology was employed, and the autoregressive models of the monthly and annual average flows were established. Merzi, Nurunnisa [5] performed the monthly stochastic modeling of the flows measured in Station 2323 on Oltu River in Çoruh Basin. It was reported that the best model was the ARMA(1,1) Model. Bacanlı and Baran [6] conducted another study and evaluated the AR(1), AR(2), AR(3), ARMA(1,1) and ARMA(1,2) models that are used mostly in hydrology. Can, Tosunoglu [7] conducted a study and used Autoregressive Moving Average and Artificial Neural Network models in order to model the daily river flows in Çoruh Basin. They produced 100 synthetic series by using these models.

Many studies were conducted on determining the drought period lengths with the run analysis of the stream flows. In these studies, it was ensured that precautions were taken in areas where there were drought risks by determining the relation between drought components (i.e. drought length, magnitude and severity). Şen [8] conducted a study and showed that the statistical features of the run length depended only on the dependency character of the process. Bayazit and Şen [9] obtained the analytic expressions of the probability distribution of the run length for first degree Markov Processes. They showed that the negative run length and the probability mass function were the geometric distributions. An equation was formed for an average repetition range and for the run severity expected in the threshold level for annual data whose Normal, Lognormal and Gamma distributions were fit [10, 11]. Şen and Sharma developed a separate distribution for the run length and magnitude; and merged these distributions for the purpose of computing the severity distribution. Shiah and Shen [12] developed a method to simulate the conditioned distribution of the run severity for a run time given for monthly data that were distributed in an independent and similar manner.

The basic issues of drought that have been resolved till our present time as the drought period, its amount, the frequency of drought, its period, and the area it covers. In this study, the drought duration and amount (severity) of it was determined for the Murat River in Euphrates River valley, and inferences were made for possible future droughts. To do this, the Autoregressive Moving Average ARMA(2,1) Model was established for the historical series monthly flow data of the Murat River. With the help of the established model, 100 synthetic flow series were produced that have the same features with historical series flows. The produced synthetic series were subjected to run analysis by cutting from the median threshold level (q=0.5) to determine the drought period and severity. In this way, maximum possible drought duration and severities were determined, dry and wet periods were defined; and information was obtained on droughts that may appear in the future. In addition, statistical tests were made to determine the probability distribution function that best fit the produced drought durations and severities.

### STUDY AREA AND DATA

In the present study, the stream flows that were measured monthly between the years 1968-2008 in the Palu streamflow gauging station 2102 for the Murat River in Euphrates River valley were used. The streamflow data were received from General Directorate of Electrical Power Resources Survey and Development Administration (EIEI) records. These data covered 41 years and 492 monthly flows. The Palu (2102) station on the Murat River, which was selected as the study area, is shown in Figure 1.

The Murat River, which was selected as the study area, constitutes the southern branch of the upper part of the Euphrates River valley, and corresponds to the Middle Euphrates Part. The total area of the basin, which is one of the widest basins of Turkey streams, is 25856,8 km², and has an annual streamflow of 8,1 billion m³. The streamflow decreases in a fast manner in July, and reaches the lowest level in September-October months [13]. The drainage field of the Euphrates River corresponds to 16% of the surface area of Turkey and to 17% of the streamflow in terms of area [14].

### MATERIALS AND METHODS

**Statistical analysis of the dry periods.** The precipitation being less than the expected level is defined as dry period.

The following factors must be determined in examining the dry periods:

1. Drought Severity: Defines how low the precipitation value is below the expected level;
2. Drought Period: Defines how long the dry period lasts;
3. Drought Frequency: Defines with which intervals the dry period examined is repeated in average; and
4. Drought Local Distribution: Defines whether or not the dry period in a certain area is also observed in surrounding nearby areas (stations).

The features of dry periods are defined as mentioned above, and it is necessary that a magnitude is defined to measure drought for statistical analyses. This magnitude must have the quality of involving the difference between the actual and expected precipitation and the sequence of precipitations in time. The magnitude used for this purpose is “run” [15].

The series of events that follow one another, that have the same characteristics, and that have different events before and after them are called as “run”. The lengths of the runs that are defined in this
way may be dealt with as a random variable and may be examined with statistical methods [16].

**Run analysis.** Runs analysis is the method used to measure the drought characteristics like the severity, frequency and area coverage of the droughts experienced in the past by making use of the recorded data.

By making use of the drought magnitudes that are determined as a result of the comparison of the run line with a predefined constant level, the evaluation of droughts may be made. The variables like the dry periods in a certain time period, the starting and ending dates of drought, the number of drought periods, drought periods, drought magnitudes, drought severity and drought probability may be obtained by comparing the run level with a predefined constant level. The change of values may be useful in understanding the features of drought. Such changes may be defined in time according to a certain level that will be defined according to a constant level by making use of drought index that is computed for a certain area or by making use of the precipitation series in an area [17].

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**FIGURE 1**

Murat River Observation Station 2102 located in Middle Fırat Basin.

**FIGURE 2**

Definition of run characteristics.
A duration where the X variable stays below a given $x_0$ value is called as the negative run length (or shortly, as the run length). The run length determines the drought period in a given $x_0$ level (since drought periods were the subject matter in the present study, only the negative runs were considered, and $X>x_0$ positive runs were not focused on. For this reason, instead of “negative run”, the term “run” was used). Along the drought period, the difference between the actual precipitation amount and the precipitation in $x_0$ value is called “run sums”. Run length and run sums show run characteristics. Run characteristics are shown in Figure 2.

$D=\sum_{x=x_0}^{\infty}(x - \bar{X})$  \hspace{1cm} (1)

Run sums refers to the drought severity in question. The run length and the run sums are not independent; they have a high correlation among themselves. The value of the correlation was found as 0.8 for the independent normal variable [18].

**Probability distribution functions of the fitness tests.** Some statistical tests are applied for the purpose of checking whether any of the sampling that has finite elements selected from a main body of a random variable fits the selected distribution function or not. The most frequently used ones are $X^2$ (Chi-Square) Test, Kolmogorov-Smirnov (K-S) Test, Anderson-Darling Test and Probability Line Correlation Coefficient (PPCC) Test.

Graphical comparison of the fitness of a distribution that is based on cumulative observation with the cumulative intensity function of the foreseen theoretical distribution is a fast way of checking whether a dataset fits the given theoretical distribution. If two functions do not show excessive deviation, it is accepted that the theoretical distribution fits the raw data. The Kolmogorov-Smirnov (K-S) Test, which may be applied only to random variables and which is one of the fitness tests; and the Cramer von Misses (CvM) Test, which may be applied to both continuous and discrete variables, are used to determine the acceptance or rejection of the hypothesis distribution at a certain significance level. The Chi-Square Test, which is another statistical test, may be applied both to discrete variables and continuous variables, and is based on the principle of comparison of probability intensity functions instead of adjacent intensity functions, which is the case in the other two tests. In addition, especially for small samples, both the K-S Test and the Chi-Square Test are not strong in terms of accepting accurate while the hypothesis is wrong in reality [19].

**RESULTS AND DISCUSSION**

Pre-analysis. Firstly, the skewness coefficient of each month was found with the “skewness” command of the Matlab R2014a Software in order to determine whether monthly flow values of the historical series ($x_{ij}$) were distributed normally or not. The average of the skewness values of each month was found and the skewness coefficient range table was used for alpha significance levels. It was checked whether our average skewness value was within the desired range or not. The average of the skewness values in the historical series was $\gamma=0.94$ (Table 1). However, $\gamma=0.94 >0.87$ means that our series has skewness (Figure 3). For this reason, the $y_{ij}=\log(x_{ij})$ conversion was made to reduce the skewness of the historical series ($x_{ij}, \gamma$), and the skewness was reduced.

**Parameter prediction and confidence interval.** In this step, the parameters are predicted for the foreseen model and the stability conditions of these parameters are checked. By using the “present” command of the Matlab R2014a Software, the parameters and standard deviations were found and the 95% Confidence Intervals were determined. The lower and upper limits of the 95% Confidence Interval of the foreseen ARMA (2,1) Model parameters:

$\phi_1 \pm 1.96 * 0.0062 = \{-1.3127, -1.3372\}$

$\theta_1 \pm 1.96 * 0.0055 = \{0.7588, 0.7371\}$

$\phi_1 \pm 1.96 * 0.0048 = \{-0.3715, -0.3903\}$

Since the ARMA(2,1) Model Parameters ($\phi_1, \theta_1$) do not include zero in 95% Confidence Interval, this shows that the model is statistically significant and may be used (Table 2).

**Determining the best model.** After the model parameters that are similar to AR (p), ARMA(p,q) that might fit a hydrological process are determined, the parameters of the model are tested for stability and reversibility, and it is tested whether the remains are independent series or not [4, 20]. The criteria that are defined by Akaike and that are computed basically as residual variance were used a wide usage area in selecting the best model [21, 22]. The first criterion that is used as the information criterion was recommended to test the level of the internally dependent (AR) models [23].

### TABLE 1

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<td>117.59</td>
<td>94.82</td>
<td>119.78</td>
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<td>953.14</td>
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<td>39.90</td>
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<td>56.78</td>
<td>60.63</td>
<td>31.35</td>
<td>46.14</td>
<td>168.89</td>
<td>412.54</td>
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<td>127.53</td>
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<td>127.53</td>
<td>38.65</td>
<td>15.66</td>
<td>12.54</td>
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</tbody>
</table>
FIGURE 3
Normal distribution probability graphics for the monthly flows of historical series.

TABLE 2
The Autoregressive (\(\phi\)) and Moving Average (\(\theta\)) parameters of the ARMA(2,1) Model, and the 95% Confidence Interval limits.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Confidence Interval limits</th>
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<tr>
<td>(\phi_1)</td>
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<tr>
<td>(\theta_1)</td>
<td>(0.7588; 0.7371)</td>
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<tr>
<td>(\phi_1)</td>
<td>(-0.3715; -0.3903)</td>
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</table>

TABLE 3
Comparison of FPE and AIC values of all models.

<table>
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<th>Model</th>
<th>AIC</th>
<th>FPE</th>
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<td>0.555</td>
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<td>ARMA(1,1)</td>
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<td>0.5525</td>
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<td>AR(2)</td>
<td>-0.5896</td>
<td>0.5545</td>
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<td><strong>ARMA(2,1)</strong></td>
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<td><strong>0.5495</strong></td>
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<td>AR(3)</td>
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<td>ARMA(3,3)</td>
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<td>0.5586</td>
</tr>
<tr>
<td>ARMA(4,3)</td>
<td>-0.5742</td>
<td>0.5629</td>
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<tr>
<td>ARMA(5,3)</td>
<td>-0.5666</td>
<td>0.5671</td>
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</table>

Final Prediction Error Criterion [FPE] is based on the principle that the error variances are corrected by considering the observed data length and the number of the parameters of the model. The minimum [min FPE] of the Final Prediction Error Criterion Values that are computed for different models are used as a criterion that helps to decide on the proper model. The Final Prediction Error Criterion is formalized in Equation 2.

\[
\text{FPE}(\phi) = \frac{\hat{\sigma}^2}{N} \sum_{i=p}^{N-p} \frac{e_i^2}{N-p}
\]  \hspace{1cm} (2)

The criterion that was proposed by Akaike [24], and which is used more commonly is defined as the Akaike Information Criterion [AIC]. By using the sample length (N), distribution parameter count (k) and residue variance (\(\hat{\sigma}^2\)) Maximum Probability Prediction, Akaike Information Criterion is expressed as follows:

\[
AIC = N \ln[\hat{\sigma}^2] + 2k
\]  \hspace{1cm} (3)

As seen in Table 3, when Final Prediction Error Criterion (FPE) and Akaike Information Criterion (AIC) for possible models are compared, the model with the lowest value was chosen as ARMA(2,1) Model, and it was also chosen as the best model.
The ARMA(2,1) model was established by using the “ARMA” command of the Matlab R2014a Software. The Equation of the ARMA(2,1) Model (Equation 4) was obtained by using the “present” command.

\[ Z(t) = 0.5783 z(t-1) - 0.3971 z(t-2) + \varepsilon(t) \]  

(4)

Additional tests of the model. In this section, synthetic series will be generated and the statistical characteristics of the produced series and the statistical characteristics of the historical series will be compared. Because the model user generally wants to establish a model that produces statistical parameters of a historical series. These may be characteristics such as historical average, standard deviation, correlogram, the distribution of the average and average length.

Generation of synthetic series. In the analysis and running of many water projects, adequate information and data must be obtained for the stream in question. However, in some situations, synthesis and simulation methods are made use of to produce synthetic flow data when there are missing points in data. Stochastic models are used mostly in generating synthetic series and in finding predictions for the future [25, 26].

At this stage, synthetic series are produced, and the statistical characteristics of the time series such as average, standard deviation and correlograms are compared with the produced series. Then, the standard normal random numbers are obtained from the random numbers that fit the uniform distribution that are produced easily with the help of the computer.

Historical average standard deviation confidence interval protection. By using the established models, 100 synthetic series were produced that have the N length with the observation period of each station, and to check whether these series protect the characteristics of the historical series (average, standard deviation), the 95% Confidence Interval was computed with the averages of the produced synthetic series and standard deviations. It was seen that historical series characteristics were within the reliability limits (Figure 4, Figure 5).

For all stations, the full protection of two important characteristics such as average and standard deviation shows that the model was selected accurately, and the produced synthetic series may be made use of in other studies.

Drought analysis. Since drought analysis is believed to be a timewise concept, a time series (precipitation, flow and soil losses) are considered and various drought characteristics such as drought period, severity, magnitude etc. are computed. To predict these magnitudes, statistical, probability or stochastic-based different models may be developed. Commonly, drought analysis intensifies on point analysis where timewise change occurs in a selected area. This method gives information only about the value of the maximum drought severity that might occur without providing any information on the areal magnitudes or drought period [15].

Our country is located in a sub-arid area on earth. This means that we may always be faced with drought in different periods. For this reason, it is necessary for our country to determine the periods and severities of maximum drought periods and to take necessary precautions. In this study, run analysis, which is used widely in determining drought features, was made use of. Drought parameters are determined with this method (drought and magnitude). The most basic factor in deriving these is the threshold or truncation level that may be a timewise function [2]. The threshold level, which

![Figure 4](image-url)

**FIGURE 4**

The 95% Confidence Intervals of the monthly averages of the produced synthetic series.
FIGURE 5
The 95% Confidence Intervals of the monthly standard deviations of the produced synthetic series.

TABLE 4
The median threshold levels of the historical series monthly average flows.

<table>
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<tr>
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<tr>
<td>Median (m³/s)</td>
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<td>98.8</td>
<td>93.9</td>
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<td>62.8</td>
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<td>38.6</td>
</tr>
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</table>

FIGURE 6
The cut-off of the median threshold level of the historical series monthly flows (q=0.5).

may be a deterministic valuable, may also be a stochastic variable or a combination of these. Various statistical parameters, which are related to the drought period and magnitudes at various threshold levels, are useful for drought characterization [27].

The median threshold level, which is one of the critical threshold levels used in water resources, was used in this study (q=0.5) (Table 4).

The average flow data and median threshold levels for 492 months of the Murat River for the period between 1968 and 2008 are given in Figure 6.
FIGURE 7
Dry and wet periods seen in historical series.

TABLE 5
Runs analysis results of historical series monthly average flows for $q = 0.5$.

<table>
<thead>
<tr>
<th>Historical series droughts</th>
<th>Duration (Month)</th>
<th>Severity ($m^3/s$)</th>
<th>Duration (Month)</th>
<th>Severity ($m^3/s$)</th>
<th>Duration (Month)</th>
<th>Severity ($m^3/s$)</th>
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<td>3591.4*</td>
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</tr>
</tbody>
</table>

*Maximum droughts

In Figure 7, dry and wet periods are given obtained by subtracting the median threshold levels from the historical series monthly flows. The level below zero denotes the dry period, while the level above zero denotes wet period. The maximum drought is shown with thick red line. This drought period was 33 months, and the severity was 3591.4 m$^3$/s.

In Table 5, the drought periods and severities obtained by cutting the historical series monthly average flows from median threshold level ($q=0.5$) are shown.

The maximum drought durations and severities of the 100 synthetic series obtained by cutting the monthly average flows ($q=0.5$) are shown in Table 6.
TABLE 6
Run analysis results of synthetic series maximum droughts for $q = 0.5$ Synthetic series droughts.

<table>
<thead>
<tr>
<th>Duration (Month)</th>
<th>Severity ($\text{m}^3/\text{s}$)</th>
<th>Duration (Month)</th>
<th>Severity ($\text{m}^3/\text{s}$)</th>
<th>Duration (Month)</th>
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<td>7</td>
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<td>664.87</td>
<td>27*</td>
<td>2965.2*</td>
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<td>730.75</td>
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<td>8</td>
<td>1032.4</td>
<td>10</td>
<td>871.45</td>
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</tr>
</tbody>
</table>

*Maximum droughts

The best distribution function that fits the drought characteristics. There are many probability distributions used for drought parameters. In this study, the well-known 11 probability distribution model (Gamma, Weibull, 3-parameter Weibull, Nakagami, Generalized Extreme Value (GEV), Exponential, Inverse Gaussian, Normal, 3-parameter Lognormal, Log-Logistic, Log-Logistic, 3-parameter Log-Logistic, Pearson Type III which are frequently used, was employed in the present study. Detailed information on these distributions may be obtained at various sources [28, 29]. In order to check the fitness of frequency function obtained from an observed sample to a selected theoretical probability distribution function, various tests were used such as the Chi-Square ($\chi^2$) test, Kolmogorov-Smirnov (K-S) and Anderson-Darling (A-D).

While the best-fitting probability distribution function to the drought durations obtained with the cutoff of historical series monthly average flows from median threshold level ($q=0.5$) was found as Gamma, the best-fitting distribution to the drought severities was found as Weibull (Figure 8 and Figure 9).

The best-fitting distribution to the drought characteristics of the synthetic series produced by using the free software Easyfit 5.6 was determined by using the Kolmogorov-Smirnov, Anderson-Darling and Chi-Square tests (Table 7).

The distributions that best fit the drought durations of the produced synthetic series are visualized as graphics (Figure 10 a-b-c). As seen in the graphics, the best-fitting distribution function to the drought durations is the Gamma distribution.

The distributions that best fit the drought severities of the produced synthetic series are visualized as graphics (Figure 11 a-b-c). As seen in the graphics, the best-fitting distribution function to the drought severity is the Generalized Extreme Value (GEV) distribution.
FIGURE 8
The distribution function that best fits the historical series drought durations.

FIGURE 9
The distribution function that best fits the historical series drought severities.

TABLE 7
Determining of the distribution that best fits the drought characteristics obtained for the median threshold level of the produced synthetic series.

<table>
<thead>
<tr>
<th>Distributions</th>
<th>Drought Duration</th>
<th>Drought Severity</th>
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<tbody>
<tr>
<td></td>
<td>Kolmogorov-Smirnov</td>
<td>Anderson-Darling</td>
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<tr>
<td>Gamma</td>
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<td>Weibull</td>
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<td>Nakagami</td>
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<td>Gev</td>
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<td>Exponential</td>
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<td>-</td>
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<td>Inverse Gaussian</td>
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<td>Log-Logistic (3p)</td>
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<tr>
<td>Pearson Type III</td>
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<tr>
<td>Gev</td>
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<tr>
<td>Log-Logistic</td>
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<td>Log-Logistic (3p)</td>
<td>2</td>
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<tr>
<td>Weibull</td>
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<td>Weibull (3p)</td>
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<tr>
<td>Lognormal (3p)</td>
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</table>
FIGURE 10
The best-fitting distributions for the drought durations seen in synthetic series;
a) According to the Kolmogorov-Smirnov Test; b) According to the Anderson-Darling test;
c) According to the Chi-Square test.

FIGURE 11
The best-fitting distribution to the drought severities seen in synthetic series;
a) According to the Kolmogorov-Smirnov Test; b) According to the Anderson-Darling test;
c) According to the Chi-Square ($X^2$) test.
CONCLUSIONS

As a result of the study, it was determined that:

1. The stochastic model that best represents the monthly average flows of the Murat River was found to be ARMA(2,1). Stochastic models are used as an important component of the decision-making processes to produce data for planning and design, to predict the values of the processes in the future or to produce scenarios.

2. 100 synthetic series were produced that were similar to the historical series in terms of statistically features by using the ARMA(2,1) Model. These synthetic series allow specialists to make predictions like in running multipurpose reservoirs, determining water potentials of streams, announcing flood warnings, planning hydroelectric production, city water and irrigation water in drought periods, and planning the transportation in streams.

3. When the historical series and synthetic series are subjected to run analysis for median threshold level, the biggest drought period length for historical and synthetic series were found to be 33 and 27 months, respectively; and the most severe drought was found to be 3591.4 and 2965.2 m³/s, respectively. By using these drought characteristics found, it is possible to make early warnings for drought, and to investigate about the precautions and the risk status.

4. From the median threshold level, it was determined that the best-fitting distribution for the drought durations was the Gamma distribution and the best-fitting distribution for drought severities was the GEV distribution by applying probability distribution function fitness tests to the drought period lengths obtained for 100 synthetic flow series subjected to run analysis. Determining the best distribution facilitated drought and flood frequency analysis.

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IN DIFFERENT MATURATION STAGES OF *ARUM ITALICUM* MILER (ARACEAE) FRUITS, DETERMINATION OF THE ELLAGIC ACID, HESPERIDIN, RESVARATROL AND QUERCETIN QUANTITIES BY HPLC-DAD

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ABSTRACT

*Arum italicum* Mill. fruits that have antioxidant effect and some phenolic compounds were investigated at its different maturation stages. Observing of some phenolic compounds like ellagic acid, hesperidin, resveratrol and quercetin varied from depending on their ripening stages in harvest as immature, half-mature and mature crops. These compounds can be recovered from *Arum italicum* Mill. fruits by using the Soxhlet extraction method in methanol for four hours. And, the phenolic compounds in these fruits can easily be identified by using HPLC-DAD device. It is obvious that the amounts of ellagic acid, hesperidin, resveratrol and quercetin in half-mature fruits of *Arum italicum* Mill. decrease and even hesperidin, resveratrol and quercetin disappear altogether. While the antioxidant effect was determined by using DPPH and TEAC, the total phenolic compounds of the fruits in all harvest periods were defined by using standards such as gallic acid and catechin.

KEYWORDS:
*Arum italicum* Mill., phenolic compounds, HPLC-DAD, ellagic acid, hesperidin, resveratrol, quercetin

INTRODUCTION

All plants in the ecological balance can be used in food, agriculture, textile, medicine and landscape applications according to their specific characteristics. About 200 of plants growing species in Turkey contain toxic components. These plants, which contain toxic substances at harmful and/or lethal levels, are called poisonous plants [1]. Moreover, it is known that poisons and medicines have been prepared from these plants since the ancient civilizations [2,3]. When these toxic substances are taken into the body, they disrupt body functions and cause poisoning. However, the poisoning effect of the plants can vary according to their species, different parts, maturation stages and the structural differences of living things [4].

*Arum italicum* Mill. (Araceae family), being in the poisonous plant class, is a perennial, tuberous and herbaceous plant with a height of 30-50 cm [5]. The plant is characterised by spadix harbouring singular flower with surrounding by a spathe and includes long stem, dark green colour and arrow shaped leaves [6-10]. These flowers that open in the spring season are malodorous [8, 11]. On a stem that resembles a corn cob in the autumn, the maturing fruits are reddish-orange in pea-size. These wild plants grow up in Turkey, Europe, and Balkans as well as in North Africa and Caucasus [12]. The toxic compounds such as oxalic acid and calcium oxalate crystals and saponin exist in its chemical contents [13-16]. For this reason, when the plant is freshly used, it is poisonous for the livings. But this toxic effect disappears when it is dried or boiled [15]. Despite toxic effects, the fresh leaves and roots of the plant are used externally as a blood carrier for skin and a wound curative for hemorrhoids and abscess in the traditional medicine [17-19]. Also, dried roots are used internally as expectorant and laxative [12].

The phenolic components like ellagic acid, hesperidin, resveratrol and quercetin (as shown in Figure 1A-1D) in the toxic *Arum italicum* fruits are investigated in this study (Figure 1A-D). These polyphenolic compounds have antioxidant effects and unique features in prevention of cancer types as it is known [20-23]. Hence, the amounts of them were determined by using HPLC-DAD method in the *Arum italicum* fruits. And the antioxidant effect of them was also carried out.

MATERIALS AND METHODS

Chemicals and reagents. Methanol, acetic acid, acetonitrile, phosphoric acid, sodium carbonate and 6-hydroxy-2,5,7,8-tetramethylchromane-2-hydrate carboxylic acid (Trolox=T) were brought from Sigma-Aldrich. The Ellagic acid (EA) was supplied from Alfa Aesar while the hesperidin (H), quercetin hydrate (Q) and resveratrol (R) were bought from...
Acros Organics, Aldrich, and ROTH, respectively. Gallic acid (GA), catechin (C), 1,1-diphenil-2-pycril-hidrazil (DPPH) was provided from Merck and Folin-Ciocalteu’s phenol reagent was ensured from Fluka. Used chemicals and reagents were at HPLC-analytical grade purity.

**FIGURE 1**
Ellagic acid (A), Hesperidin (B), Resveratrol (C) and Quercetin hydrate (D)

**Apparatus.** The HPLC instrument performing on a 1260 Infinity Agilent HPLC system was used for the determination of the amounts of phenolic compounds. The diode array detector (DAD) and an Inertsil ODS-3 C18 column (5 μm μm, 250x4.6 mm ID) were attached on it.

The total phenolic compounds and the antioxidant capacity of the fruits were determined by performing with Perkin Elmer Lambda 35 UV/VIS spectrophotometer.

**Samples.** Arum italicum fruits were collected from Manisa in Turkey during summer and autumn 2017. Freshly fruits were kept at –18°C in the deep freezer until extraction time. Immature, half-mature and matured fruits were carried out in the Soxhlet extraction separately.

**Extraction procedure of the fruits.** Each of fruits in different maturations (2.00 g) was refluxed with MeOH (50 mL) by simple Soxhlet apparatus under stirring for 4 hours. Each extraction was done four times (n=4). Then all extraction mixtures were cooled at room temperature for 20 min and filtered off through Whatman no. 1 filter paper to get the crude extracts. They were retained at 4°C until HPLC and UV/VIS analyses were performed.

**Conditions for HPLC-DAD analysis.** The prepared methanolic extracts of the fruits were injected to the HPLC system to determine the ellagic acid (EA), hesperidin (H), resveratrol (R), quercetin (Q).

The proposed condition for HPLC analysis of EA by Aguilera-Carbo et al. [24] was performed on HPLC with DAD detector system. The operated DAD detector was at 254 nm wavelength that was related with the absorption maximum value of EA standard. Solvent A (acetonitrile) and solvent B (aqueous solution of H3PO4, pH at 2.5), in the ratio of 30:70 were used as an isocratic mobile phase and the flow rate was operated with 0.7 mL/min. The injection volume was 10 μL. Other chromatographic conditions were defined as 25°C temperature and 254 nm wavelength. Retention times of standards and extracts were compared by using their HPLC chromatogram curves for identification of the EA amounts in the samples. Amount of EA in the extracts was calculated by using linear calibration graph generated with EA standard solutions at a concentration range of 6.875-55 μg/mL.

The determination of the other phenolic compounds (H, R and Q) on the HPLC was operated according to the Wang et al. procedure [25]. Solvent A (2% aqueous acetic acid) and solvent B (0.5% aqueous acetic acid-acetonitrile; 50:50 v/v) were used as a mobile phase and the flow rate was determined as 1 mL/min. The injection volume was adjusted to 10μL. Gradient elution on the H, R, Q analysis was performed as following conditions: 0 min, 60:40; 5 min, 60:40; 20 min, 45:55, 25 min, 20:80; and 30 min, 0:100. The absorbances of UV for H, R and Q were measured at 280 nm, 320 nm and 360 nm, respectively. The column temperature was kept at 30°C. Quantitative determination for H, R, Q was established from their calibration graphs. The linear concentration range of the standard solutions for H was found as 25.00-100.00 μg/mL. These assigned ranges for R and Q were 1.375-11.00 μg/mL and 15.00-120.00 μg/mL, respectively.

**Determination of the total phenolic compounds.** The determination of total phenolic compounds in the contents of all prepared samples was carried out using the Folin-Ciocalteu method. The standard phenolic compounds were chosen as GA and C in this application. The standard solutions of GA were prepared in five different concentrations as 20, 40, 60, 80, 100 mg/L while the standard solutions of C were also prepared in five different concentrations as 5, 10, 15, 20, 25 mg/L. Then, Folin-Ciocalteu reagent (2 mL, 10%) and sodium carbonate (Na2CO3) solution (1 mL, 7%) was added to each standard solutions of GA (0.5 mL) to get the standard graphics. These mixtures were kept in a dark place during 30 minutes before getting their UV/VIS absorbance. The two calibration graphs were created for both GA and C by measuring of the absorbance.
The values of the standards at 750 nm wavelength against the blank solution. The calibration graph was created with the absorbance against to the concentration with 5 standard solutions to get the linear y equations given as following equation 1, where y=absorptions, x=concentrations, and R²=0.9962.

\[(y=0.016x+0.0063)\]  
Equation 1

The same calibration graph was also constructed for C standard to obtain the linear y equations as follows in equation 2, where y= absorptions, x=concentrations, and R²=0.9985.

\[(y=0.0347x+0.196)\]  
Equation 2

The total phenolic compounds are identified in 100-gram of arum fruits as the equivalent of milligram to GA and also to C standards with giving the simple formulas as GAE/100 g and CE/100 g, respectively (GAE/100 g is expressed as milligrams of GA equivalent per 100 gram of peel weight and CE/100 g is expressed as milligrams of C equivalent per 100 grams of peel weight).

Antioxidant capacity of the Arum Italicum fruits. The DPPH stock solution was prepared as 0.1 mM concentration to get the antioxidant capacities of Arum Italicum fruits. To calibrate the absorbance at 0.900 this solution was diluted with MeOH. DPPH solution (2.9 mL) was put to samples (0.1 mL) and it was incubated for 30 minutes at room temperature in a dark place. UV/VIS spectrometer was calibrated at 517 nm and MeOH was used as a blank solution. Samples and DPPH solutions were measured in thirtieth minute by UV/VIS. DPPH inhibition percentage of the sample was calculated by using the following equation 3, where Acontrol=at the thirtieth minute measurement of DPPH solution, and A sample=at the thirtieth minute measurements of all sample solutions.

\[(\text{Inhibition } \%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right)\times 100\]  
Equation 3

Trolox equivalent antioxidant capacity. The antioxidant capacities of samples were identified with the Trolox. The stock Trolox solution was prepared to 1000 ppm concentration. Its concentrations were arranged as 1000 μM, 800 μM, 400 μM and 200 μM. The solution of DPPH (2.9 mL) was put to Trolox standards (0.1 mL) and they were kept for 30 minutes in a dark place at room temperature as previously. And the UV/VIS absorptions were measured at 517 nm wavelength with using the MeOH as a blank. All measurements were done at thirtieth incubating minute to get the calibration graph. The antioxidant capacities of Arum Italicum fruits in Trolox equivalent were calculated as mM/g by using linear equation of y given as the following equation 4, where y=inhibition percentage of DPPH, x=concentrations of Trolox, and R²=0.9982.

\[(y=0.0843x+0.1522)\]  
Equation 4

RESULTS AND DISCUSSIONS

In this study, Arum Italicum Miller fruits were collected in three different maturation stages, immature-green color, half mature-orange color and mature-red color. All extracts of the fruits were analyzed with HPLC to obtain the chromatograms of phenolic compounds like EA, H, R and Q. The standard of EA was recorded at 254 nm where the UV absorption maxima is. The retention time of EA was 6.9 min. The chromatograms of EA standard shown in Figure 2 and all the samples shown in Figures 3-5, are presented as follows.
The calibration curve for EA was showed a good linearity, using as following equation 1, over the test ranges such as 6.88, 13.75, 27.50 and 55.00, where y=peak area, x=concentration as μg/mL, and R²=0.9959. The amount of EA in *Arum Italicum* fruits, calculated with the Equation 5, is presented in Table 1. The quantities of EA in fruits have been moderately yielded.

\[y = 134.39x - 132.32\]  
*Equation 5*

The calibration curve for H was showed a good linearity, using as following equation 6, over the test ranges such as 25.00, 50.00, 75.00 and 100.00, where y=peak area, x=concentration as μg/mL, and R²=0.9995. The retention time of H was 14.9 min. The amount of H in *Arum Italicum* fruits, calculated with the Equation 6, is also presented in Table 1. The quantities of H in green fruits have been moderately yielded when the quantities of it in half matured-orange color fruits was not found.

\[y = 16.43x - 2.245\]  
*Equation 6*

The calibration curve for R was showed a good linearity, using as following equation 7, over the test ranges such as 1.38, 2.75, 5.50 and 11.00, where y=peak area, x=concentration as μg/mL, and R²=1. The retention time of R was 22.9 min. The R with small quantities was only observed in matured-red color fruits and there were not any quantities of R in other two types of fruits like half maturate-orange color and immature-green color.

\[y = 36.36x + 0.3385\]  
*Equation 7*

The calibration curve for Q was also showed a good linearity, using as following equation 8, over the test ranges such as 15.00, 30.00, 60.00 and 120.00, where y=peak area, x=concentration as μg/mL, and R²=0.9998. The retention time of Q was 27.3 min. The amount of Q in *Arum Italicum* fruits, calculated with the Equation 8, is also presented in Table 1. The quantities of Q in green and red color fruits are very similar value and reasonable yield.

\[y = 46.46x - 66.401\]  
*Equation 8*

The amounts of phenolic compounds (EA, H, R and Q) were calculated in μg/g equivalent value from their HPLC spectrums and given in Table 1. As demonstrated by the results in Table 1, the quantity of phenolic compounds depends on the fruits maturation. The immature-green color fruits of *Arum Italicum* have the highest quantities of EA, H and also Q. On the other hand, the R amounts are only observed in matured-red color fruits of *Arum Italicum*.

The total amounts of phenolic compounds in all fruits, shown in Table 2, were determined by the Folin-Ciocalteu’s method as explained in the experimental part. Both Gallic acid (GA) and catechin (C) can be used for the determination of the total phenolic compounds in any samples. In this work, we have studied the determination of total phenolic compounds in extracts of the *Arum Italicum* fruits by using both GA and C standards to evaluate and to compare the results. Calculated values of total phenolic compounds were given in Table 2.

The total values of phenolic compounds were 522.97 in half-mature, 612.03 in mature and 926.09 mg of GAE/100g in immature fruits.

The same discussions can also be constructed on the results obtained using the C standard, as shown in Table 2. The total values of phenolic compounds were 104.47 in half-mature, 145.53 in mature and 290.35 mg of CE/100g in immature fruits.
The antioxidant effect and Trolox equivalent antioxidant capacity were defined by the free radical’s absorption activity in vitro DPPH and TEAC. The present percentages of DPPH and TEAC are given in Table 3. The percentages of DPPH inhibition of the prepared fruit extracts were calculated as 27.31% in half-mature, 51.14% in mature and 66.35% in immature fruits. Additionally, the Trolox equivalent antioxidant capacities of the fruits extracts were calculated as 8.06 in half-mature, 15.12 in mature and 19.63 μM/g in immature fruits.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>The DPPH inhibition and TEAC values in all samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>DPPH Inhibition (%)</td>
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<tr>
<td>Green arum fruits</td>
<td>66.35±1.38</td>
</tr>
<tr>
<td>Orange arum fruits</td>
<td>27.31±2.55</td>
</tr>
<tr>
<td>Red arum fruits</td>
<td>51.14±2.47</td>
</tr>
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</table>

The highest DPPH inhibition was 66.35% in green fruit samples, but the lowest was also observed at 27.31% in orange fruit samples. Similarly, the highest and lowest values of TEAC in the same samples (green and orange fruits) were obtained as 19.63 and 8.06μM/g, respectively. As a result, it has been proved that the green fruit samples used in this study have the higher antioxidant activity than their orange and red fruit samples.

CONCLUSION

Foods containing substances such as oxalic acid, oxalate and saponin are known to be toxic to humans. It is known that *Arum Italicum* fruits have the high amounts of the oxalic acids, the oxalate crystals and saponins. Notwithstanding, these fruits have been consumed by people internally and externally without any fear in anywhere of the world. Especially, in the province of Manisa-Turkey, it is well known that the green fruits of *Arum Italicum* are effective in the treatment of hemmorhoids with swallowing. And, these people have been swallowed the *Arum Italicum* fruits as a raw. Although this traditional behavior of the people is very dangerous the consumption of the *Arum Italicum* fruits does not exceed the daily intake level. Hence, there have not been encountered poisoning in Turkey. On the other hand, when these fruits are dried or boiled, the toxic effects of *Arum Italicum* fruits disappear. Thus, their high-level consumptions are possible and their rich phenolic contents may be beneficial to human health.

REFERENCES


ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF ORIENTAL HACKBERRY (CELTIS TOURNEFORTII L.) AGAINST CCl₄ INJURY IN RAT LIVER

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ABSTRACT

This study aimed to investigate the antioxidant and hepatoprotective effects of Celtis tournefortii leaf extract (Ct) against CCl₄-induced liver damage in rats. A total of 32 Wistar albino rats were divided into four equal groups (n = 8): control, CCl₄ group, CCl₄ + Celtis group (CCl₄ + Ct), and Celtis group (Ct). Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities, glutathione (GSH) level, malondialdehyde (MDA) content, total antioxidant status (TAS), and total oxidant status (TOS) were analyzed in the liver tissues. Liver enzymes were assayed in the serum. The liver histopathology was also evaluated. The levels of alanine aminotransferase and lactate dehydrogenase significantly decreased in the CCl₄ + Ct group in comparison with those in the CCl₄ group. SOD and GPx activities and TAS increased significantly with CCl₄ + Ct administration when compared with the administration of CCl₄ alone. The oxidative stress parameters, such as MDA and TOS, showed a reduction when Ct treatment was applied to the CCl₄-induced group. The results revealed that Celtis remarkably attenuated the degenerative and necrotic changes caused by CCl₄ in the liver tissues. Consequently, the Ct may augment the antioxidant status and moderated the severity of CCl₄-induced liver damage. The therapeutic use of Celtis tournefortii might be effective in the treatment of liver injuries.

KEYWORDS:
Celtis tournefortii, Oriental Hackberry, CCl₄, Antioxidant, Hepatoprotective

INTRODUCTION

The liver is the first organ that metabolizes nutrients, drugs, and environmental toxicants that have entered the body through the digestive system and enter the liver through the portal vein after absorption. The liver contains metabolizing enzymes that can biotransform xenobiotics, thus converting the toxins to low-toxicity compounds for easier excretion. However, excess toxic substances or converted active metabolites might occasionally not be eliminated, thus detrimentally altering the liver function by acute or chronic exposure to toxicants [1].

CCl₄ is the most commonly used chemical agent for the oxidative stress-mediated hepatotoxicity and one of the best ways to develop liver damage by xenobiotics on animal models [2, 3]. The CCl₄-induced hepatotoxicity is mediated through the formation of reactive oxygen species. Therefore, CCl₄ causes hepatotoxicity, including necrosis, apoptosis, autophagy, and mitochondrial defects. The biotransformation of CCl₄ is performed by cytochrome P450 of the liver microsomes to convert trichloromethyl radical (CCl₃•) and its derivative trichloromethyl peroxy radical (CCl₃OO•), thereby causing lipid peroxidation [3].

Antioxidants function in scavenging free radicals and/or in inhibiting the production of free radicals. Therefore, antioxidants play important roles in the protection from the tissue damage caused by oxidative stress. Phytochemicals are versatile plant compounds; they feature potentially beneficial effects, one of which is as antioxidants in terms of disease prevention and amelioration [4]. Therefore, the prophylactic and therapeutic uses of phytochemicals have nutraceutically become widespread. Recent studies have shown that the antioxidant and hepatoprotective activities of plants have increased their importance in the discovery of new medications with less side effects and their use in the treatment of hepatic disorders. Celtis tournefortii, commonly known as the “oriental hackberry,” is a palatable and sweet, juicy, and although mealy, thin-fleshed with a thick outer coat. This plant is also used as a green bait for livestock, because its fruits are rich in nutrients and without tannin. Several Celtis species are used in the epileptic seizure, foot perspiration, and wound healing. Celtis tournefortii seeds are used by the native population against kidney sand, whereas its leaves are used for the stomach pain, menstrual bleeding, sedative purpose, and facilitating digestion. C. tournefortii fruits are consumed for the treatment of diarrhea, dysentery, and peptic ulcer [5]. A few studies exam-
ined the antioxidant property of *Celtis tournefortii* fruit but not its leaves. However, the protective effects against hepatotoxicity have not been previously investigated. This study exhibits an authentic value due to the acquisition of the primary scientific data. Based on this assumption, this study aimed to determine the antioxidant and hepatoprotective effects of *Celtis tournefortii* leaf extract (Ct) on rats.

**MATERIALS AND METHODS**

**Plant Material.** *Celtis tournefortii* leaves were collected from Siirt, Turkey in August 2016. Then they were dried in outdoor under the shadow and powdered.

**Celtis tournefortii Leaf Extraction.** One g powdered celtis leaf was added to a 100 mL beaker containing 50 mL solvent (water, ethanol-water (1:3 v/v), and ethanol-water (3:1 v/v), separately) and extracted by stirring for 3 h, 750 rpm. The extract was filtrated and centrifuged for 5 minutes at +4°C, 3500 rpm in falcon tube. The obtained extract was evaporated under the reduce pressure at 37oC for 30 minutes. 

**Determination of Total Phenolic and Total Flavonoid Content.** Total phenolic content (TPC) in the Ct extract were determined with Folin-Ciocalteau reagent according to the method modified from Singleton and Rossi [6] using gallic acid as a standard. TPC was calculated mg gallic acid equivalent for 100 g sample (mg GAE/100 g sample). Total flavonoid content (TFC) was determined on the basis of AlCl3 method [7] using quercetin as a standard. TFC was calculated mg quercetin equivalent for 100 g sample (mg QE/100 g sample). Analyses were triplicated.

**LD50 Study.** In order to determine whether C. tournefortii leaf extract has acute toxicity, LD50 study was performed on rats for 7 days. LD50 doses were administered as 250, 500, 1000, and 2000 mg/kg bw Ct extract (n=6).

**Animals and Experimental Protocol.** Experiments were performed using 32 *Wistar albino* male rats (150 – 250 g; 2 months of age) obtained from Experimental Application and Research Center, Yuzuncu Yil University (Van, Turkey). Rats were kept at 22°C, under a 12:12 h light/dark cycle in separate stainless cages. They were fed standard chow and tap water *ad libitum*. The groups (n=8) for the experiment were randomly formed as follows:

Control group received 1 mL physiological saline via gavage every day for 28 days and was administered 2 mg/kg bw single dose olive oil.

Group II rats (CCl4 group) were injected with single dose of 2 mg/kg bw CCl4/olive oil (1:1 v/v) and received 1 mL physiological saline via gavage every day for 28 days.

Group III rats (CCl4+Ct group) were injected with single dose of 2 mg/kg bw CCl4/olive oil (1:1 v/v) and were administered 100 mg/kg bw Ct extract via gavage every day for 28 days.

Group IV rats (Ct group) were injected with single dose of 2 mg/kg bw olive oil and were administered received 100 mg/kg bw Ct extract via gavage every day for 28 days.

The present study was conducted with Yuzuncu Yil University Animal Researches Local Ethic Committee (no. 27552122-604.01.02).

**Sample collection.** The rats were anesthetized with ketamine-xylazine at the end of the experiment. Blood was collected from the heart of each rat and transferred to EDTA tubes. The tubes were centrifuged for 10 minutes at 850×g to prepare serum for enzyme analysis. Serum marker enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were analyzed using an auto-analyzer (Roche Modular P, Germany). Liver tissues were dissected and stored at −80°C until analysis.

**Biochemical Analyses.** Liver tissues were homogenized in ice-cold phosphate buffered saline (pH 7.4) using titanium probe homogenizer (Bandelin Sonopuls HD 2200) for 3 min and then centrifuged at 8570×g for 30 min at +4°C. The resulting supernatants were used for antioxidant enzymes and reduced glutathione and malondialdehyde analysis. Malondialdehyde (MDA) was measured at 532 nm using the method of Slater [8], based on TBA reactivity. Reduced glutathione (GSH) concentration was measured at 412 nm by the amount of yellow color formed as a result of the reaction of sulfhydryl groups in the tissue samples with DTNB (5, 5′-dithio-bis-(2-nitrobenzoic acid)), according to the method described by Rizzi et al. [9]. Superoxide dismutase (SOD) activity was assayed by calculating percentage inhibition of formazan dye at 505 nm [10]. Glutathione peroxidase (GPx) activity in the liver homogenates was determined at 340 nm based on the catalytic oxidation of cumene hydroperoxide to reduced glutathione as described by Paglia and Valentine [11]. Catalase activity was estimated by using the Aebi method [12] based on at 240 nm by breaking down H2O2 and transforming it into water and oxygen. Total antioxidant status (TAS) and total oxidant status (TOS) was carried out using commercially available kit (Rel Assay Diagnostic, Turkey) as described by Erel [13, 14]. The TAS method is based on antioxidants in the sample converting the ABTS+ radical (2,2′-Azino-
bis(3-ethylbenzothiazoline-6-sulfonic acid)) into the ABTS form. The total antioxidant level of the sample is related to the change in absorbance at 660 nm. The analysis is calibrated with Trolox Equivalent [13]. The TOS method is based on the conversion of the ferrous (Fe^{2+}) ion complexes of the oxidants present in the sample to the ferric (Fe^{3+}) form by oxidation [14]. The assay is calibrated using hydrogen peroxide and the results are expressed as micromolar hydrogen peroxide equivalent per liter (μmol H₂O₂ Equiv./L). Oxidative Stress Index (OSI) is a ratio of TAS and TOS parameters used to express the status of oxidative stress in tissues. OSI was calculated regarding to the following formula:

OSI (arbitrary unit) = (TOS/TAS)×100. The protein content of the homogenates was determined using the modified Lowry method [15].

Histopathology. Liver tissue samples were routinely embedded in paraffin and stained with hematoxylin and eosin (HE). The stained sections were evaluated by imaging with a Nikon 80i-DS-R12 microscope. Histopathological results were evaluated semiquantitatively according to degree of the lesion such as (-): none; (+): mild; (++): moderate; (+++): severe.

Statistical Analysis. Data were expressed as mean ± standard deviation (SD). Significant differences between groups were assessed using one-way analysis of variance followed by Tukey’s test and Tamhane’s T2. p-Value ≤ 0.05 was accepted to be statistically significant.

RESULTS

Figs. 1(A–B) present the TPC and TFC results of Celtis tournefortii leaf water, 1:3 v/v ethanol–water, and 3:1 v/v ethanol–water extracts. The highest amounts of TPC and TFC in the extract were determined as ethanol–water (3:1 v/v) in comparison with water and ethanol–water (1:3 v/v).

The serum enzyme levels were used as biochemical markers for early acute hepatic damage (Table 1). According to the results, the levels of serum enzymes (AST, ALT, LDH, and ALP) were remarkably higher in the CCl₄ group compared with the control group (p < 0.05). However, these activities decreased with the administration of Celtis in the CCl₄ + Ct group in comparison with those in the CCl₄ group. Yet, only the decreases in ALT and LDH levels were found to be statistically significant (p < 0.05).

The results showed liver malondialdehyde (MDA) content and total oxidant status (TOS; as an indicator of oxidative stress) increased in the CCl₄ group compared with those in the control groups (p < 0.05). On the other hand, the MDA level and TOS were significantly lower in the CCl₄ + Ct-administered groups compared with those in the CCl₄ group (Figs. 2A–2B). CCl₄ administration decreased total antioxidant status (TAS), but Celtis supplement results in an increase (Figure 2C).

![Image of TPC and TFC results](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Serum parameters of rats</th>
<th>CG</th>
<th>CCl₄</th>
<th>CCl₄+Ct</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>89.4±8.1</td>
<td>129.1±17.7*a</td>
<td>112.3±21.8*a</td>
<td>96.3±12.6*b</td>
</tr>
<tr>
<td>ALT</td>
<td>37.5±8</td>
<td>45.4±4.4*a</td>
<td>38.1±4.3*b</td>
<td>33.4±3.7*b</td>
</tr>
<tr>
<td>LDH</td>
<td>758.4±85.5</td>
<td>1001.3±97.7*a</td>
<td>821.9±65.3*b</td>
<td>763±83.3*b</td>
</tr>
<tr>
<td>ALP</td>
<td>154.5±25</td>
<td>200.3±27.4*a</td>
<td>170.5±23.2</td>
<td>162.9±16.3*b</td>
</tr>
</tbody>
</table>

a: It was significantly different from control (p<0.05).
b: It was significantly different from CCl₄ (p<0.05).
Glutathione (GSH) content and superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities significantly decreased in the CCl4-treated group ($p < 0.05$). However, the combination of CCl4 with C. tournefortii treatment showed a significant increase in the SOD and GPx activities in the liver. In addition, only the Ct supplementation caused significant increases in the aforementioned activities and GSH level in the CCl4 and CCl4 + Ct groups (Figs. 3A–3D).

**FIGURE 2**

MDA content (A), TOS (B), TAS (C) and OSI (D) levels of groups.

a: It was significantly different from control ($p<0.05$).
b: It was significantly different from CCl4 ($p<0.05$).
c: It was significantly different from CCl4+Ct ($p<0.05$).

**FIGURE 3**

GSH content (A) and SOD (B), GPx (C), CAT (D) activities of groups.

a: It was significantly different from control ($p<0.05$).
b: It was significantly different from CCl4 ($p<0.05$).
c: It was significantly different from CCl4+Ct ($p<0.05$).
Table 2 shows the results of histopathological changes and lesions for both the control and treated rats. Normal histological appearance of the liver was observed in all cases of the control and Ct groups (Figs. 4A and 4E, respectively). Severe histological changes were observed in the livers of all rats in the CCl₄ group. In all cases, common coagulation necrosis in hepatocytes, fibrosis formation in broad bands in the portal area, bile duct proliferation, and inflammatory cell infiltration were determined in the liver tissue sections (Figure 4B). Cloudy swelling and vacuolar–hydropic degeneration were observed in the cytoplasm of hepatocytes, especially in the subcapsular region (Figure 4C). Intrahepatic cholestasis was also detected in the cytoplasm of hepatocytes. On the other hand, the microscopic results for the CCl₄ + Ct group showed significantly diminished in comparison with those of the CCl₄ group and exhibited similar histological appearance with the control group. No sign of extensive vacuolar degeneration nor necrotic changes were observed in the liver of rats in the CCl₄ + Ct group. The lesions were confined to several lobules, whose structure showed no deterioration. One case exhibited a rare occurrence of necrotic hepatocytes (Figure 4D), whereas two cases presented mild periportal fibrosis.

TABLE 2
Histopathological changes of Control, CCl₄, CCl₄+Ct, and Ct groups.

<table>
<thead>
<tr>
<th>Changes/lesions in liver</th>
<th>Control</th>
<th>CCl₄</th>
<th>CCl₄+Ct</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hydropic degeneration</td>
<td><img src="image1.png" alt="A" /></td>
<td>8/8ᵇ</td>
<td>2/8</td>
<td>-/6ᵇ</td>
</tr>
<tr>
<td>Mild</td>
<td>*</td>
<td>*</td>
<td>2</td>
<td>*</td>
</tr>
<tr>
<td>Moderate</td>
<td>*</td>
<td>5</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Severe</td>
<td>*</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2. Necrosis</td>
<td><img src="image2.png" alt="B" /></td>
<td>8/8ᵃ</td>
<td>1/8ᵇ</td>
<td>-/6ᵇ</td>
</tr>
<tr>
<td>Mild</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Moderate</td>
<td>*</td>
<td>5</td>
<td>1</td>
<td>*</td>
</tr>
<tr>
<td>Severe</td>
<td>*</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>3. Bile duct proliferation</td>
<td><img src="image3.png" alt="C" /></td>
<td>8/8ᵃ</td>
<td>2/8ᵇ</td>
<td>-/6ᵇ</td>
</tr>
<tr>
<td>Mild</td>
<td>*</td>
<td>2</td>
<td>2</td>
<td>*</td>
</tr>
<tr>
<td>Moderate</td>
<td>*</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Severe</td>
<td>*</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>4. Periportal fibrosis</td>
<td><img src="image4.png" alt="D" /></td>
<td>8/8ᵃ</td>
<td>2/8ᵇ</td>
<td>-/6ᵇ</td>
</tr>
<tr>
<td>Mild</td>
<td>*</td>
<td>2</td>
<td>2</td>
<td>*</td>
</tr>
<tr>
<td>Moderate</td>
<td>*</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Severe</td>
<td>*</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*ᵃ: None

*b*: It was significantly different from control (p<0.05)
*c*: It was significantly different from the CCl₄ (p<0.05)
*c*: It was significantly different from the CCl₄+Ct (p<0.05)

FIGURE 4
A: Control group, Normal histological appearance of the liver; B: CCl₄ group, widespread necrotic hepatocytes (arrows), proliferative bile ducts (arrow-heads), and fibrosis (asterisk); C: CCl₄ group, cloudy swelling (arrows) and vacuolar-hydropic degeneration (arrow-heads) of hepatocytes; D: CCl₄+Ct group, necrosis in some hepatocytes (arrows); E: Ct group, Normal histological appearance of the liver. H.E.X20
DISCUSSION

The prophylactic and/or therapeutic effects of phytochemicals indicate the importance of numerous medicinal plants; the pharmacological effects of medicinal plants can be attributed to the presence of these valuable constituents [16]. Therefore, certain medicinal plants are preferred owing to their specific biological activities and low toxic effects. The present study investigated for the first time the antioxidant and hepatoprotective effects of {\textit{Celtis tournefortii}} leaf against the hepatic damage in rats.

According to the results of TPC and TFC in {\textit{Celtis tournefortii}} extract, the present study revealed the considerable amounts of polyphenolic compounds present in the extract. In the study of Yildirim et al. [17], the amounts of TPC and TFC reached 6.67 mg GAE/g and 1.93 mg/g QE in the methanolic extract of fresh {\textit{C. tournefortii}} fruit, respectively. They also observed that the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition by methanolic extract of {\textit{C. tournefortii}} reached 52.46% [17]. The {\textit{C. tournefortii}} fruit contains rutin, quercetin, kaempferol, naringin, gallic acid, caffeic acid, ferulic acid, chlorogenic acid, ellagic acid, rosmarinic acid, p-coumaric, catechin, myricetin, retinol, and α-tocopherol [5, 17]. Similar phenolic contents might be present in the fruits and leaves, but the leaves might also contain higher levels of these compounds [18, 19].

In the results of the lethal dose (LD$_{50}$) in the study, no mortality was observed in any of the Ct groups. The results showed no reduction in the physical activity of the rats, change in the color of their fur, nor molting. In addition, no unusual changes were observed in the eye coloration, urine, and stool of all the treated rats. The oral LD$_{50}$ value was estimated to be >2000 mg/kg bw. In a previous study conducted on {\textit{Celtis durandii}}, the mean LD$_{50}$ and total lethal dose (LD$_{100}$) were estimated to reach 14.10 and 18 g/kg, respectively, with a mortality rate of 42% [20]. Therefore, the high doses of Ct might feature low toxicity and possibly safe and non-toxic.

The previous investigations have indicated that CCl$_4$ administration leads to increased AST, ALT, and ALP levels in the serum [1, 21]. The disruption of the liver cell integrity and the degeneration of parenchymal cells result in the rise in transaminase activities, as biomarkers of hepatotoxicity, in the serum. However, the {\textit{Celtis}} treatment of CCl$_4$-induced liver damage in rats has contributed to recovery, which is proven by the decreased activities of transaminases and LDH within 4 weeks of treatment. Limited data are available for the use of {\textit{Celtis}} species on liver injury. The methanolic extract of {\textit{Celtis timorensis}} leaves was reported to cause a significant reduction in the serum biochemical parameters against CCl$_4$ [21].

Lipid peroxidation is assumed to be related to the pathophysiology of CCl$_4$-induced hepatic damage. In the current study, the MDA level, as an indicator of oxidant/antioxidant imbalance, significantly increased in the CCl$_4$-administered rats. Another indicator of the oxidation and antioxidant status TOX and TAS that exhibited significant change with administration of CCl$_4$ group. The findings of this study showed that treatment with Ct reduced oxidation and increased the antioxidant status. In a previous study, El-Alfy et al. [22] reported that the aqueous and ethanolic extracts of {\textit{C. australis}} and {\textit{C. occidentalis}} leaves significantly reduced MDA formation in the presence of FeSO$_4$$-\text{H}_2\text{O}_2$ in rat tissue homogenates (brain, heart, and liver), indicating anti-lipid peroxidation activities in vitro. Antioxidant molecules in internal and external environment of the cell act synergistically and form TAS. Therefore, the measurement of the total antioxidant capacity provides more valuable information than the individual measurement of antioxidants. Hismiogullar et al. [23] reported the elevated hepatic TOS and suppressed TAS induced by CCl$_4$, these conditions were ameliorated by the curcumin treatment. Several previous investigations have specified that phenolic and flavonoid compounds, such as quercetin, kaempferol, gallic acid, caffeic acid, ellagic acid, rutin, p-coumaric, catechin, myricetin, and α-tocopherol, could prevent cell oxidation and damage [1, 24, 25]. Ct also contains these compounds and other aforementioned phenolic antioxidants [5]. The decreased lipid peroxidation and TOS in the treatment with Ct might be due to the presence of scavenger compounds. Furthermore, in the current study, SOD, GPx, and CAT activities and GSH content diminished the liver damage caused by CCl$_4$. The GSH depletion caused by CCl$_4$-induced toxicants affected adversely both the elimination of toxic metabolites, which are the main cause of liver injuries, and the activity of GSH-dependent enzymes [26]. However, Ct treatment prevented the toxic effects of CCl$_4$ by restoring the activities of antioxidant enzymes, such as SOD and GPx. Previous studies confirmed that these improvements in antioxidant enzymes can be attributed to the antioxidant properties of the {\textit{Celtis}} species [27-29]. Zanchet et al. [30] reported that {\textit{Celtis iguanae}} exerted protection by increasing SOD activity against plasma lipid peroxidation in cholesterol-fed rats.

In this study, CCl$_4$ administration caused severe histological changes and hepatic damage as manifested by significant increases in MDA, serum enzymes, and TOS, which are indicators of impaired cell integrity and liver function. Conversely, the treatment with Ct ameliorated liver injury, which was detected with less degeneration and necrotic changes. The Ct may prevent hepatic damage or lead to the reconstruction of damaged liver parenchyma. Several plants possess hepatoprotec-
tive properties due to phenolic and flavonoid compounds. Moreover, previous studies indicated that various phytochemicals, such as rutin, quercetin [31, 32], gallic acid [33], ferulic acid [34], vanillic acid [35], and naringin [36], effectively prevent the liver damage caused by CCl4 intoxication. Therefore, these phytochemicals might be responsible for the hepatoprotective effects of Celitis extract. These compounds might also synergistically modulate fibrosis and necrosis in the liver.

CONCLUSION

The results revealed that Celitis tournefortii might possess hepatoprotective effect against CCl4-induced liver damage given that it diminished the oxidative stress parameters and enhanced antioxidant status, also attenuating the degeneration and necrotic changes. Celitis tournefortii might be nutraceutically used to achieve oxidant/antioxidant balance for liver injury.

ACKNOWLEDGEMENTS

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ABSTRACT

Excessive accumulation of heavy metals in tea has certain health risk to the consumers. To discuss the spatial relationship between heavy metals in tea leaves and soil, 160 samples of tea leaves and corresponding subsoil (15–30 cm) were collected in the tea plantations of Zhejiang and Jiangsu. The total Al, Mn, Zn, Pb, As, Cd, Cu, Ni, Cr, and Se in both tea leaves and soil samples, as well as pH value in soil were analyzed. The results showed it is necessary to control the pH of soil in tea plantations which are close to villages or metal industries, as well as, to search more appropriate areas, where are far away from villages or metal industries, to plant tea should be taken into consideration. It is significant to construct a targeted standard of heavy metals contents that could applicable throughout the world for reduce the health risks to the consumers.

KEYWORDS:
Heavy metals, Tea leaves, Surface soils, Enrichment patterns, Health risk

INTRODUCTION

Tea, made from the leaves and buds of tea plant (Camellia sinensis L.), has a long history of over 5000 years in dietary and medicinal applications especially in Asian countries, such as China, Japan, India, and Thailand [1]. The bioactive components of tea mainly include proteins, polysaccharides, polyphenols, chlorophyll, minerals and trace elements, volatile compounds, amino and organic acids and alkaloids [2, 3]. Tea polyphenols are effective antioxidants and have pharmacological activities, but the content of polyphenols in the low-grade coarse tea is low. Tea polysaccharides (TPS) are the main bioactive components of tea, especially in the low-grade tea with a content from 0.8% to 1.5% [3-5]. In the high-grade tea, TPS levels are only from 0.4% to 0.9% [6].
the potential risk of tea consumption on metal intake by heavy tea drinkers.

MATERIALS AND METHODS

Collection of tea samples. A total of 160 samples representing four types of tea, which originate from Tea Garden in Jiangsu and Zhejiang.

Laboratory analysis method. Tea samples were dried at 65 °C to constant weight and ground into fine powder to obtain a representative sample. About 0.5 g of the sample was digested with HNO₃/H₂O₂ using USEPA Method 3050B on a hot block (Environmental Express, Ventura, CA). All elements from the digested samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Perkin-Elmer Corp., Norwalk, CT).

Tea and soil infusions were prepared by adding 100 mL of boiling double DI-water to 1.0 g of tea leaves in a 50 mL conical flask. The tea infusion was mixed using a glass rod to ensure adequate wetting, then covered and allowed to boil for 5 min based on tea industry's recommended brew time. The solution was filtered through a Whatman N. 40 filter, cooled, and diluted with double DI-water to 50mL. Concentrations of Al, Mn, Zn, Pb, As, Cd, Cu, Ni, Cr, and Se were then determined via ICP-MS. A standard solution was prepared by dilution of a 1000 mg L⁻¹ stock solution (Merck) prior to use. Double DI-water was used throughout this experiment. A standard solution is added to the sample to control the accuracy and accuracy of the test.

Al in the soil adopts the method [17], and Cu, Zn, Ni, Cd, Cr, Mn and Pb in the soil are based on the method of HJ 803-2016. The determination of As and Se in soil is based on the method of HJ 680-2013. The determination of Cu, Zn, Ni, Cd, Cr, Mn, Pb, Al, As and Se in tea is carried out by the method of GB 5009.268-2016.

Health risk assessment. In this study, potential risk of metal exposure from tea ingestion was assessed. The weekly intake of metals from tea ingestion was estimated following USEPA 1992 [18]:

\[ \text{TWI} = C \times \frac{W I}{B W} \]  

(1)

where TWI is the tolerable weekly intake (mg kg⁻¹ BW per week), C is the metal concentration in tea infusion (mg L⁻¹), WI is the average weekly intake rate of tea (L week⁻¹), and BW is body weight (kg). For children and adults, default BW is 10 kg and 70 kg.

Potential health risks from ingesting metals in teas were estimated for both children and adults. As per Sofuoglu and Kavcar [18], an estimated of weekly tea intake of 0.525 L by children <15 and 5.25 L by adults >35 years were consumed.

RESULTS AND DISCUSSIONS

The As contents in teas were 9.30–71.67 ug/g, averaging 33.73 ug/g (Fig.1-3). The contents of Al, Mn, Cr, As and Pb in the old leaves were significantly higher than those in the young leaves, especially the difference in Al content. The Al content in the old leaves reached 10 times the Al content in the young leaves; on the contrary, Cu, Zn and Ni is larger than the old leaves, indicating that different metal elements have different transfer and enrichment rules in different parts of tea. Except for Pb, the coefficient of variation of other elements in the old leaves is greater than that of young leaves, which may be due to the age of the tea tree and the difference between the old leaves collected in the sampling process and the old leaves and the earlier leaves. Its growth cycle is short and the coefficient of variation is relatively small. Because the tea leaves are prone to accumulate Pb in the atmospheric deposition, the difference in Pb distribution in the atmosphere leads to the variation of Pb in the young leaves compared with the old leaves.
For Fig. 4, Contour Plot of Zn (ug/g) vs B, A is shown. Tea garden soil is the main source of metal elements in tea. The metal element migrates from the soil to the tea leaves through the roots of the tea tree and accumulates. The enrichment factor (the ratio of the metal element content in the tea to the metal element content in the tea) is usually used to reflect the degree of enrichment and migration. The larger the enrichment factor is, the larger the enrichment factor is. The more easily the crop absorbs the element from the soil, the more mobile it is.

**FIGURE 2**
Surface Plot of Se (ug/g) vs C, B

**FIGURE 3**
Surface Plot of Se (ug/g) vs C, A
For Fig. 5-7, Contour Plot of Cu, Cd and As are shown. The enrichment ability of metal elements is stronger in areas with large slopes than in areas with small slopes. It may be because the area with small slope is more likely to accept the accumulation of foreign matter, such as atmospheric deposition and precipitation. Therefore, the source of metal elements in tea leaves is more than the area with large slope, and the metal elements absorbed from the soil are smaller than the area with large slope. The area with large slope is strongly affected by precipitation, and the foreign matter is difficult to accumulate. The metal elements in the tea are mainly derived from the soil, so the enrichment coefficient is large.

The enrichment factors of tea plants with different planting years vary greatly. The enrichment factors of the other 9 elements except Pb in the old leaves were more than 40a. The enrichment ability of tea trees for metal elements increases with the increase of planting years. Therefore, in the environmental quality monitoring of tea gardens, special attention should
be paid to the monitoring of metal elements in tea trees. The enrichment coefficient of Cu was the largest among teas of different planting years. The enrichment coefficients of old leaves and young leaves above 40 years were 6.5 times and 4.6 times lower than 40a, respectively. In general, the difference of enrichment coefficient of old leaves with different planting years is greater than that of young leaves, indicating that the effect of planting years on the enrichment of metal elements in old leaves is greater than that in young leaves.

**Health Risk Analysis of Tea Metal Elements.** Because the tea is not eaten directly, but by soaking and drinking, the metal elements in the tea can not be transferred into the human body. The transfer coefficient of the metal element between the tea and the tea soup needs to be calculated to enter the human body. The leaching rates of Cu, Zn, As, and Cd were 28.7%, 19.3%, 16.2%, and 40%, respectively. Assuming that the toxic effects of various metal elements in tea on human health are additive, rather than synergistic or antagonistic, the total health risk of tea in Jiangsu and Zhejiang is the sum of the health risks of four pollutants.
The average annual risk level of personal health caused by chemical carcinogens As and Cr is quite different, and the cancer risk level of Cd is 20 times that of As. The personal annual risk of Cd and As accounted for 95.5% and 4.5% of the total annual risk of the individual, respectively. It can be seen that Cd is the main risk pollutant. The average annual risk of chemical non-carcinogens Cu and Zn is lower than the maximum acceptable risk level recommended by the relevant agencies. The average annual risk of Zn health hazard is much lower than that of the Dutch Ministry of Construction and Environment and the Royal Society. Ignore the level of risk. The health risks caused by metal elements in tea garden tea mainly come from chemical carcinogen Cd, which should be paid attention to by relevant departments.

CONCLUSION

To discuss the spatial relationship between heavy metals in tea leaves and soil, 160 samples of tea leaves and corresponding subsoil (15–30 cm) were collected in the tea plantations of Zhejiang and Jiangsu. We found that As, Cd did not exceed the limit for medicinal plants recommended by WHO (2007) excluding one herbal tea for As.

The content of metal elements in old leaves and young leaves has a large difference, especially the difference in Al is the most significant. The effect of planting years on the content of metal elements in old leaves is greater than that in young leaves. The longer the tea age, the more the metal elements are enriched in the old leaves, but the effects on the young leaves are different.

ACKNOWLEDGEMENTS

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SOME DRYING PARAMETERS OF WATERCRESS  
(*NASTURTIIUM OFFICINALE R.*)

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**ABSTRACT**

In this research, drying characteristics, energy requirement and colour changes of Watercress (*Nasturtium officinale R.*) in hot-air drying with glass cabin were reported. Samples of freshly harvested Watercress were dehydrated in three different temperatures, 50°C, 60°C and 70°C. Selected drying air velocity was 0.65 m.s⁻¹ for all temperatures. Watercress were dehydrated from the initial moisture content of 390 (percentage dry basis) to a final moisture content of 7...9%. The minimum specific energy requirement was determined as 25.54 kWh.kg⁻¹ for 70°C. 60°C drying air temperature was found to yield better quality product in terms of colour retention of Hunter L, a, b and ΔE. As a result, in order to reduce drying energy consumption and to keep better colour retention, this hot-air drying can be recommended for this kind of application. Drying data were modelled with 8 different drying models. Models with the highest coefficient of regression (R²) and lowest standard error of estimation (SEE) were determined to be the most appropriate models. According to this, Aghbashlo model has been determined to be the best model at 50°C, 60°C and 70°C hot-air drying with glass cabin in terms of the closest values between experimental data and predicted data.

**KEYWORDS:** Watercress, hot-air drying with glass cabin, drying characteristics, energy requirements, colour retention, drying models.

**INTRODUCTION**

Aquatic plants lives in Wetlands. Wetlands, "natural or artificial, continuous or seasonal, sweet, bitter, or salty, stagnant or stream masses, swamps, turfishes and the immediate depth of the tide are defined as” sea waters that do not exceed 6 m and the areas in which they are located “. Wetlands are among the most productive environmental systems. These areas are the cradle of the water and biodiversity that countless species of plants and animals depend on to Survive.

There are many different drying methods that are used widely today, but the most common method in global terms is sun drying [1, 2]. Drying is the oldest method of food preservation and it requires high amounts of energy. The hot air drying is the most frequently used drying technique for fruit and vegetable [3]. Since the energy source is the cheapest, free and renewable, significant savings can be achieved with this type of drying. However, this technique is dependent on weather conditions, it has a longer drying duration, and is associated with issues such as dust and microbial attack etc. In addition, the required drying duration is about 2-4 times longer than that of greenhouse, cabinet and parallel air flow type dryers [4, 5].

In addition, the drying methods and dryer types used strongly affect the colour retention of the product. In recent years, much attention has been paid to the quality of foods during drying. The method used for drying affects various material properties such as colour, texture, density, porosity, and sorption characteristics [6]. Colour plays an important role in appearance, processing, and acceptability of food materials. Colour is one of the most important quality attributes, as the first quality judgment made by a consumer is based on the colour of the product and colour influences consumers’ food choices, perceptions, and purchase behaviours [7].

Watercress is a highly common herbal plant. This plant is a herbaceous flowering plant native to Europe, Asia, and North America, and is the best known member of the Watercress genus Brassicaceae. Besides, watercress grows in water in all regions of Turkey and has various local names: “gerdeme, acı gerdeme, kordamot, cırcır, çayır teresi, istapan, kerdeme, hoş kiran, sezab, tuzık, su mancası”. 100 grams of fresh watercress only has about 10 calories. 70 grams of watercress contains approximately 1.65 grams of protein, 0.1 grams of fat, 0.3 grams of fiber and 0.9 grams of carbohydrates. 70 grams of fresh watercress is very rich in vitamins and it provides 212% of daily vitamin K need, 50% of vitamin C and 45% of vitamin A. It is also rich in calcium, magnesium, potassium, vitamin E, vitamin B [8].

There is a theory that consumption of watercress can be linked to a reduced risk of cancer via decreased damage to DNA and possible modulation of antioxidant status by increasing carotenoid concentrations [9]. Watercress (*N. officinale R.*) is
ecologically and economically one of the most important freshwater macrophytes. It has been used by humans for many years as both a food and a medicinal plant. It is also used for the treatment of wastewater. The use of the consumed plant as nutrients should be widespread in rural areas [10]. This plant with heart-shaped leaves and little white flowers grows in wetlands and it is used in place of terenine. It is used in salads, in soups, in pastry mixtures, roasted with other herbs [11, 12]. Therefore, fresh and cleaned watercress were dehydrated in a computer connected hot-air drying with glass cabin at various temperatures and selected most suitable velocity to determine the drying kinetics, energy requirement and colour retention for drying in this experimental investigation. Besides, another aim of this study was to also investigate the effects of various drying methods and drying temperatures on the colour of dehydrated Watercress.

MATERIALS AND METHODS

Watercress grown in the South East Anatolian Region of Turkey were harvested manually and used for the investigation. Watercress were cleaned and separated from all foreign materials such as dust, dirt, pieces of branches and foreign leaves. Watercress were dried in a computer connected parallel flow type dryer. The hot-air dryer was equipped with an electric heater (air heating duct), temperature adjuster, centrifugal fan (blower), air speed adjuster (regulator of variable transformer), corrosion resistant chromium mesh, corrosion resistant chromium sheet, glass wood insulator, a 0.01 g sensitive HZQ Precision Weighing Balance digital balance, drying air inlet and outlet channels as well as a thermostat, temperature indicators and wattmeter (Fig. 1). The products were placed on the chromium mesh acting as a thin layer. In order to produce different temperatures and fix up the velocity, the electric current of the heater and the rotation of the fan were adjusted manually. The system was also automatically controlled by the thermostat. To measure the power consumption, air speed, relative humidity and drying air temperatures at different points, several digital devices such as watt meter, hot-wire anemometer with a measurement sensitivity of 0.1 m.s⁻¹, PeakTech 5060 humidity and temperature sensors and thermocouple were connected to the drying system. It should also be noted that our experimental drying studies show that the volume of the drying chamber is approximately 0.6m x 0.4m x 0.3m (0.072m³), depending on the temperature distribution of the drying air over the dryer length. Thus, the drying chamber was selected to be less than 1m in length. During these studies, it was also observed that when the length of the drying chamber was over 1m, there were important temperature and relative humidity differences between the beginning and the end of the drying chamber [13, 14, 15]. The moisture content (percentage dry basis) of fresh products, at harvest was approximately 390% (Eq. 1) [16]. Moisture content of the products were determined by using an air oven set at 105 °C, with a sensitivity of 0.01 an AND MX-50 humidity meter which kept going until reaching a constant weight [17, 18].

For safe long-term storage, the moisture content should preferably be less than 10%. For that reason, the fresh products with a moisture content of 390% were dehydrated until the moisture content became 7…9% in the dryer. During drying time, the mass of watercress samples were weighed automatically by the balance in every 30 minutes and all tests were repeated three times. The dryer was installed in a relative humidity of 40% (±3) and a temperature of 22°C (±1). This air was heated by the heater and directed to the drying chamber. Three different temperatures, 50, 60 and 70°C, and a selected air velocity of 0.65 m s⁻¹ were used for experimentation. This is due to the fact that it was observed in the preliminary studies that a temperature below 50°C and an air speed over 0.65 m/s is extremely increasing the drying time and energy requirement for this product [19]. The products were placed on the tray of the oven dryer for which the technical specifications are given in Table 1. During experiments, drying characteristics, total drying time, total energy needed for drying of one charge of the dryer, total energy requirement for drying 1 kg of wet product (specific energy requirement) and colour retention for different convective drying temperatures were found (Eq. 2 and 3) [20] (Holman, 1994). When drying was complete, the average moisture content of each sample was analysed in accordance with the vacuum oven method [17] (Eq. 1) and the Hunter L, a, b values of dehydrated Watercress were determined to study the colour of the samples. Colour was evaluated by measuring Hunter L (brightness, 100=white, 0=black), a (+, red; -, green) and b (+, yellow; -, blue) parameters by means of a reflectance colourimeter (CR 410, Chromometer, Minolta, Japan). A white tile was used to standardize the instrument. From the instrumental Hunter L, a, b values and the colour difference (ΔE) were calculated according to the Eq. 4.

$$PM_{db} = \left[ \frac{W_o - W_d}{W_d} \right] \times 100$$  \hspace{0.5cm} (1)

$$E_{t(c)} = A v \rho c \Delta T \cdot D$$  \hspace{0.5cm} (2)

$$E_{kg(c)} = \frac{E_{t(c)}}{W_o}$$  \hspace{0.5cm} (3)

$$\Delta E = \left[ (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \right]^{1/2}$$  \hspace{0.5cm} (4)
TABLE 1
Mathematical models applied to drying curves

<table>
<thead>
<tr>
<th>Model No</th>
<th>Model Name</th>
<th>Model Inequality</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Page</td>
<td>$M_e=\exp(-k't')$</td>
<td>[25]</td>
</tr>
<tr>
<td>2</td>
<td>Henderson &amp; Pabis</td>
<td>$M_e=a \exp(-kt)$</td>
<td>[21]</td>
</tr>
<tr>
<td>3</td>
<td>Thomson</td>
<td>$t=a.(\ln(M_0)+b.(\ln(M_e))^2$</td>
<td>[26]</td>
</tr>
<tr>
<td>4</td>
<td>Aghbashlloo et al.</td>
<td>$M_e=\exp(-k_1.t/(1+k_2.t))$</td>
<td>[27]</td>
</tr>
<tr>
<td>5</td>
<td>Jena &amp; Das</td>
<td>$M_e=a.exp(-kt+b)\sqrt{c}$</td>
<td>[28]</td>
</tr>
<tr>
<td>6</td>
<td>Demir et al.</td>
<td>$M_e=\exp(-kt+c)$</td>
<td>[23]</td>
</tr>
<tr>
<td>7</td>
<td>Alibas</td>
<td>$M_e=a.exp(-kt)+c$</td>
<td>[22]</td>
</tr>
<tr>
<td>8</td>
<td>Midilli et al.</td>
<td>$M_e=a.exp(-kt)+bt$</td>
<td>[29]</td>
</tr>
</tbody>
</table>

The differentiable Humidity (MR) (equality 5), regression coefficient ($R^2$) was the main criterion for the selection of the most suitable mathematical model for drying the Watercress with hot air. The standard error of the estimate (SEE) provides the difference between the measured and estimated data in all measurement intervals during drying processes and the ideal value should be "0". The regression coefficient of estimation ($R^2$) Equation 7, standard error (SEE) (Equality 8) and drying rate (DR) (Equality 6) are calculated using the following equations:

$$MR = \frac{M - M_e}{M_0 - M_e}$$  \hspace{1cm} \text{(5)}

$$DR = \frac{M_t + d_t - M_0}{d_t}$$  \hspace{1cm} \text{(6)}

$$R^2 = \frac{\sum_{i=1}^{N}(M_{exp,i} - M_{pre,i})^2 - (\sum_{i=1}^{N}(M_{exp,i} - M_{pre,i}))^2}{\sum_{i=1}^{N}(M_{exp,i} - M_{pre,i})^2}$$  \hspace{1cm} \text{(7)}

$$SEE = \sqrt{\frac{\sum_{i=1}^{N}(M_{exp,i} - M_{pre,i})^2}{N-n_i}}$$  \hspace{1cm} \text{(8)}

Mathematical modelling of drying curves was utilized from experimental data in order to provide a relationship between moisture content and drying time, and 8 different model equations were statistically compared (Table 1).

Nonlinear regression analyses were performed using the NLREG Advanced statistical program. The constants and coefficients (k1, k2, a, b, c, g, and n) for the equations defined in Table 1 were calculated by non-linear regression analyses. The statistical data related to 8 different drying methods defined in Table 1 as well as the constants and coefficients obtained with different thin layer patterns for hot-air drying are given in Table 3. [21, 22, 23, 24]
RESULTS AND DISCUSSION

During a drying process, two periods can be distinguished. The first is called constant drying rate period. The second drying stage is called the falling drying rate period. During the first period, the surface of the product behaves like a water surface. The rate of moisture removal during this period is mainly dependent on the surrounding conditions and affected only slightly by the nature of the product. The end of the constant drying rate period is marked by a decrease in the rate of moisture migration from within the product below to sufficient level to replenish the moisture being evaporated from the surface. The falling drying rate period is dependent essentially on the rate of diffusion of moisture from within the product to the surface and also on moisture removal from the surface. For agricultural products, the duration of each of the drying regimes depends on the initial moisture content and the safe storage moisture content. In particular, for fruits and most vegetables the drying would take place within both the constant and falling rate periods that can be seen easily. Both the external factors and the internal mechanisms controlling the drying processes in the two main rate regimes are important in determining the overall drying rate of products [16, 30]. For these reasons, the changing of the moisture content of Watercress must have two periods depending on the drying time. The moisture content of the products as a function of drying time are presented in Fig. 2 for different hot-air drying temperatures. As seen from these figures, all lines have two stages. The moisture content rapidly reduces and then slowly decreases with rising of the drying time. In addition, it is obvious from the figures that drying temperature has an important role in the total drying time (Fig. 3, 4). The lowest drying time (3.5 h) was obtained at 70 °C. The highest drying time (11.5 h) was observed at 50°C. The total energy requirement for a charge of dryer and energy needed for drying 1 kg of the product at a temperature of 70°C for watercress. The maximum energy (42.17 kWh/kg) is needed at the temperature of 50°C.

![FIGURE 2](image)

**FIGURE 2**
Moisture content as a function of hot-air drying time for temperatures of 50°C, 60°C and 70°C

![FIGURE 3](image)

**FIGURE 3**
Total drying time of product at different temperatures and power levels

Colour is an important quality attribute in food for most consumers. In addition, colour analysis is important for foods, especially as a quality criterion for the production and for the trade. It is an index of the inherent good qualities of a food product and associating colour with acceptability of a food product is a universal thing [31]. L, a, b and ΔE values are commonly used as an index to report the

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### TABLE 3

<table>
<thead>
<tr>
<th>Model No</th>
<th>50 °C</th>
<th>60 °C</th>
<th>70 °C</th>
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<tbody>
<tr>
<td>k</td>
<td>n</td>
<td>k</td>
<td>n</td>
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<td>0.4811</td>
<td>0.1356</td>
</tr>
<tr>
<td>2</td>
<td>0.0067</td>
<td>0.1751</td>
<td>0.0199</td>
</tr>
<tr>
<td>3</td>
<td>0.2786</td>
<td>0.6101</td>
<td>0.7190</td>
</tr>
<tr>
<td>4</td>
<td>0.0248</td>
<td>0.0075</td>
<td>0.0349</td>
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<td>0.9035</td>
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<td>0.1340</td>
</tr>
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<td>10</td>
<td>0.0679</td>
<td>0.0030</td>
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</tr>
</tbody>
</table>
colour quality. The changes in colour parameters of dehydrated watercress are presented in Figure 6. Samples dried at 50°C showed the highest Hunter L value, whereas the samples dried at 60°C gave the lowest L values. Different authors have reported that a decrease in L value correlated well with increases in browning of foods [32, 33]. The redness (a) value decreased in comparison to fresh samples and the highest decreases were found in the drying temperatures of 50°C. The yellowness (b) value was highest for the samples dried at 70°C. The colour changes in ΔE was also obtained for hot-air drying temperatures. The colour difference was relatively high for the samples dried at 50°C. Finally, the colour changes in samples dehydrated at 60°C were lower than the other drying temperatures.

With regards to the drying of watercress plant with glass cabin hot air, the time-dependent moisture content diagram is given in Figure 7, and the separable water content based on drying speed is given in Figure 8.

CONCLUSIONS

Watercress was successfully dried in hot-air drying with glass cabin dryer at different temperatures such as 50, 60 and 70°C, and an air speed of 0.65 m/s. It is concluded by the results of the experimental investigation that the drying air temperature has an important role on the total drying time, specific power consumption and Watercress colour changes. The main conclusion of this study is that watercress must be dried in hot-air drying at temperature of 70°C and an air velocity of 0.65 m/s to minimize the energy consumption and to keep the higher quality and colour retention for drying of watercress. Drying data were modelled with 8 different drying models. Models with highest coefficient of regression (R²) and lowest standard error of estimated (SEE) values were chosen to be the most appropriate models. According to this, the best model at 50°C, 60°C and 70°C hot-air drying with glass cabin in terms of the closest values between experimental data and predicted data was observed to be Aghbashlo model.
NOMENCLATURE

\( A \) : Drying air flow surface area, m²
\( c \) : Specific heat of air under adiabatic conditions, kJ/(kg.K)⁻¹
\( D_t \) : Total drying time, h
\( E_{kg(c)} \) : Energy requirement for drying 1 kg of product and for hot-air drying, kWh/(kg)⁻¹
\( E_{te(c)} \) : Total energy requirement for a charge of the hot-air dryer, kWh
\( PM_{db} \) : The moisture content on dry basis expressed as percentage, %
\( v \) : Drying air speed, m.s⁻¹
\( W_d \) : Weight of dry matter in product, kg
\( W_o \) : Initial weight of non-dried product, kg
\( \Delta T \) : Temperature differences, K
\( \rho \) : Air density, kg.m⁻³
\( MR \) : Separable humidity
\( M \) : Moisture content at any moment [(kg of water) (kg dry matter)⁻¹]
\( M_{b} \) : Balance moisture content (kg water) (kg dry matter)⁻¹
\( M_{f} \) : First moisture content of the product (kg of water) (kg dry matter)⁻¹

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MATERIAL AND MECHANICS PERFORMANCE OF FULL DEPTH ASPHALT PAVEMENT

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ABSTRACT

Full depth asphalt pavement is to evaluate the laboratory results of stabilizing the blend. The unconfined compressive strength (UCS) tests and response surface methodology were carried out. Results show that UCS increase by increasing cement or decreasing RAP content. By assuming a constant percentage of RAP, by increasing one percent of the cement, the UCS value for the stabilized layer increased by an average of 400 kPa, respectively. Only in the sample containing 15% of reclaimed asphalt pavement (RAP), maximum dry density increases when the cement content increases from 0 to 4 percent. Furthermore, at a fixed percentage of cement, increase in RAP content result in decreasing optimum moisture content.

KEYWORDS:
RAP, Stabilization, Full-depth reclamation, Unconfined compressive strength

INTRODUCTION

Various methods of pavements recycling are used at present as cost-effective ways to improve flexible pavements. Generally, there are three methods of pavement recycling, including cold recycling, hot recycling, and full-depth reclamation [1].

If the damage to the pavement is related to the upper layers and the pavement has good conditions in respect of bearing capacity, hot recycling is a good alternative. If the pavement has structural weaknesses with serious damages, full-depth reclamation is suggested, and if the case lies between these two states, cold recycling can be effective [2]. It is important to determine whether pavement damage is structural or not. The main damage, indicating structural weakness of pavements, includes fatigue in wheels path, rutting, and reflecting cracking [3].

In full-depth reclamation, all the asphalt thickness, together with parts of the aggregates beneath it, is used to create a stabilized base course [4]. Application of full-depth reclamation results in an increase in a pavement’s bearing capacity, structural strength and stability, extending of lifetime, and improvement of pavement conditions. In cases where the pavement structure has low bearing capacity, full-depth reclamation is very effective. The costs of full-depth reclamation are 25–50 percent lower than normal pavement reconstruction [5, 6]. Full-depth reclamation and cold recycling are more economic compared to other in-place recycling methods; however, full-depth reclamation is considered more desirable because it is able to remove structural problems as well [7, 8]. Full-depth reclamation has been successfully employed by various departments [9-12] and has been reported to have some advantages such solving pavement problems deeply and eliminating structural problems of the pavement; In this method, asphalt concrete layer and the layer beneath it are re-used and there is no need for material depots; Environmentally friendly and keeping the thickness of the pavement constant and preventing the increase in the thickness of the repaired pavement.

Full depth asphalt pavement consists of five main steps including pulverization, blending the materials, formation, compaction and application of the surface course [13].

Full depth asphalt pavement is a pavement rehabilitation technique in which all of the asphalt pavement section, as well as a predetermined amount of underlying base material, are treated, pulverized, mixed and compacted to produce a thicker, stabilized base course [14]. Full Depth Asphalt Pavement is typically performed to a depth of 100–300 mm. There are several major advantages to this technique. It completely eliminates and corrects pavement distresses extending below the surface layer, unlike many other maintenance techniques, and can actually increase the structural capacity of the pavement [15]. Additionally, the use of in-situ material can result in about 30–50 percent cost savings and cut down on greenhouse gas emissions by about 50 percent as well. Along with cost and emission savings, in-place recycling procedures, such as full depth asphalt pavement, can be constructed in less time than a full reconstruction project.

The present study is aimed at investigating the effect of various percentages of cement and reclaimed asphalt pavement on optimum moisture content, maximum dry density, unconfined com-
pressive strength (UCS) of the treated base course in full depth asphalt pavement.

MATERIALS AND METHODS

Fabrication of samples. 72 h before the test, soil samples and RAP materials were exposed to room temperature in order to eliminate the moisture; furthermore, particles with the size above 2.5 cm (1 inch) were removed. According to the thickness of asphalt concrete layer in Sirjan streets and literature review, four different RAP to soil ratio of 0/100, 20/80, 40/60 and 60/40 were treated with four different percentages of Portland cement (3, 4, 5 and 6 percent). At first, optimum moisture content and dry density were obtained using modified proctor compaction test in accordance with the ASTM D-180 method C. The height and diameter of the mould in this method are 116.43 and 101.16 mm respectively. The soil was poured into the mould in 5 layers and each layer was compacted with 56 blows. After 24 h, the samples were dismantled from the mould, and wrapped in vinyl bags for curing. Unconfined compression tests were conducted on the samples after 7 and 28 days of curing.

Unconfined compressive strength (UCS) test. UCS is a common test in assessment of stabilized materials and has been used by many researchers to investigate the improvement in strength properties of treated or stabilized soil. According to Method A, ASTM D1633, the test was carried out using a machine with loading rate of 1 mm/min. The moulds used for compression were the same as the moulds in modified proctor compaction test (ASTM D-180 method C). The UCS was determined based on the average of two measurements. For this purpose, two samples were cured for 7 days and two samples were cured for 28 days. The final amount of UCS for each specific curing time (7 or 28 days) was assumed as the average of the two UCS measurements.

RESULTS AND DISCUSSION

Effect of moisture and soil content on UCS is shown in Fig.1. It is evident that at a fixed RAP/soil content, with increase in cement content, optimum moisture content and maximum dry density decrease. Only in the soil-RAP mixture containing 20% of RAP, by increasing cement from 0 to 4 percent, maximum dry density increases.

Effect of cement content and soil content on UCS is shown in Fig.2. After full-depth reclamation, the stabilized soil-RAP mixture will be used as a stabilized base course. The minimum allowable value of 7-days UCS for the stabilized base course has been considered to be 2100 kPa (300 psi). Previous researchers have stated that any UCS smaller than 2100 kPa leads to decreasing pavement-loading capacity under traffic loading. In the present research, the minimum allowable value of 7-days UCS for the stabilized soil-RAP mixture was considered as 2100 kPa.

Effect of cement content and moisture on UCS is shown in Fig.3. As can be seen from Fig. 3, in a constant amount of cement, by increasing RAP, optimum moisture content decreases. This is because water absorption is decreased as the amount of particles coated with bitumen is increased.
peak of stress–strain diagram and is the mean of many tests. As can be observed, for both types of soil, at a fixed RAP/soil ratio, the unconfined compression strength of the treated material increases by increasing the percentage of cement. So that by increasing one percent of the cement in a constant RAP, the UCS values for the stabilized layer increased by an average of 400 kPa.

Effect of moisture and RAP content on UCS is shown in Fig. 5. Fig. 5 show the changes in optimum moisture content and maximum dry density versus cement percentage, respectively. At a fixed soil/RAP ratio, by increasing the percentage of cement, optimum moisture content and maximum dry density decrease. So that in a constant amount of RAP, by one percent of increasing of cement content.

Effect of RAP content and cement content on UCS is shown in Fig. 6. It should be noted that the proposed equation and its coefficients are just valid in case of the materials utilized in the present research. In order to propose a comprehensive equation for predicting UCS of soil-RAP blend stabilized with the cement, more researches are needed.

CONCLUSION

The unconfined compressive strength (UCS) tests and response surface methodology were carried out. Results show that UCS increase by increasing cement or decreasing RAP content. By assuming a constant percentage of RAP, by increasing one percent of the cement, the UCS value for the stabilized layer increased by an average of 400 kPa, respectively. Only in the sample containing 15% of reclaimed asphalt pavement (RAP), maximum dry density increases when the cement content increases from 0 to 4 percent. Furthermore, at a fixed percentage of cement, increase in RAP content result in decreasing optimum moisture content.

REFERENCES


LIMITED DISSOLVED OXYGEN CSTR NITRIFICATION: MICROBIOME ANALYSIS

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ABSTRACT

Operating process at low dissolved oxygen (DO) concentrations is a good way to reduce the energy costs in wastewater treatment. Considering the limited DO concentrations (0.2~0.3 mg/L), it remains unclear whether nitrification will be achieved and which microbial groups are affected during the process. In the study, nitrification was realized in the nitrifying reactor at limited DO concentrations (0.2~0.3 mg/L), and the bacterial community both in the nitrifying sludge and the seeding sludge almost has the same phyla after over two months operation. However, autotrophs of Nitrosomonadales instead of heterotrophs of Burkholderiales and Rhodocyclales finally became the dominant microbes in nitrifying sludge. Regarding nitrifiers, the respective abundance of nitrite oxidizing bacteria (NOB) of Nitrospira and ammonium oxidizing bacteria (AOB) of Nitrosomonas were 2.41% and 37.4% in nitrifying sludge. In spite of the low ratio of NOB to AOB, the reactor performed nitratation process rather than nitritation process. The study characterized the microbial community shift, evidenced that efficient nitrification can occur at limited DO concentrations (0.2~0.3 mg/L), and demonstrated the relationship between performance and microbial structure in the nitrification reactor.

KEYWORDS:
Nitrification, Microbial community, Limited dissolved oxygen, Nitrosomonas, Nitrospira

INTRODUCTION

It has been well documented that the stable and efficient lab-scale nitrification can be achieved at dissolved oxygen (DO) concentrations even below 0.5 mg·L⁻¹ [1-4]. Also, sludge cultivated under long-term low DO conditions had a greater ammonia oxidation capacity compared with the high DO enrichment [1, 2, 5], which suggested that if efficient nitrification in low DO condition extended to full-scale plants, which would be feasible and possible to minimize the operational cost without lower the effluent quality.

Nevertheless, previously studies have conflicting perspective on the mechanism of nitrifying systems at low dissolved oxygen that whether improving ammonia oxidation capacity [5]. Recently, studies demonstrated that increasing sludge nitrification capacity under long-term low DO conditions was due to the elevated abundance of AOB in activated sludge [1, 2]. Moreover, based on the findings by Fitzgerald et al. [6], it was implied that there were significant numbers of unidentified ammonia-oxidizing microorganisms participated in ammonia oxidation via autotrophic and heterotrophic ways in low DO condition. They proposed Pseudomonas, Xanthomonadaceae, Rhodococcus, and Sphingomonas are potentially involved in low-DO nitrification. Overall, there are many documents to explain the mechanism of efficient nitrification in low DO; however, it is difficult to support them with conclusive experimental evidence. Regarding the bacterial community in low DO nitrification process, so far as we know, only very few studies have been launched [6, 7]. Therefore, a better understanding of the structure and function of nitrifying sludge communities with good performance at low DO may be essential for improving and optimizing wastewater treatment process.

The main objective of this study was to investigate whether nitrification will be achieved and how the microbial community shift during the limited DO concentrations (0.2~0.3 mg/L), and thus to provide the comprehensive insight into the relationship between key microbial groups and nitrification performance in the limited DO (0.2~0.3 mg/L) nitrification process by Illumina MiSeq sequencing technology.

MATERIALS AND METHODS

The experiment set-up and origin of the biomass. One bench scale continuously stirred tank reactor (CSTR) with a working volume of 2.4 L was set up (Fig. 1). The reactor was fed continuously with synthetic wastewater to achieve a hydraulic retention time (HRT) of approximately 6 h.
Throughout the duration of this study, sludge was periodically wasted from the reactor to maintain an solids retention time (SRT) of 20 d. The DO concentrations were maintained in the reactor 0.2–0.3 mg L⁻¹ by continuous DO measurement and air supply control. Air was provided using air diffusers connected to air pump, and mixing was achieved through continuous overhead mechanical stirrers. Synthetic wastewater, containing 190 mg/L NH₄Cl, 50 mg/L K₂HPO₄, 20 mg/L CaCl₂·2H₂O, 25 mg/L MgSO₄·7H₂O, 1 mg/L of trace element solution as described by Bellucci et al. [2]. In order to focus on the study of the nitrification process and communities, no organic matter was put into the influent. The pH in the reactor ranged from 7.0 to 7.5, controlled by a buffer containing NaHCO₃. Operational temperature was approximately 30 °C. The reactor was seeded with activated sludge from Quyang WWTP (Shanghai, China) operated an anaerobic/anoxic/aerobic (A²O) process.

Physical and chemical analyses. All water samples were filtered by a 0.45 μm filter before analyzing. Concentrations of NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N were all measured according to standard methods. DO and pH were monitored by pH/oxi1970i meter with DO and pH probes (WTW Multi1970i, Germany).

Illumina MiSeq Sequencing Technology. In order to analyze the microbial community structure in the nitrify system, sludge samples collected on Day 0 and 65 were marked as ‘seeding sludge’ and ‘nitrifying sludge’ respectively, which were taken from the CSTR for DNA extraction using FastDNA Spin Kit for Soil (MP Biomedicals, LLC, Solon, OH). DNA samples were amplified in triplicate by PCR using primer set F515 (5'-GTGCCAGCMGCCGCGG-3') and R907 (5'-CCGTCATATGCAGTTCAGTCT-3') for the V4 region of bacteria 16S rRNA gene. The 12-nucleotide barcodes were added to the 5' end of R907 to allow multiplexing. PCR reactions were performed as described by Wang et al. [8]. A mixture of the amplicons was sequenced on an Illumina MiSeq platform according to the standard protocols. To minimize the effects of random sequencing error, low-quality sequences were removed by eliminating those without an exact match to the forward primer. The sequences were clustered into operational taxonomic units (OTUs) by setting a 0.03 distance limit (equivalent to 97% similarity) using the MOTHUR program. OTU evenness was estimated by Functional organization indices (Fo) [9]. Sequences were also phylogenetically assigned to taxonomic classifications using an RDP classifier Bayesian Algorithm.

RESULTS AND DISCUSSION

Reactor performance. The nitrifying reactor was operated for more than two months under a limited DO (0.2–0.3 mg/L) condition. The ammonium removal loading rate achieved in the reactor was considerably high (approximately 0.11 g/(m³·d)) compared with other reported low-DO nitrifying systems [1, 2, 6]. It should be noted that nitrate was the dominating product in the effluent, whereas nitrite concentrations maintained at less than 1.5 mg/L throughout the duration of the study (Fig. 2), indicating that the continuous process performed nitrification process (ammonium conversion to nitrate) rather than nitritation process (ammonium conversion to nitrite). These results are consistent with the findings of previous studies treating low-strength wastewater [1, 2, 6], they demonstrated that efficient nitrification without nitrite accumulation in stabilized effluent could be achieved under low DO (DO <0.3 mg/L) conditions.
Biodiversity of the bacterial community. Two 16S rRNA gene libraries (Table 1) were constructed from Illumina MiSeq sequencing of seeding sludge and nitrifying sludge with 12304 and 11546 high-quality reads, respectively. The coverages were more than 99%, revealed that most communities were employed to sequence. A total of 491 and 397 OTUs were acquired respectively at 3% divergence (97% similarity). ACE and Chao1 estimators dropped from 517 and 518 in the seeding sludge to 462 and 449 in the nitrifying sludge, respectively. The Shannon diversity index also decreased from 5.00 to 3.75. All these indices demonstrated that the bacterial community decreased in the nitratation reactor.

Previous studies indicated that community evenness was a key factor in keeping the functional stability of a bio-ecosystem [10, 11]. Thus, Functional organization (Fo), graphically represent by Pareto-Lorenz (PL) evenness curves, was introduced in this study to assess changes in community evenness visually [9]. As show in Fig. 3A, 20% of the cumulative proportion of OTUs corresponded to 77% and 88% of the cumulative relative abundance of the OTUs from the seeding sludge and nitrifying sludge, respectively. The higher Fo index value of nitrifying sludge represented a much more specialized community of the nitrifying sludge, in which species were distributed more unevenly than those in seeding sludge. In a word, the biodiversity in terms of community evenness in nitrifying sludge obviously decreased and a highly functionally organized microbial community gradually formed. Furthermore, the Fo index of the shared and unique bacterial communities based on OTUs between the seeding sludge and the nitrifying sludge also evaluated (Fig. 3B).

The microbial communities in the shared OTUs of the two samples showed nearly identical PL-curves and similar Fo index, indicating that the shared microbiome had similar distribution patterns. However, regarding the Fo of the unique OTUs, the Fo index of S-U was at the medium-level nearly 60%, whilst the Fo index of N-U was above 90%, indicating significant functional organization shift occurred in the unique OTUs. As immigration can be precluded in this experimental system, detectable changes in OTUs must represent selective growth under the conditions imposed. In other words, the microbial community in the nitrifying reactor became highly functionally organized in the limited DO condition.

### TABLE 1
Richness and diversity estimators of the bacteria phyotypes

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<tr>
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</table>

![Figure 2](image2.png)

**FIGURE 2**
Reactor performance with time

![Figure 3](image3.png)

**FIGURE 3**
Pareto-Lorenz curves derived from the seeding sludge and the nitrifying sludge based on OTUs at 3% divergence. (A) the overall bacterial communities, and (B) the shared and unique communities between the seeding sludge and the nitrifying sludge.

The 45° diagonal represents the perfect evenness of a community. Fo is quantified as the cumulative proportion of OTU abundances when the cumulative proportion of species is 0.2. S-U and N-U represent the unique OTUs of the seeding sludge and the nitrifying sludge, and S-S and N-S refer to the shared OTUs.
Bacterial community composition. Fig. 4 shows the relative bacterial community abundance at the phylum level. Compared with seeding sludge, the bacterial community in nitrifying sludge distributed almost over the same phyla after over two months operation, except for a small number of minor phyla accounting for no more than 0.6% of total community found only in seeding sludge (Student’s test, p>0.05). Moreover and interestingly, recent high-throughput studies showed that bacterial communities of municipal WWTP reactors operated under diverse conditions (e.g. different configurations, different geographic locations or different seasons) were highly similar at the phylum level [12-14]. 

Within the predominant phyla of Proteobacteria, Alpha-proteobacteria (2.1-5.7%), Beta-proteobacteria (28.5-50.3%), Gamma-proteobacteria (4.5-7.7%) and Delta-proteobacteria (2.3-4.5%) were four classes detected from the nitrifying sludge and the seeding sludge (Fig. 5A). Among the four classes, Beta-proteobacteria was the most abundant class, which is in agreement with previous investigations on the community structure of Proteobacteria phylum in low DO bioreactors [7, 16] and sewage treatment plants [13, 14]. Since Beta-proteobacteria were also shown the greatest enrichment in nitrifying sludge, we further analyzed the taxonomic structure down to the order level for a more detailed characterization of the Beta-proteobacteria community evolution. As show in Fig. 5B, Burkholderiales (18.6%) and Rhodocyclales (8.3%) were the two major orders in the seeding sludge, whereas Nitrosomonadales (38.4%) became the most abundant in the nitrifying sludge and followed by Rhodocyclales (5.5%), Burkholderiales (4.2%) and SC-1-84 (1.4%). Since most of the Burkholderiales and Rhodocyclales were heterotrophs and thus both of them depleted might due to the absence of organic matter in influent. Reciprocally, the highly enriched Nitrosomonadales in nitrifying sludge possibly attributed to the growth of ammonia oxidation bacteria in the low DO reactor, since ammonia was the only substrate in influent, and Nitrosomonadales include the well-known AOB genera of Nitrosomonas and Nitrospira.

Inconsistent with the aforementioned result at the phylum level, the microbial communities of the nitrifying sludge and the seeding sludge were quite different at the genus level (Student’s test, p<0.05, Fig. 6), indicating that community differences were more evident at lower taxonomic levels [7, 13, 14]. The abundances of Saprospiraceae_uncultured, Azospira, Thermomonas, Alcaligenaceae_ uncultured dominated in seeding sludge were remarkably reduced, while Nitrosomonas, Rhodocyclaceae_ uncultured, NS9_marine_norank and Nitrospira were highly enriched in nitrifying sludge. This phenomenon indicated that the microbial community was shaped over time in the limited DO concentration (0.2-0.3 mg/L).
Genera making up less than 1% of the total composition in both libraries are defined as “Minor genus”.

Regarding the well-known nitrifiers, it was found that Nitrospira and Nitrosomonas were the dominant nitrite oxidizing bacteria (NOB) and AOB in nitrifying sludge, accounting for 2.41% and 37.4% respectively. Nevertheless, the process performed nitratation process rather than nitritation process at the low ratio of NOB to AOB. Except Nitrosomonas and Nitrospira, the other known AOB and NOB genus were very rare in the reactor. Nitrosococcus, another genus belonging to Gammaproteobacteria AOB, accounted for only 0.4% in the nitrifying sludge. For NOB, only 0.2% Nitrobacter
was found in the nitrifying sludge and 0.1% was found in the seeding sludge. These results suggested that expect *Nitrosomonas* and *Nitrospira*, all other well-known nitrifiers play only a very limited role in the limited DO nitrification process.

**CONCLUSIONS**

In this study, nitrification was achieved in the limited DO concentrations (0.2–0.3 mg/L), and Illumina MiSeq high-throughput sequencing was applied to investigate the bacterial community in the nitrifying reactor. The results showed that the biodiversity including species richness and community evenness in nitrifying sludge decreased compared with that in seeding sludge. *Beta-proteobacteria* was always the predominant class in the reactor. However, *Nitrosomonalales* instead of *Burkholderiales* and *Rhodocyclales* became the dominant order after over two months operation. *Nitrospira* and *Nitrosomonas* were responsible for the nitrification in the limited-DO reactor. The limited DO concentration (0.2–0.3 mg/L) shaped the microbial community and selected for a community that performs nitrification process.

**ACKNOWLEDGEMENTS**

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ESTIMATING QUANTITATIVE TRAITS BASED PHENOTYPIC DIVERSITY IN DIVERSE RICE GERMPLASM

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ABSTRACT

Rice is one of the important staple food crops and cultivated worldwide. In present study we have evaluated 49 diverse rice germplasm for economically important quantitative traits. Thirty key yield related traits including days to heading and maturity, percent lodging, ligule length, number of nodes, peduncle length, grain yield, leaf length/width, plant height, stem height and diameter, primary and secondary branches/panicle, total tillers and productive tillers/plant, 1,000 grains weight, grain length, breadth and thickness, etc. were measured. All the genotypes showed varied response to different traits. Maximum grain yield/plant (23g) and seed yield/plant (25g) was recorded in IR-84675-7-3-2-B-B and IRGC 33498 genotypes, respectively. The elite genotypes IR-OSF101, IR80416-B-32-3, IR-84678-25-5-B, IR-84677-21-1-B, IR-84675-7-3-2-B-B, IRGC 29008, IRGC 25926, IRGC 33498, IR-83140-B-32-B, HH25-SAL-9Y3-Y1, IRO-4L-191 and IR-83142-B-79-B were also noted that showed better morphological performances than other genotypes. The high yielding elite rice genotypes are recommended for breeding program.

KEYWORDS:
Genetic variability, Elite lines, Rice, Quantitative traits, Yield related traits

INTRODUCTION

Rice, *Oryza sativa* L., is one of the important model species and commonly grown tropical, subtropical and other monsoon regions of the world [1]. It is the second major staple food crops for the Asian countries including Pakistan [2]. The Asian countries produce maximum rice (~92%) [3] and cultivated in 112 different countries. In Pakistan it is one of the major sources of income for people and foreign export and named as “Golden Grain of Pakistan” [4]. The rapid increase of population needs further improvement in the rice yield. It is important to developed new high yielding rice genotypes [5]. Hence, agro-morphological study is important for further improvement of any breeding program [6-12].

Morpho-biochemical and molecular based diversity help in identification and characterization of elite genotypes [13-17]. The agro-morphological based variability of rice has been reported by many researchers. Li et al. [18] reported the correlation between the morphological traits and genetic differentiation of 111 germplasm of two important rice groups i.e. Japonica and Indica. Yawen et al. [19] found significant morphogenic differences among 5285 indigenous rice genotypes of China. Patra and Dhua [20] also explored the morphological based differences in some important rice genotypes of India. Yibo et al. [21] found maximum iter-specific and itra-specific morphological variability in eleven *O. rufipogon* Griff populations of Hainan Island, China. Nascimento et al. [22] recorded high level of genetic diversity for both qualitative and quantitative traits among 146 rice germplasm. They recorded five highly diverged genotypes having maximum spicklets/plant. Bibi et al. [23] reported significant level of morphological diversity among 116 rice genotypes of Northern areas of Pakistan. They recorded maximum variability for three important quantitative traits i.e. yield/plant, flag leaf length and sterile culms/plant. They also reported seven different groups through phylogenetic analysis. In recent studies, Ahmed et al. [24] reported morpho-biochemical and molecular based variability among 31 rice genotypes of Bangladesh and recorded maximum variability in different cultivar groups. Pachauri et al. [25] evaluated 124 rice germplasm for 19 morphological traits. They
recorded maximum variability in two important quantitative traits i.e. number of effective tillers and 100 grain weight. They also noted 72.48% variability in first four principal component groups. The present study was conducted to explore highly diverse 49 rice genotypes for various economically important quantitative traits and to identify promising genotypes for future breeding program.

MATERIALS AND METHODS

Plant Materials and Experimental Design. The present experiment was conducted at Plant Genetic Resource Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The fresh seeds of 49 rice germplasm were acquired from PGRI, gene bank. The seeds were than grow in small pots with optimum water supply. After twenty days of germination, the young plantlets were shifted to field condition by using augmented design. The length of row (4.5 meters), row x row distance (30 cm) and plant x plant distance (15cm) was used. All the required cultural practices were applied throughout season to maintain optimum growth and development. The water stress was avoided by applying optimum water treatment at proper time.

Assessing Economically Important Quantitative Traits. A total of 30 quantitative traits i.e. days to heading and Maturity, percent lodging, ligule length of, total number of nodes, panicle, ligule and peduncle length, plant, seed yield/plant, straw yield, harvest index, leaf length/width/area, height of plant, stem diameter and height, total tillers or productive tillers per plant, grain related traits, primary branches and secondary branches/panicle, total/visible or sterile spikelet/plant, grain weight/length/width/thickness and grain chalkiness percentage were measured.

Data analysis. The quantitative data of basis statistics was analyzed by using statistical software “Statistica version 7”.

RESULTS

Diverse Rice Germplasm Shows Variation for Yield Related Traits. A total of 49 rice germplasm were assessed for some key morphological traits. All the traits show direct relation with one another. In addition, the morphological response among genotypes varied with type of genotype. Some genotypes showed better performance than three check cultivars i.e. Super Basmati, JP-5 and IR-6. Maximum (30) quantitative traits were measured and all the genotypes showed variable response to each traits. Our findings showed significant variability among all germplasm (Table 1-5).

Days to Heading (DH) and Days to Maturity (DM). Days to heading is an important quantitative trait and having direct relation with potential and average yield. It values range from 59 to 79 days. The genotype IR-84678-25-5-B and IR-84677-21-1-B had lowest days to heading (59 days). The highest days to heading (79 days) was noted in genotypes IR6 and Super-Basmati (79 days). The mean value (66.7), standard deviation (SD) (4.6 %) and coefficient of variation (CV) (7%) was recorded for this trait (Table1). The early maturity is useful to gain maximum yield. For economically point of view the early mature genotype give maximum yield than late mature genotypes. Our results showed that days to maturity value ranged from 95 to 115 days. The genotypes IR-84677-21-1-B, IR-OSF101, IR-83142-B-8-B-B, IR-84678-25-5-B and IR80416-B-32-3 mature early than other genotypes. The mean value measured by this trait was 101 with CV (4.8%) (Table 1).

Percent lodging (%Lg). The high level of lodging has negative impact of yield. Our results showed that lodging frequency was very low and recorded in only few genotypes. The percent lodging values ranged from 0-10% with CV (31.95%) (Table 1).

Ligule length (LL) and Peduncle Length (PL). High level of variability was observed among genotypes for ligule length and its value ranged from 0.7 to 5.7mm. The highest LgL (5.7mm) and lowest LgL (0.7mm) LgL was recorded for genotype IR-83142-B-6-B and IRGC 28986, respectively. The mean value for this character was 1.7 with CV 38.8% (Table 1). Similarly, the maximum (51.2cm) and minimum (22.3cm) PL values were noted for genotypes IRGC 29008 i.e. and RSP-2 respectively. The mean value 30cm with CV 15.2% was recorded for this trait (Table 1).

Number of Nodes (NN). Moderate level of variability was noted for number of nodes in all 49 tested genotypes. Number of nodes value varied from 3-7 with mean value 4.6 and CV 15.2 % (Table 1).

Grain Yield/Plant (GY/P), Seed Yield/plant (SY/P) and Straw Yield/Plant (SY/P). Grain yield is one of the key quantitative traits for determination of elite genotype. In present study maximum genotypes showed better Grain Yield/Plant (GY/P) and its value among genotypes varied from 14.4-22.8g. Genotype IR-84675-7-3-2-B-B showed maximum GY/P (22.8g), minimum GY/P was recorded for genotype HH25-
SAL-9Y3-Y1. The CV 30.9% with mean value of 14.4g was determined for this trait (Table 2). While maximum seed yield/plant was recorded for genotype IRGC 33498 (25g) followed by IRGC 25926 (24g) (Table 2). Similarly the straw yield/plant values ranged from 5.5-24.9g with mean value of 13.7g and CV 34.5%. The highest SY/P (24.9g) was observed for genotype IRGC 33498 (Table 2).

### TABLE 1

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Mean: 66.7 101.1 0.7 1.7 4.6 30
Minimum: 59 95 0.7 3.2 22.3
Maximum: 79 115.3 10 8.7 7 51.2
SD: 4.6 4.9 2.3 0.7 0.7 4.6
CV: 7 4.8 319.5 38.8 15.2 15.2
Variance: 21.6 23.6 5.2 0.4 0.5 20.9
Harvesting Index (HI). Harvesting Index (HI) was measured by dividing economic yield by biological yield. High level of variability was observed for this trait. The Maximum HI value 3% was recorded for IRO-4L-191. The mean value 1.12% with CV of 34.5% was noted for this character (Table 2).

### Table 2

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**Mean**: 14.4  
**Minimum**: 6.6  
**Maximum**: 22.8  
**SD**: 4.5  
**CV**: 30.9  
**Variance**: 19.8

Harvesting Index (HI) was measured by dividing economic yield by biological yield. High level of variability was observed for this trait. The Maximum HI value 3% was recorded for IRO-4L-191. The mean value 1.12% with CV of 34.5% was noted for this character (Table 2).
Leaf length (LL), Leaf width (LW) and Leaf Area (LA). Leaf Length (LL) trait also shows significant divergence among all rice genotypes. Its values ranged between 16-40cm and highest LL (40cm) was recorded for genotype IRGC 29014 with CV of 18.3% (Table 2). The Leaf Width (LW) values among genotypes ranged from 0.8-1.5 cm. The highest value (1.5 cm) was noted for IRGC 31674 and IRGC 29151 (Table 2). Similarly, maximum variability was recorded for Leaf Area (LA). Its values ranged from 11.2-34.1 cm. The highest value (34.1cm) was recorded for genotype IRGC 29014. The mean value for this economically important trait was 24.3cm with CV of 24.3% (Table 2).

Plant height (PH). All three types (dwarf, semi-dwarf and maximum height) genotypes were recorded in present study. The Plant Height (PH) value ranged between 57-127cm. Genotypes IRGC 26390 and IR-OSF101 gave maximum (127cm) and minimum (57.4cm) plant height, respectively. The CV of 19.7% with mean value of 86.7% was recorded for this trait (Table 3).

Panicle Length (PL). The Panicle Length (PL) was measured from the tip to base of panicle and showed maximum divergence among genotypes. The maximum PL (27.8cm) was noted in genotype IRGC 25926 and minimum (18.9cm) in IRGC 33498 (18.9cm) with CV and mean of 8.2% and 22cm, respectively (Table 3).

Stem Height (SH) was measured from the top and base of plant by excluding the panicle after proper heading. Its value ranged from 37.2-103.5cm. The maximum and minimum values were recorded for genotypes IRGC 26390 and IRGC 31618. The mean value and CV for this trait was 64.7cm and 16.7%, respectively (Table 3). Similarly, maximum (5.4mm) Stem diameter (SD) value was observed for genotype IRGC 26390 and minimum (3.1mm) for genotype 83140-B-11-B. The CV and mean value of 10% and 3.9cm was recorded for SD (Table 3).

Total Tiller/Plant (TT/P) and Productive Tiller/Plant (PT/P). Total Tiller/Plant (TT/P) and Productive Tiller/Plant (PT/P) have direct impact on yield improvement of plant. Maximum (13.8) and minimum (4.2) TT/P was observed in genotype IR-83140-B-32-B and IRGC 27575, respectively. The CV and mean value was found 9 and 25.4%, respectively (Table-3). From Table 3 we also noted that maximum PT/P (13) was found in genotype IR-83140-B-32-B. The CV and mean value of 26.6% and 8.7 was noted for this key trait (Table-3).

Primary Branches per panicle (PB/P) and Secondary Branches per Panicle (SB/P). The Maximum Primary Branches/Panicle (PB/P) (14.2) and Secondary Branches/Panicle (SB/P) (30.05) were recorded in genotypes IRGC 25926 and HH25-SAL-9Y3-Y1, respectively. The high level of variability and CV was found for both traits (Table 4).

Measurement of Total Spikelet per Panicle (TS/P), Fertile Spikelet/panicle (FS/P) and Sterile Spikelet/panicle (SS/P). The Total Spikelet/Panicle (TS/P) value ranged from 58-156. The highest TS/P (156) was observed in genotype IRGC 25926. The lowest TS/P (58) value was found for genotype IRGC 31618. The mean value (93.4) and CV (22.7%) was recorded for this character (Table 4). The similar results were noted for fertile spikelet per panicle and its value ranges from 49-135 with CV of 18.9% (Table 4). Sterile spikelets were counted to check the spikelet by pressing via finger. The genotype HH25-SAL-9Y3-Y1 showed maximum (75) sterile spikelets. The mean value and CV for this trait was 15.1 and 78.2% (Table 4).

Measurement of 1000 Grains Weight, Grain Length (GL), Grain Breadth (GB), Grain length / breadth Ratio, Grain Thickness (GT), Grain Chalkiness (GC). 1000 Grain Weight (1000 GW) is one of the important yield related trait and the maximum weight of rice grain give high yield. Our results shows maximum variability among genotype for 1000GW and its value ranged from 3.7-29.8g. Maximum 1000 SW (29.8g) was noted for genotype IRO-4L-191 followed by 17g in line IRGC31699. The mean value and CV for this trait was 11.2 and 345.3% (Table 4). The Grain length value ranged between 5.1 to 7.2 mm with mean value and CV 6.3 and 9.3%, respectively (Table 5). Similarly, JP5 and IRGC 31611 gave maximum grain breadth (2.9mm). The mean and CV was 2.4 and 23.5%, respectively (Table 5). The maximum value (3.8mm) for grain length/breadth was noted for check cultivar Super-Basmati with mean and CV of 2.8 and 20% (Table 5). The genotype JP5 showed maximum (1.6mm) grain thickness. Very low level of variability variation was found for this trait with CV (7.6%) (Table 5). Chalkiness is very useful trait for marketing point and it determine the both quality and price of rice. Our findings shows that it value ranged from 0-3.3 with mean value of 0.2. Only few genotypes have maximum grain chalkiness percentage and rest of genotypes had no or very low percent chalkines grains (Table 5).
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Mean: 86.7, Minimum: 57.4, Maximum: 127, SD: 17.1, CV: 19.7, Variance: 291.9

Mean: 22, Minimum: 18.9, Maximum: 27.8, SD: 1.8, CV: 8.2, Variance: 3.3

Mean: 64.7, Minimum: 37.2, Maximum: 103.5, SD: 16.7, CV: 25.9, Variance: 279.7

Mean: 3.9, Minimum: 3.1, Maximum: 3.4, SD: 0.4, CV: 2.3, Variance: 0.2

Mean: 9, Minimum: 4.2, Maximum: 13.8, SD: 2.3, CV: 26.6, Variance: 5.3
# TABLE 4

Analysis of the quantitative traits i.e. Primary Branches/Panicle (PB/P), Secondary Branches/Panicle (SB/P), Total Spikelet per Plant (TS/P), Fertile Spikelet /Plant (FS/P), Sterile Spikelet/Plant (SS/P) and 1000 Grains Weight (1000 GW)

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**Maximum:** 7.2 2.9 3.8 1.5 3.3 59.5 7.2 2.9 3.8 1.5 3.3 59.5 7.2 2.9 3.8 1.5 3.3 59.5

**SD:** 0.6 0.6 0.5 0.1 0.6 10.2

**CV:** 9.4 23.5 20 7.6 362.8 378.8

**Variance:** 0.3 0.3 0.3 0 0.4 105
DISCUSSION

In this study 49 diverse rice germplasm were evaluated under field condition. Maximum variability was observed for all traits. On the basis of high performance, twelve genotypes showed excellent morphological performances than other genotypes. However the yield response varied with type of genotype. Some genotypes showed better performance than check varieties and these genotypes will be useful for future rice breeding program. Siddique et al. [26] carried out similar study, they collected 475 varieties from different parts of the country, and they also investigated seed traits and found that Pakistani rice varieties were dominated by long grain type. In present study high level of variability was observed for all 30 quantitative traits. However the genotypes response varied with type of trait (Table 1-5).

In present study high level of genetic variability was observed among 49 rice genotypes for thirty important yield related traits. All the studied characters showed direct relation with yield trait. Twelve elite genotypes were recorded showing better performance than others. The high yield genotypes are recommended for future breeding programs. However, morphological screening of these genotypes is not enough; hence, further biochemical and molecular evaluation methods are needed for further selection of improved rice genotypes.

ACKNOWLEDGEMENTS

We are thankful to acknowledge the cooperation of gene bank, Plant Genetic Resources Institute (PGRl), NARC, Islamabad, Pakistan, for providing seed samples of germplasm used.

Muhammad Ishaq Ibrahim and Sohail Ahmad Jan are equally contributed principal authors.

REFERENCES


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TREATMENT OF CHEMICAL SYNTHESIS-BASED PHARMACEUTICAL WASTEWATER

Ruoling Jia*

College of Chemistry and Chemical Engineering, Xinxiang University, Xinxiang, 453000, China

ABSTRACT

Adopting catalytic wet hydrogen peroxide oxidation to treatment of chemical synthesis-based pharmaceutical wastewater. Effects of temperature, resident time and peroxide amount, etc. on degradation efficiency of TOC were investigated. The catalytic wet hydrogen peroxide oxidation experimental results were analyzed and optimized using response surface methodology. Pharmaceutical wastewater degradation through the catalytic wet hydrogen peroxide oxidation process was found to strongly depend on two key parameters, i.e., residence time and reaction temperature. Optimization result is calculated that the optimized conditions are present: temperature is 294°C, amount of oxygen is 101.68%, and residence time is 47 s.

KEYWORDS:
Chemical synthesis-based pharmaceutical wastewater, response surface methodology, catalytic wet hydrogen peroxide oxidation, TOC removal

INTRODUCTION

In recent years, pharmaceutical wastewater has become a serious source of water pollution with the rapid development of the pharmaceutical industry. Wastewaters in a pharmaceutical industry generally originate from the chemical synthesis and formulation of the drugs. The chemical synthesis-based pharmaceutical wastewaters have high chemical oxygen demand (COD), total suspended solids (TSS) value and a wide pH range of pH (1–11), due to various organic and inorganic constituents including solvents, additives, catalysts and reactants [1-3]. In addition, water consumption and wastewater generation in the chemical synthesis process are larger than in other pharmaceutical manufacturing processes. Some pharmaceutical active compounds were added to the priority pollutant list of the European Union Water Framework Directive in 2014. In this context, it should be focused on the removal of the active compounds in each pharmaceutical wastewater in addition to organic matter [4-6].

Pharmaceutical wastewater has traditionally been treated using physico-chemical and biological processes [7].

Recently, the advanced oxidation processes (AOPs) are receiving more attention and used for the degradation of organic compounds in water/wastewater. Compared to the other conventional techniques, AOPs can generate highly reactive hydroxyl radicals (·OH) with a redox potential of 2.80 eV at a moderate temperature and pressure that aid in mineralizing the organic molecules non-selectively into carbon dioxide and water. Among various AOPs, catalytic wet peroxidation (CWPO) is a potent alternative for treatment of non-biodegradable organic compounds in wastewater under the atmospheric pressure and mild operating temperature (<80 °C) in the presence of catalyst and oxidant [8-10]. CWPO, also called as a Fenton-like process, converts organic compounds into harmless organic/inorganic compounds using homogeneous or heterogeneous non-ferrous active species as a catalyst in the presence of hydrogen peroxide at a moderate temperature and atmospheric pressure. While the Fenton process has a restricted pH range (pH 3–4), the CWPO process can also perform well at neutral pH based on the type of support and active metal used. In addition, sludge generation by CWPO is minimum. Recently, CWPO process was widely applied in various effluent treatment processes such as pharmaceuticals, dyes and dye intermediates, nitrogenous aromatic compounds, refractory organic compounds [11], etc.

Currently, one of the major challenges of CWPO technique is the development of new catalyst with higher efficacy and separation of the catalyst after a reaction. Usually, transition group metals are used as active metal species (i.e. homogeneous catalyst) and are conventionally added in the form of fine particles in order to enhance reaction by providing maximum specific surface area. However, dispersion of active metal species into the reaction mixture leads to an unstable state because the surface tension favors a smaller interfacial area for a given mass. The aforementioned challenges can be overcome through heterogeneous catalyst where the active metal species are dispersed on the porous support, which has the advantages of producing a stable state of active metal and enhancing the catalytic reaction rate by favoring the adsorp-
tion of organic species on the support. In addition, using porous media as a catalyst support can be shortened to the reaction time, and therefore, reduce both investment and operating cost. Many researchers have explored various heterogeneous catalysts where active metals such as Cu, Ce, Fe, Mo, nFe0 are supported on various materials (e.g., GAC, ZMS-5, SBA-15, MCM-41, Clay).

Among various supports, activated carbon (AC) material received immense attention in material science because of its high surface area, strong adsorption capacity, abundant porous structure, possess multi-functional groups and easily available. AC is widely used in many industrial applications including separation, adsorption and as a catalyst support [12]. In this context, granular activated carbon (GAC) has been used as an active sorbent to absorb organic/inorganic pollutants for their later degradation via other mechanisms. However, the GAC performance often experiences a decreased adsorption capacity after the first cycle because of blocking of pores due to the incomplete regeneration of GAC (e.g., the residuals and formation of phenolic oligomers during the absorption/oxidation process reported) [13]. Nevertheless, several works have suggested the modification of AC with metal oxides incorporation, including iron oxide, zinc oxide, zero-valent iron, manganese oxide, aluminum oxide and copper oxide to enhance the catalytic activity.

Among all metal oxides tested, copper oxide impregnated AC (Cu/AC) catalyst has been regarded as a promising catalyst for the oxidation of nitrogenous aromatic compounds; the catalyst can be easily separated from treated water with the filtration. In addition, Cu as active species in catalyst has gained more attention in recent days due to its non-toxic/low toxicity, easy availability and good catalytic properties. However, to the best of authors knowledge, there have been a limited number of studies regarding CWPO with Cu/AC as the catalyst [14]. The current knowledge gaps are that copper impregnated GAC (i.e., Cu/GAC) was not tested for resorcinol bearing wastewater and degradation mechanism was not reported. Resorcinol (=1,3-dihydroxybenzene) was selected as a targeting pollutant because it is one of the major non-biodegradable pollutants in wastewater owing to its high toxicity, high oxygen demand and low biodegradability [15]. It has been listed as one of the potent endocrine disrupting chemicals. The major source of resorcinol is from various industrial effluent streams such as textile, steel, petrochemical, petroleum refinery, rubber, dyes, plastics, pharmaceutical, cosmetics, paper and pulp etc. [16]. Further, it is highly poisonous as compared to phenol, and found to show a negative impact on cardiovascular and central nervous system. Resorcinol is very detrimental to the living beings even at very trace amount because of its toxicity and carcinogenic nature [17]. Therefore, it drives to develop the efficient and effective method for the removal of resorcinol from water and wastewater.

In this work, the treatment of a pharmaceutical wastewater by heterogeneous catalytic wet hydrogen peroxide oxidation (CWHPO). The heterogeneous CWHPO system has been evaluated in terms of increasing the biodegradability of the pharmaceutical wastewater.

**EXPERIMENT**

**CWPO experimental set-up.** The CWHPO system consists of a fixed bed reactor made of glass with an inner diameter of 1.2 cm and 15 cm of length. As catalyst, crystalline iron oxides supported over a mesoporous SBA-15 silica matrix was used. The catalyst was conformed as extrudates of 2.0 mm × 1.5 mm following a methodology described elsewhere. The pellets show crystalline hematite entities of ca. 14 wt.% of iron content and the main properties of SBA-15 topology, such as mesoscopic order and narrow pore diameter distribution, with a BET surface of about 264 m²/g. The catalyst particles are packed between glassy beads to enable a better distribution of the inlet solution inside the catalytic bed.

**Analytical methods.** Total organic carbon (TOC) content of the solutions was analyzed using a combustion/non dispersive infrared gas analyzer model TOC-V Shimadzu, which was calibrated with a standard solution of potassium phthalate prior to the TOC analyses.

**RESULTS AND DISCUSSION**

Effect of temperature is an important and crucial parameter in CWPO. The degradation of pharmaceutical wastewater over AC was studied by varying the CWPO process temperature in the range of 280 °C–310 °C. It can be seen from Fig. 1, the temperature has a strong effect on the TOC removal. When temperature increased from 280 °C to 310 °C the removal enhanced from 39.1% to 99.5%. At lower temperature (280 °C) the lower TOC removal was claimed, presumably due to slow generation of hydroxyl radical. TOC removal increases with temperature due to increasing kinetic constants according to Arrhenius law.

In order to increase CWPO process efficiency it is necessary to optimize the hydrogen peroxide dose as it has a significant impact on the operating cost, and the concentration of H₂O₂ is directly propositional to the generation of hydroxyl radicals that are responsible for the degradation of pharmaceutical wastewater in CWPO process. In this study, the effect of H₂O₂ was observed in terms of
the stoichiometric ratio of H$_2$O$_2$/pharmaceutical wastewater from 0 to 2 times at different concentrations of pharmaceutical wastewater in the range of 50–500 mg/L. As seen in Fig. 2, the TOC removal percentage increased with the increase in the amount of H$_2$O$_2$ ascribed to an increase in generation of hydroxyl radicals. At the stoichiometric ratio of H$_2$O$_2$/pharmaceutical wastewater = 1, the TOC removal of 81.7%, 87.1%, 46.5% and 39.1% was observed for the pharmaceutical wastewater concentration of 50, 100, 200, and 400 mg/L, respectively. For a particular stoichiometric ratio of H$_2$O$_2$/pharmaceutical wastewater, the TOC removal decreased gradually with increasing the initial concentration of pharmaceutical wastewater, which could be due to unavailability of active sites for the production of hydroxyl radical.

Effect of residence time is another important and crucial parameter in CWPO. The degradation of pharmaceutical wastewater over AC was studied by varying the CWPO process residence time in the range of 20–60 s. It can be seen from Fig. 3, the residence time has a strong effect on the TOC removal. When residence time increased from 20 to 60 s, the removal enhanced from 39.1% to 99.5%.

Contour plot of TOC removal (%) vs C, B is shown in Fig.4. The reaction temperature was maintained at 295°C; the TOC removal rate was affected by the amount of peroxygen as a peak curve. When the peroxidation amount is less than 100%, the TOC removal rate increases with the increase of the peroxygen content; when the peroxygen content is in the range of 100% to 200%, the TOC removal rate increases slowly.

Contour Plot of TOC removal (%) vs C, A is shown in Fig.5. The residence time is 40 s. The removal rate of TOC is a peak curve affected by the amount of oxygen. The main reason is that the activation energy of the reaction becomes the main factor affecting the reaction and the excessive concentration of H$_2$O$_2$ may reduce the existence of free radicals, which is not conducive to the reaction;

Contour plot of TOC removal (%) vs B, A is shown in Fig.6. The amount of peroxygen is kept at 100%. The TOC removal rate increases with increasing residence time and temperature, but the TOC removal rate increases with residence time by much greater than the temperature.

Objective function of Minitab’s response surface provides an intuitive tool for objective optimization and its unique response optimizer is a powerful tool for multi-objective problem to the solution encountered in the experimental design. And the target function is optimized by Minitab’s response optimizer. The result is shown in Fig. 7. It is calculated that the optimized conditions are present: temperature is 294°C, amount of oxygen is 101.68%, and residence time is 47 s.

FIGURE 1
Surface Plot of TOC removal (%) vs C, B

FIGURE 2
Surface Plot of TOC removal (%) vs C, A

FIGURE 3
Surface Plot of TOC removal (%) vs B, A
The AC catalyst exhibits high efficiency in the treatment of chemical synthesis-based pharmaceutical wastewater by CWPO in a batch reactor. Chemical synthesis-based pharmaceutical wastewater degradation through the CWPO process was found to strongly depend on two key parameters, i.e., residence time and reaction temperature. Optimization result is calculated that the optimized conditions are present: temperature is 294°C, amount of oxygen is 101.68%, and residence time is 47 s.

CONCLUSIONS
REFERENCES


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ABSTRACT

Pesticides are useful tools in agriculture, but they have also harmful effects on environment. There are several methods to remove pesticides from the polluted soils. One of them is phytoremediation. It has emerged in recent years as an environment-friendly method. In this study, pumpkin plant (Cucurbita pepo L.) was used for the phytoremediation of imidacloprid. Pumpkin seeds were planted into pot soil treated with 3 different doses of imidacloprid pesticide. After 14 days, distribution of imidacloprid in soil, root, stem and leaves were examined. Bioconcentration factors and translocation factors respectively varied between 0.46-1.35 and between 0.50-0.58. For recovery test, imidacloprid was applied to the untreated soil at 500 ng/g spiking level. Mean recoveries was 81.40% by SC and 86.88% by matrix-matched calibration with RSD of 4.75% and 5.64%, respectively. Overall recovery of the method was 84.23% with RSD of 6.01% (n = 14). These values comply with SANTE Guidelines. Imidacloprid concentration in soil decreased with time. The greatest imidacloprid concentration was observed in root, followed by leaf and stem parts. Plants up took imidacloprid by phytoextraction and kept around the root by the rhizofiltration. Imidacloprid was phytoremediated from soil by 25.2% in this study.

KEYWORDS: Cucurbita pepo L., phytoremediation, imidacloprid

INTRODUCTION

Pesticides have negative effects on both environment and other living organisms. For example, while some pesticides leach into groundwater, some others may accumulate in agricultural lands [1].

Imidacloprid is an example of long-lasting pesticides in agricultural lands (Table 1) [2]. Scientists have tried different methods to remove persistent pesticides from the soils [3]. Phytoremediation is an ecological cleaning method, in which plants are used to remediate soil. Unlike other methods, the method allows in-situ purification [4]. Phytoextraction, phytovolatilization, rhizofiltration are other mechanisms of phytoremediation [5]. They can be used for remediation of contaminated soils, wastewater treatment sludge, sediment and water. Organic compounds, pesticides and heavy metals can be remediated by plants [6].

Romeh [6] used Plantago major for phytoremediation of imidacloprid from water and reported that imidacloprid mostly accumulated in roots (15.74 μg/g), leaves (37.21 μg/g) and fruits (5.74 μg/g). The greatest remediation from the roots, leaves and fruits was achieved respectively 6, 1 and 3 days after the treatments. The accumulated quantities in roots, leaves and fruits decreased respectively to 6.95, 1.46 and 0.12 μg/g 10 days after the treatments. White et al. [7] carried out a phytoremediation study with pumpkins to remove p,p'-DDE from the soil. Pumpkins were planted on soil, polluted at 200-1200 ng/g with p,p'-DDE. The p,p'-DDE varied between 250-9240 in roots and between 12.7-4970 ng/g in stems. Bioconcentration factors were 7.22, 5.40, 0.87 and 0.28 for root, stem, leaf and fruit, respectively. Translocation factor was 0.74. Romeh [8] tested the efficiency of P. major in remediation of azoxystrobin from the soils and indicated that azoxystrobin mostly accumulated in roots. The QuEChERS method, could reliably be used for pesticide residue analysis in soils, roots, stems and leaves [6, 9, 10, 11].

MATERIALS AND METHODS

Chemicals Reagents and Instrumentation. Imidacloprid to be used in experiments was supplied from Dr. Ehrenstorfer Laboratories. The other reagents (hepta hydrate magnesium sulfate, sodium chloride, tri-sodium citrate dehydrate and acetoni-trile) were supplied from Merck, with the purities of 99.0-100.5%, 99.5%, 99.0-101.0%, and 99.95%, respectively. Bondesil- Primary Secondary Amine (PSA) was supplied from Varian. The di-sodium salt sesquihydrate (99%) and acetic acid (99.8-100.5%) were supplied from Acros Organic and
Some characteristics of imidacloprid [2].

<table>
<thead>
<tr>
<th>International Chemical Identifier</th>
<th>ChH5OClN5O2</th>
<th>GUS Leaching Potential Index</th>
<th>3.74</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-concentration Factor (BCF)</td>
<td>0.61</td>
<td>ARID-Acute Ref.D (mg/kg/bw/day)</td>
<td>0.08</td>
</tr>
<tr>
<td>ADI–Accept.Daily Intake (mg/kg/bw/day)</td>
<td>0.06</td>
<td>Degradation in Soil (Day)</td>
<td>191</td>
</tr>
<tr>
<td>Mammals – Acute LD50 (mg/kg)</td>
<td>131</td>
<td>Water</td>
<td>610</td>
</tr>
<tr>
<td>Birds – Acute LDso(mg/kg)</td>
<td>31</td>
<td>Dichlororathene</td>
<td>67000</td>
</tr>
<tr>
<td>Fish – Acute 96 hour LC50(mg/l)</td>
<td>&gt;83</td>
<td>Toluene</td>
<td>690</td>
</tr>
<tr>
<td>Vapour Pressure at 25°C (mPa)</td>
<td>4.0 X 10⁻⁶⁷</td>
<td>Hexane</td>
<td>100</td>
</tr>
<tr>
<td>Henry’s Law Constant at 25°C(Pa.m³/mol)</td>
<td>1.7 X 10⁻¹⁰</td>
<td>Propanol</td>
<td>2300</td>
</tr>
</tbody>
</table>

TABLE 1

Properties of soil used in the experiment

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>83.10</td>
<td>Sandy-loam</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>12.80</td>
<td></td>
</tr>
<tr>
<td>Silt (%)</td>
<td>4.10</td>
<td></td>
</tr>
<tr>
<td>Organic matter</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.16</td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Fortification Experiment (Recovery Test). Pesticide-free soil (blank) was spiked by using the following principles [13, 14]: About 250 g sieved soil was spiked with 250 mL 0.5 μg/mL imidacloprid standard solution (in MeCN) corresponding to 500 ng/g level. Soil samples were air-dried at room temperature for 7 days to get “aged-soil”. Following the evaporation of the solvent, samples were oven-dried at 30°C for a night. Method recovery and precisions were tested in accordance with SANTE European Guideline [15]. Method linearity was tested in MeCN within the range of 25–3000 pg/μL. Limit of detection (LOD) was assessed with the instrument software.

Analytical methods. Modified QuEChERS method of Lesueur et al. [13] was used for soil samples extraction and cleanup. QuEChERS method modified by Liu et al. [10] was used for the analyses on plants parts. Details of the analytical steps of the QuEChERS method for both soil and plant samples are illustrated in Figure 1. Chromatographic analyses were performed with a LC-MS/MS.

RESULTS AND DISCUSSION

Method Performance. The linearity of solvent calibration (SC) and matrix-matched calibration (MC) curves were assessed through r and S_{2/y} values (Eq 1). Both curves were found to be linear over the range of 25-3000 ng/mL since r >0.99 and S_{2/y} < 0.1 [16, 17] (Table 3, Fig. 2).

\[ S_{2/y} = \frac{\sum(Y_i - \bar{Y})^2}{n-2} \]

Where:

- \( Y_i \): Response obtained from injecting standard,
- \( \bar{Y} \): Point corresponding to standard on the regression line,
- \( n \): Number of injections.

Matrix-matched calibration solutions were used to assess the repeatability of retention time (rt). The rt of imidacloprid ranged between 8.354 and 8.221 (RSD 0.38%, n=20). Co-extracted compounds may induce matrix effect (ME) and thus influence quantitation of analytical results. Equation 2 was used to calculate ME and the value was calculated as 12.39%. The MC standards for soil matrix were prepared with blank soil extract and imidacloprid standards at 6 different concentrations (25, 50, 250, 750, 1500 and 3000 pg/μL). Differences in imidacloprid standard responses in solvent and matrix-matched standard are illustrated in Figure 3.
FIGURE 1
Analytical steps of the QuEChERS method for soils (a) and plants (b)

(a) Soil Sample Preparation
- 10 g soil
- Add 20 ml MeCN
- Shake with Vortex, 1 min
- Adding with 3.5 g MgSO4·7H2O, 1 g NaCl, 1 g potassium persulfate and 0.5 g diatomaceous earth
- Mix by Vortex, 1 min
- Centrifuge 10 min, at 4500 rpm
- Transfer supernatant into centrifuge tube containing 150 ml PSA and 1.5 g MgSO4·7H2O
- Centrifuge 5 min, at 4500 rpm
- Transfer 1.1 ml extract to GC Vial for LC-MS-MS analysis

(b) Plant Sample Preparation
- 2 g Plant sample
- Add 10 ml distilled water and 10 ml MeCN containing 0.1% AA
- Shake with Vortex, 1 min
- Adding with 12.27 g MgSO4·7H2O and 1.5 g NaAc
- Shake with Vortex, 30 sec
- Centrifuge 5 min, at 10000 rcf
- Transfer 1 ml supernatant into centrifuge tube
- Add 109 ml MgSO4·7H2O and 50 ml PDA
- Shake with Vortex, 3 min
- Centrifuge 5 min, at 10000 rcf
- Transfer 1 ml Extract to GC Vial for LC-MS-MS analysis

FIGURE 2
Solvent calibration (a) and matrix-matched calibration (b) curves

TABLE 3
Calibration parameters for the imidacloprid for LC-MS/MS detection with six-level calibration, in solvent and sample matrix.

<table>
<thead>
<tr>
<th>Calibration</th>
<th>Calibration and/or analytic function, a</th>
<th>Correlation coefficient, r</th>
<th>Relative residual standard deviation SΔy/ŷ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent calibration, SC</td>
<td>y = 29.161 + 8.6926x</td>
<td>0.99</td>
<td>0.098</td>
</tr>
<tr>
<td>MCSoil (0.5 g/mL SEQ)b</td>
<td>y = 80.863 + 71.847x</td>
<td>0.99</td>
<td>0.097</td>
</tr>
</tbody>
</table>

a) x = injected amount to LC-MS/MS, y = detector response as area
b) 1 μL is equal to 0.5 mg soil, Sample equivalent, SEQ
Matrix Effect (ME) % = \frac{\text{Peak area of matrix standard} - \text{Peak area of solvent standard}}{\text{Peak area of solvent standard}} \times 100 \tag{2}

Student T test was used to assess significant differences between SC-MC (Equation 3) [19].

\[ t = \frac{\bar{d}\sqrt{n}}{S_d} \tag{3} \]

Where: \( \bar{d} \) = Mean differences (\( d_i \))
\( S_D \) = Standard deviation of differences (\( S_{d_i} \))

According to Student’s t-test calculated value was less than tabulated value (for 95% confidence and n-1 degrees of freedom, 6-1), i.e., \( 1.57 < 2.57 \), matrix effect was not significant. But, generally MC is recommended in method validation studies [20]. All calculations, including fortified samples, were done based on MC.

The analyses of 7 replicate-spiked (500 ng/g level) soil samples were performed in LC-MS/MS system and the results were assessed through MC (0.5 g/mL sample equivalent). The analytical function of \( y = 80.863 + 71.847x \) was used for calculations (Table 3). Recovered imidacloprid ranged between 78.68-93.24% with a mean recovery of 86.88% and RSD of 5.64%. Present findings were parallel to the values specified for mean recovery (70–120%) and repeatability (RSD ≤ 20%) [15]. These figures were also similar with the findings of White et al. [21], Lesueur et al. [13], Albright and Coats [22], Alagic et al. [12] and Liu et al. [23] with the recovery rates of 75%, 70-110%, 73.23%, 95.50–104.70% and 75.01–118.89, respectively.

**Field Experiment Findings.** Pesticide quantities in the soil sampled in different days are provided in Table 4. There was a clear decline in pesticide concentrations day by day for all application doses. These declines may be resulted from absorption of imidacloprid by *Cucurbita pepo* (phytoremediation). White et al. [24] found that p, p’-DDE concentrations in soil samples were between 110-670 ng/g.

Imidacloprid concentrations in plant samples, harvested 14 days after pesticide application, are given in Table 5. Imidacloprid concentrations varied between 217.00-491.25 ng/g in roots; between 191.00-475.00 ng/g in leaves and between 128.00-246.25 ng/g in stems.

**FIGURE 3**
Chromatogram of imidacloprid (50 pg/μl); in solvent (a) and in matrix (b)

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Imidacloprid concentrations in the soil, ng/g (mean of 5 replications)</strong></td>
</tr>
<tr>
<td><strong>Sampling day</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>14</td>
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</table>

<table>
<thead>
<tr>
<th>TABLE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Imidacloprid concentrations in the plant parts, ng/g (mean of 5 replications).</strong></td>
</tr>
<tr>
<td><strong>Plant part</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Root</td>
</tr>
<tr>
<td>Stem</td>
</tr>
<tr>
<td>Leaf</td>
</tr>
</tbody>
</table>
Bioconcentration factors (BCF = Concentration in plant part / Concentration in soil) and translocation factors (TF = Stem BCF / Root BCF), which indicate the pesticide uptake rate of the plant, were found to be between 0.46 - 1.35 and the TF values were found to be between 0.50 - 0.58 (Figure 4). White et al. [7] reported BCF values for p,p’-DDE as between 2.37 - 7.22, and TF values as between 0.19 - 0.74. In another study, White et al. [21] reported TF values as between 0.04 - 0.37.

Regardless of the application dose, phytoremediation rate was calculated by proportioning the average concentration value of the 14th day of the plant samples to the average soil concentrations of the zero time soil samples. This value was 0.252. Similar findings were reported by White et al. [7] as 0.301 for p,p’-DDE and Cucurbita pepo ssp pepo.

**CONCLUSIONS**

In plant parts, imidacloprid was found mostly in the roots and later on the leaves and stem. The reason for this may be adsorption of imidacloprid in the soil by means of roots of pumpkin plants (rhizofiltration mechanism) and accumulation of imidacloprid in the harvestable parts (phytoextraction mechanism). In present experiments, imidacloprid phytoremediated by 0.252 from the soil with Cucurbita pepo L. It can be concluded that more accurate results could be achieved through phytoremediation of the pesticides by the sampling approach at harvest time.

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In this paper, both environmental and anthropogenic variables into GWLR model are incorporated to study the distribution of grassland fire occurrence in Hulunbuir over the period 2001-2015. The results show that mean annual temperature, elevation, distance to nearest settlement, distance to nearest road, and distance to nearest agricultural area are correlated with grassland fire occurrence, while these explanatory factors are not spatially stationary in the whole study area. Thus, the consideration of non-stationarity in fire modeling is important to better understand fire regimes and to more efficiently prioritize critical area to fire occurrence.

**KEYWORDS:**
GWLR, fire modelling, explanatory factors, elevation

**ABSTRACT**

In this paper, both environmental and anthropogenic variables into GWLR model are incorporated to study the distribution of grassland fire occurrence in Hulunbuir over the period 2001-2015. The results show that mean annual temperature, elevation, distance to nearest settlement, distance to nearest road, and distance to nearest agricultural area are correlated with grassland fire occurrence, while these explanatory factors are not spatially stationary in the whole study area. Thus, the consideration of non-stationarity in fire modeling is important to better understand fire regimes and to more efficiently prioritize critical area to fire occurrence.

**INTRODUCTION**

Fire occurrence is an essential component of fire risk [1], which refers to a detected active fire that happens when the fire begins to spread [2]. Fire occurrence knowledge can be used to identify areas with high ignition risks [3, 4], target fire management actions [5].

Nearly one-fifth of the Earth’s land is covered with grasslands, they can be found on all continent except Antarctica [6], and approximately 80–86% of the global area burned occurs in grassland and savannas [7]. According to the statistics, there are approximately 7256 grassland fires in China during 1991 and 2005, with fire-prone areas accounting for $2.06 \times 10^7$ km$^2$ [8]. Extensive fire occurs in these grass-dominated regions caused heavy land degradation, loss of life and property, reduced agricultural and livestock production, and brought instability to ecological security in these regions [9].

Understanding the spatial patterns of grassland fire occurrence and their relationships with underlying factors is a key issue to improve fire management decision-making [10]. Under the background of global warming, increases in temperature may lengthen the fire season and increase the combustibility of grass [11]. In African savanna, most fires occurrence in dry periods (June-August), and moisture availability has a strongly positive relationship with fire. However, these relationships appear weaker in Australia, and less so in South America [12]. In the western of American, grassland fire tends to be associated with positive growing season moisture more prior to the fire season (June-July), while warmer temperatures might reduce the moisture available during this season [13].

Widespread fires are burnt annually in arid and semi-arid grasslands of China, with most fires in spring and autumn. The water content of dead grass is lower than other months, because the weather in these months is warm and dry [14]. Human activities are also associated with grassland fire occurrence. However, human activities are dynamic in time and space, which makes intricate the estimation of specific spatial patterns. The dynamics of grassland fire regimes in Hulunbuir of Inner Mongolia are mainly related to human factors, and the proportion of fires started by direct or indirect human causes exceed 90% [15]. Increases in grassland fire frequency may be due to the increase of human populations in grasslands. One reason is that nomadic people dominated in Hulunbuir historically, there has been a shift toward permanent settlement in recent decades, urban growth is encroaching upon grasslands resulting in more people living at the urban-grassland interface. On the other hand, traditional farming activities use fire as the main tool to clear land, or to promote regrowth of grass for breeding more herds. These fires may be escaped, often result in severe fires [16]. Furthermore, socio-economic variables reflecting land use and rural population density are also significant predictors of fire occurrence [17, 18].

The importance of the effects of socio-economic factors in modelling for fire occurrence should be given adequate attention [19, 20]. Therefore, the relationships between grassland fire occurrence and causal factors are often involve complex interactions and expected to vary among ecosystems and across spatial and temporal scales under climate change.
A range of methodologies have been employed to determine spatial factors influencing grassland fire occurrence. These include Analytic Hierarchy Process [20], logistic regression analysis [21, 22] and simulation models [23]. Most previous studies are global with the assumption that the model parameters are valid and homogeneous for the entire study area, these assumptions do not apply for factors with spatial differences, and mislead the significance of hypothesis testing on the model coefficients [24, 25]. This spatial variation was better analyzed by the Geographical Weighted Regression (GWR) methods, which can be used to deal with spatial heterogeneity and autocorrelation in model error [26, 27]. Although GWR is applied to the occurrence of in forest fires some studies [28, 29], few studies have conducted to evaluate the relationship between grassland fire and the spatial heterogeneity of explanatory variables.

The objective of this study was to analyze the spatial heterogeneity of the factors influencing grassland fire occurrence in Hulunbuir over the period 2001-2015. Our hypothesis is that the explanatory factors are spatially variable, and their contributions with grassland fire occurrence change significantly over the space. Thus, we apply binary logistic regression within the framework of GWR models to explore the spatial variation. Grassland fire occurrence points are identified from MODIS active fire product [30], and explanatory variables are selected based on experience of models.

**MATERIALS AND METHODS**

**Study area.** Hulunbuir is located in the Inner Mongolia Autonomous Region (47.08°–53.23°N, 115.22°–126.06°E) in northeastern China, and encompasses approximately 252,948 km². This study area is crossed from north to south by the Great Xing’an Mountain, with elevation gradually decreases from the center to the east and west; the elevation ranges from 200 to 1500 m. Hulunbuir has a typical temperate continental monsoon climate, annual mean air temperature ranges from -3°C to 0°C with a generally decrease from southeast to northwest, while annual mean precipitation varies from 250mm to 408mm with a gradually decrease from southeast to northwest. This region is cold and dry in winter under the Mongolian high-pressure weather system, mostly rainy in summer, and rainless and windy in spring and autumn. The natural vegetation in Hulunbuir region is diverse, including Filifolium sibiricum, Stipa baicalensis and Leymus chinensis. There are approximately 3000 villages and towns in the study area, and mainly distributed in the southern part. Fire mostly occurs in spring and autumn in this region, and nearly 80% of fires were human-caused.

**Data.** Dependent variable. In this study, fire occurrence is a binary dependent variable, i.e. ‘high’ or ‘low’. Most of previous fire occurrence models used ‘fire occurrence’ as 1 value and ‘non-fire point’ as 0 value, which assumed that all of the no fire locations could not support a fire. However, ‘no fire’ areas only mean that they did not experience a fire occurrence during the temporal span of the data set [31]. For this reason, the high/low fire occurrence is constructed from the Collection 5 MODIS global monthly fire location product (MCD14ML) consists of 15 years (January 2001–December 2015).

The MCD14ML product is a combination of level 2 MOD14 / MYD14 active fire products, obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) on board NASA’s Earth Observing System (EOS) Terra and Aqua satellites [32]. This product is a monthly ASCII file of detected fires, spatial resolution of this fire product is 1 km at nadir. The current Collection 5 dataset has removed persistent false detections and improved detection confidence values [33].

The high/low fire occurrence is screened from MCD14ML Collection 5 product in four stages. In the first stage, the low-confidence MODIS active fire detections was removed to avoid false detections, limiting our analysis to the more intense fires. Secondly, grassland fire occurrence points are identified from this fire detections using the Fire Spread Reconstruction approach (FSR) [34]. This method groups fire pixels into burning events with a unique area of ignition in space and time. The threshold values of spatial and temporal distance between two points were empirically determined and set at 5 km and 4 days. The earliest fire detection points within each group are assumed to be the candidate ignition points, and only the point with the smallest ID is selected to be the fire occurrence point to minimize the influence of spatial and temporal autocorrelation [35]. Furthermore, the MODIS 500m land cover data [36] are used to filter the grassland fires. Fire points which located on villages, water bodies, agricultural areas, forests and barrens are removed to reduce the commission error. Therefore, only fire detection points within grassland are used.

In total, there were 896 grassland fire occurrences between 2001 and 2015 in the study area. The locations of these fire occurrence events are shown in Figure 1.

Finally, a 1x1 km grid was overlaid with the ignition points, and fire density in each grid was estimated to build logistic regression models by tertile analysis. The grids are considered as high fire occurrence (1) if the density values above the 66th percentile, and the grids which values below the 33rd percentile are considered as low occurrence (0). Other grids are not taken into account in construction of the model [37].
The factors affecting fire occurrence used in the analysis were represented by the following explanatory variables: climate, vegetation, topography and human activity variables (Table 1).

**Climate.** Climate can influence the distribution of fire occurrence by constraining fuel moisture content. The mean annual temperature, mean annual precipitation and mean annual wind speed were selected as climatic variables. Climate data from 2001 to 2015 were collected from ten meteorological stations, which obtained from National Meteorological Information Center of China (http://data.cma.cn/). These data were interpolated to raster layers and resampled at a 1km resolution.

**Vegetation.** Fire occurrence are also influenced by herbaceous biomass quantity, the annual maximum normalized difference vegetation index (NDVI) has been employed to represent spatial variability in fuel loads. This dataset was provided by GSCLoud, Computer Network Information Center, Chinese Academy of Sciences (http://www.gscloud.cn). Then the average growing season NDVI (AGSNDVI) dataset from 2001 to 2015 was obtained and resampled at 1 km resolution using the majority algorithm.

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Abbreviation</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biophysical factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean annual precipitation</td>
<td>Prep</td>
<td>mm</td>
</tr>
<tr>
<td>Mean annual temperature</td>
<td>Temp</td>
<td>°C</td>
</tr>
<tr>
<td>Mean annual wind speed</td>
<td>WindSpeed</td>
<td>m/s</td>
</tr>
<tr>
<td>NDVI</td>
<td>NDVI</td>
<td></td>
</tr>
<tr>
<td>Elevation</td>
<td>Elev</td>
<td>m</td>
</tr>
<tr>
<td>Slope</td>
<td>Slope</td>
<td>degree</td>
</tr>
<tr>
<td>Aspect</td>
<td>Aspect</td>
<td>degree</td>
</tr>
<tr>
<td>Human factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance to nearest county-level road</td>
<td>DisRoad</td>
<td>km</td>
</tr>
<tr>
<td>Distance to nearest settlement</td>
<td>DisSet</td>
<td>km</td>
</tr>
<tr>
<td>Distance to nearest agricultural area</td>
<td>DisAgrArea</td>
<td>km</td>
</tr>
<tr>
<td>Distance to nearest railway</td>
<td>DisRailway</td>
<td>km</td>
</tr>
<tr>
<td>Distance to nearest river</td>
<td>DisRiver</td>
<td>km</td>
</tr>
</tbody>
</table>
**Topography.** We used three topographic variables as factors that can affect fuel loading and limit human accessibility of the area: elevation, slope and aspect. We expected that fire occurrence would be more likely at lower elevations and steeper slopes. The elevation, slope and aspect surfaces were derived from the Global Digital Elevation Model 30m data, using the surface analysis provided in the ArcGIS Spatial Analyst tool.

**Anthropogenic Data.** Humans directly influence fire regimes both by igniting fires and by suppressing the spread of those fires [38]. A number of human variables were used in this study, such as distance to county-level roads, distance to railways, distance to rivers, distance to settlements, and distance to agricultural areas. These data were digitized from topographic maps, and conducted using ArcGIS 10.5 software. All the data above were spatialized at a resolution of 1 km in Transverse Mercator projection and used the WGS 1984 datum.

**Methods.** To examine the spatial variation of factors associated with grassland fire occurrence, a geographically weighted logistic regression (GWLR) model was developed. The equation of the geographically weighted logistic regression is:

$$\hat{p}_{(u_i,v_i)} = \frac{1}{1 + e^{-\left(\beta_0(u_i,v_i) + \beta_1(u_i,v_i)x_{1i} + \cdots + \beta_k(u_i,v_i)x_{ki}\right)}}$$

Where $p_{(u_i,v_i)}$ is the dependent variable; $i$ is the regression point index, $(u_i, v_i)$ are the coordinates in space of point $i$.

GWLR models could produce local regression coefficients for each location by weighting function. The weighting function assumed that the influence of the points closer to the central point(bandwidth) have more effect on parameter estimates. There are four types of kernel functions for weighting function: Gaussian fixed kernel, bi-square fixed kernel, gaussian adaptive kernel and bi-square adaptive kernel. Gaussian kernel decreases from the center and mitigate the risk of there is no data within the kernel. Bi-square kernel has a clear-cut range, and is suitable for clarifying local extents for model fitting. Fixed bandwidth is a suitable choice, if the points are regularly in the study area. It is desirable to use adaptive bandwidth when the points are clustered and not regularly spaced. In GWLR, Gaussian adaptive kernel is more secure than bi-square kernel, due to the outcome distribution is unbalanced. The Akaike Information Criterion (AIC) is one of the common methods to identify the optimal bandwidth, and the low AIC value indicates an optimum bandwidth.

In this study, GWLR model adjustment used a random sample of 70% (491) of the fire data, remaining 30% (210) for the validation process. Gaussian adaptive kernel was used to select bandwidth, and the number of neighbors was 78 by minimizing the AIC. The multicollinearity between the independent variables was checked by using the variance inflation factor (VIF) and eigenvalue analysis. The VIF greater than 7.5 or eigenvalue greater than 30 indicates a sign of strong multicollinearity.

To test the significance of the spatial variability of independent variables, the principle of the interquartile range of the GWLR parameters is greater than two times of ±1 standard deviation range of the estimated coefficients of logistic regression model [39] and Monte Carlo test were examined in the GWLR model. The independent variables including Mean annual temperature, elevation, distance to rivers, distance to towns and distance to agricultural areas were used for adjustment of the final model.

**Model evaluation approaches.** The main results from GWLR for each observation point were the local coefficients of the independent variables. These local coefficients for independent variables could explore the spatial variability of the explanatory factors. However, GWLR does not estimate the regression coefficients in no data locations. Thus, we used inverse distance weighted interpolation method to generate parameter and t-statistic surfaces for the whole study area.

Secondly, we presented the receiver operating characteristic curve (ROC) to evaluate the predictive performance of the GWLR model. The area under the curve (AUC) indicated the model prediction accuracy, and Youden criterion (Youden criterion=sensitivity+specificity-1) was used to determine the cut-off point. Sensitivity is the proportion of high occurrence of grassland fires that predicted to be high occurrence. Specificity is the proportion of low occurrence of grassland fires that predicted to be low occurrence.

Furthermore, spatial autocorrelation statistics for the residuals of GWLR model have been estimated using Moran’s I, if the residuals were autocorrelated then the results of the GWLR model would be unreliable.

Finally, three goodness-of-fit criteria were used to compare LR and GWLR: residual sum of square (RSS), AIC, deviance and prediction accuracy. The smaller the value of AIC, RSS and deviance, the better the performance of the model fitting.

GWLR models were fitted by using the GWmodel [40], and interpolation and plotting were performed with ArcGIS software.

**RESULTS AND DISCUSSION**

The GWLR results show that grassland fire occurrence is significantly correlated to mean annual temperature, elevation, distance to nearest settlement, distance to nearest road, and distance to nearest agricultural area (Table 2).
Based on the minimization of the Akaike Information Criterion, the optimum number of neighbors is 78. AIC=531 indicates that the model is the best in terms of describing the relationship between variables. Low Variance Inflation Factor (VIF) values (< 7.5) indicate that there is no multicollinearity between these variables. The Monte Carlo test (p-value<0.01) reveals a significant spatial variation in the local parameter estimates for these variables, and the inter-quartile range of the GWLR parameters greater than two times of ± 1 SD of the equivalent logistic regression model parameters also suggest that these variables are significantly non-stationary in the study area.

Fig.2 shows the map of regression coefficients of the explanatory variables, and Fig.3 presents the significance level of these variables. These maps could measure the spatial variation and explanatory sense of the explanatory variables. Fig.2a reveals that the parameter values of mean annual temperature have the strongest positive effects on grassland fire occurrence and the significance level of mean annual temperature are highly significant in the whole study area (Fig.3a). Even though the parameter values of mean annual temperature are always positive, it can be shown that the mean annual temperature has a more positive effect in the eastern part than the western part.

As expressed earlier, mean annual temperature decreases from southeast to northwest, warmer temperature can reduce fuel moisture availability, increase the flammability of the live and dead grasses. Moreover, increased temperature also leads to a long fire season. All of the other variables except of the mean annual temperature have both positive and negative signs.

Elevation showed high negative parameter values in most of the study area, the parameters of this variable suggest that fires are more likely to occur at lower altitudes (Fig.2b). The significance level of elevation shows that the influence of elevation has stronger impact in the western region, which has high altitudes (Fig.3b). Some studies showed that lower elevations tend to be more xeric, with dryer fuels. Other studies also found anthropogenic factors are more closely related to fires with the decreasing of elevation.

Anthropogenic factors were also found to be relevant factors to explain grassland fire occurrence in our model. The factor distance to nearest settlement had both positive and negative signs, the positive parameters were found in the northwest, while negative parameters were distributed from the center to the southeast (Fig.3a). This is understandable because the characteristic of settlement distribution is dense in the southeast, grassland fire decreases as distance to nearest settlement increases. Areas with positive parameter values of distance to nearest settlement were found in the northwest part.

Another important variable was distance to nearest agricultural area, which had negative effects in the northwest and positive effects in the southeast (Fig.2d). As the wide pastures and rich farmlands in the northwest, most of grassland fires were caused by intentional or negligent agricultural burnings and other burnings to regrow or maintain pastures for livestock, while fire occurrence in small farm plots are easier to control. This factor suggests that highly agricultural properties increased the grassland fire risk, which is related to agricultural activities where fire is frequently used for eliminating stubble, clearing grasses and pasture establishment.

The roads, like the settlements, also have a negative explanatory sense in the eastern part, although most of locations are not significant (Fig.3e). Several works showed that grassland fires frequently occur along transportation corridors, however other works revealed that large fires were more likely to occur farther away from roads [41, 42]. These different results could explained that our variable such as DisRoad was not significant in most of the study area. It can be concluded that grassland fires are more likely to occur in the areas close to human activities. The closer the distance to cultivated lands, the more frequently grassland fires occurred.

<table>
<thead>
<tr>
<th>Variable (Abbreviation)</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
<th>Inter-quartile Range</th>
<th>Standard Error (LR)</th>
<th>VIF</th>
<th>Monte Carlo test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.514406</td>
<td>1.240903</td>
<td>3.732545</td>
<td>1.863470</td>
<td>0.358741</td>
<td>/</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temp</td>
<td>0.321745</td>
<td>1.543952</td>
<td>4.51428</td>
<td>1.048991</td>
<td>0.103099</td>
<td>1.184776</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elev</td>
<td>-0.008762</td>
<td>-0.001447</td>
<td>0.009864</td>
<td>0.003740</td>
<td>0.000694</td>
<td>1.295493</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DisSet</td>
<td>-0.000045</td>
<td>-0.000029</td>
<td>0.000046</td>
<td>0.000028</td>
<td>0.000007</td>
<td>1.031020</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DisAgrArea</td>
<td>-0.000095</td>
<td>0.000021</td>
<td>0.000078</td>
<td>0.000053</td>
<td>0.000009</td>
<td>1.177076</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DisRoad</td>
<td>-0.000052</td>
<td>-0.000024</td>
<td>0.000084</td>
<td>0.000072</td>
<td>0.000033</td>
<td>1.100816</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
FIGURE 2
Map distribution of GWLR parameter estimates of the factors influencing grassland fire occurrence in Hulunbuir: a. mean annual temperature, b. elevation, c. distance to nearest settlement, d. distance to nearest agricultural area, e. distance to nearest road.
Figure 3

t-value of the factors in the GWLR model: a. mean annual temperature, b. elevation, c. distance to nearest settlement, d. distance to nearest agricultural area, e. distance to nearest road
In order to evaluate the performance of GWLR model for prediction of grassland fire occurrence, ROC curve (Figure. 4) was produced.

The ROC analysis showed that the area under the curve (AUC) was 0.819 (95% confidence interval (CI) 0.7603-0.8775). The optimal cut-off point for the classification of the GWLR model according to the graph of sensitivity versus specificity is 0.522. The Moran Index measures the level of spatial autocorrelation among the residuals, and in this case the value of residuals of GWLR model indicates that there is no spatial autocorrelation (Table 3).

In this study, several approaches were utilized to compare the fitting effect of logistic regression and GWLR model according to AIC, deviance and RSS. Information about these goodness-of-fit statistics is shown in Table 4.

We can see that, compared to the LR model, GWLR model has smaller AIC, deviance and RSS. Furthermore, GWLR model also has higher

## FIGURE 4
The receiver operator characteristic (ROC) curve of the GWLR model

## TABLE 3
Moran’s I index of the residuals of the GWLR model

<table>
<thead>
<tr>
<th>Lag Increment</th>
<th>Neighbor Pairs</th>
<th>Morans I</th>
<th>VarNormalI</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 &gt; to &lt;= 0.025</td>
<td>17</td>
<td>0.317972</td>
<td>0.058578</td>
<td>1.322203</td>
</tr>
<tr>
<td>0.03 &gt; to &lt;= 0.04</td>
<td>27</td>
<td>0.218947</td>
<td>0.036878</td>
<td>1.15076</td>
</tr>
<tr>
<td>0.04 &gt; to &lt;= 0.05</td>
<td>43</td>
<td>0.243023</td>
<td>0.023136</td>
<td>1.61132</td>
</tr>
<tr>
<td>0.07 &gt; to &lt;=0.08</td>
<td>62</td>
<td>0.441115</td>
<td>0.016053</td>
<td>3.49771</td>
</tr>
<tr>
<td>0.1 &gt; to &lt;= 0.11</td>
<td>79</td>
<td>0.117513</td>
<td>0.012591</td>
<td>1.06546</td>
</tr>
<tr>
<td>0.11 &gt; to &lt;= 0.12</td>
<td>92</td>
<td>0.303208</td>
<td>0.010814</td>
<td>2.935376</td>
</tr>
<tr>
<td>0.06 &gt; to &lt;=0.08</td>
<td>118</td>
<td>0.479215</td>
<td>0.008429</td>
<td>5.241963</td>
</tr>
<tr>
<td>0.15 &gt; to &lt;= 0.16</td>
<td>130</td>
<td>0.13464</td>
<td>0.007644</td>
<td>1.563339</td>
</tr>
<tr>
<td>0.08 &gt; to &lt;= 0.1</td>
<td>140</td>
<td>0.180051</td>
<td>0.007097</td>
<td>2.161433</td>
</tr>
<tr>
<td>0.1 &gt; to &lt;=0.12</td>
<td>171</td>
<td>0.217419</td>
<td>0.005811</td>
<td>2.87893</td>
</tr>
</tbody>
</table>

## TABLE 4
Fit statistics of logistic regression and GWLR model

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>Deviance</th>
<th>Residual sum of squares</th>
<th>Prediction accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logistic regression</td>
<td>552.036757</td>
<td>538.036757</td>
<td>87.4619</td>
<td>75.24</td>
</tr>
<tr>
<td>GWLR</td>
<td>531.558863</td>
<td>485.227414</td>
<td>74.1452</td>
<td>81.89</td>
</tr>
</tbody>
</table>
prediction accuracy than LR model. It means that the fitting effect of GWLR model is better than the LR model.

Grassland fires in this area is the result of a combination of environmental and anthropogenic factors. The mean annual temperature positively influences the grassland fire in our model. This result is consistent with the conclusion from other researches. Although temperature and precipitation influence the moisture contents of fuels, mean annual precipitation in the models was not significant. This is reasonable since rain increases the moisture contents of both dead and live fuels, reducing the dry fuels available to burn in fire season, however the semi-arid regions in Hulunbuir lack the fuels necessary for a fire to start and require abundant rain to provide ample fuels for fire ignition. Thus, mean annual precipitation was eventually rejected in our final model.

In our study, topographical factors were considered, but only elevation was highly significant. This result indicates that grassland fires are more likely occur at low elevations. It might because most of grassland fires in Hulunbuir are anthropogenic, and intensive human activities tend to be concentrated at low altitudes.

It highlights that anthropogenic effect on the concentrations of grassland fire, which are strongly associated with human’s access to natural landscape, so that the proximity to settlements and roads are important factors influencing the occurrence of grassland fire in Hulunbuir. According to our findings, agricultural activities is positively correlated with grassland fire occurrence. This is because fire is frequently used in arable and crop lands, very close to or intermixed with grasslands.

Based on GWR technique, our results indicate that the relationship between the explanatory factors is not spatially stationary in the study area. The GWLR model also shows a performance improvement when compared to the global models in terms of accuracy, relative goodness of fit, and residuals.

CONCLUSIONS

In this paper, we incorporated both environmental and anthropogenic variables into GWLR model to study the distribution of grassland fire occurrence in Hulunbuir over the period 2001-2015. The results of this study show that mean annual temperature, elevation, distance to nearest settlement, distance to nearest road, and distance to nearest agricultural area are correlated with grassland fire occurrence, while these explanatory factors are not spatially stationary in the whole study area. Thus, the consideration of non-stationarity in fire modeling is important to better understand fire regimes and to more efficiently prioritize critical area to fire occurrence.

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A new method is approached to promote the recovery by using CO2 to take the place of adsorption shale gas. The method proceeds from the perspective of exploitation mechanism of CO2 molecular replacement, by means of experiment evaluation methods of shale adsorption isotherms, direct depressurization and desorption by injecting CO2 replacement. The experiment results in lab of Fu Xian suggest that the recovery by injecting CO2 increases by 7.66% and is higher than that by the way of direct depressurization and desorption. It is more suitable to injecting CO2 when the reservoir pressure exhausts to 6.559MPa, and the proper volume of the injecting CO2 is 0.22 times its pore volume. The CO2 molecular replacement method is feasible. It can improve the shale gas recovery and shorten the exploration cycle.

KEYWORDS:
Shale gas, replacement, CO2, exploration, experiment

INTRODUCTION

The development technology related to shale gas is a plateau that China must break through [1]. Although China has the key technologies for shale gas mining, such as Horizontal well drilling technology and large-scale hydraulic fracturing technology [2]-[4], etc. However, since shale gas belongs to unconventional low grade natural gas. The shale gas has low pressure drop desorption output and long cycle, and it is difficult to have economic benefits in the short term.

The adsorption gas in shale gas extracted from the United States is achieved by depressurization and desorption. The depressurization and desorption mining is a kind of understanding of the suction process, It is also the most important desorption method [5] in the shale gas adsorption process. However, due to the strong adsorption of shale gas, the difficulty of mining is increased. At present, the production of simple decompression and desorption is low and the cycle is Long, it is difficult to achieve the desired mining efficiency, so how to improve shale, so the efficiency of gas extraction is crucial. A. Kalantari Dahaghi [6] used numerical simulation techniques to analyze methods for improving shale gas recovery. In 2011, Wang Hai Zhu and others proposed the development of shale gas technology [7] by using supercritical CO2, but they did not conduct experimental research on related processes; in the same year, W. Jing et al. discussed the method [8] of enhancing oil recovery. However, in general, the current methods for improving the recovery of shale gas are still very limited. The site is mainly based on depressurization and deblocking. In 2015, Pei Peng studied [9] the theoretical principles and feasibility of using CO2 in both the stimulation stage and the secondary gas recovery stage. In 2016, Chen, Jian analysed the pore characteristics of samples from hydrolysis experiments [10] of lacustrine shale by combining of mercury intrusion, low-pressure nitrogen, and carbon dioxide adsorption. In 2018, Du, Xi dong studied the effects of CO2 injection pressure (P-CO2) [11] on CO2 dispersion and the mechanism of CO2-CH4 displacement in a shale sampled from Chang ning of China. At the same year, Zhu, Chao fan designed a new experimental apparatus [18] to test the change in the mole fractions of CO2 and oil before and after adsorption and dissolution at equilibrium conditions. For simplicity, n-dodecane (n-C-12) was used as the oil. The adsorption and dissolution amounts of CO2 and n-C-12 were obtained using a mathematical method.

CO2 is a major greenhouse gas, and reducing CO2 emissions is one of the main measures to mitigate global warming. In conventional oil and gas reservoirs, field tests using CO2 displacement or oil displacement have been made and good results have been achieved. At the same time, CO2 has better adsorption performance in shale cores. To this end, this paper proposes a method of replacing shale gas adsorption gas with CO2 and conducts experimental evaluation. Through indoor experiments, the feasibility of CO2 replacement and shale CH4 adsorption gas is explored, which provides a basis for improving shale gas recovery.
Mechanism of CO₂ replacement method used in shale gas exploration. CO₂ replacement method is injecting a new substance to replace the CH₄ which is adsorbed on the shale. Because the adsorption capacity of CO₂ gas on shale is stronger than CH₄ gas. This is fundamentally different from the gas injection displacement, which is to drive away the oil and gas that has been adsorbed or freed in the pores.

The mechanism of displacement desorption is that in the process of displacement desorption, on the one hand, other gas molecules which are not adsorbed inconstant strive for the opportunity of adsorption under the action of Van der Waals force, in order to achieve a dynamic equilibrium state. On the other hand, the thermodynamic properties of gas molecules determine that these adsorbed gas molecules are constantly competing for van der The bond- age of Huali, the state of adsorption is free, so as to achieve the purpose of mining shale gas. This process is also a process of molecular displacement analysis.

Basic information of the experimental sample. At present, the adsorption and desorption experiments in the literature are basically made by pulverizing samples, and the pulverized core samples could not reflect the properties under the formation conditions to some extent; Therefore, in this shale gas adsorption and desorption experiment, the standard core is used for displacement and desorption experiments, which will more accurately reflect the adsorption and desorption of gas by shale under formation conditions. The experiment uses the Chang 7-section shale core of the Upper Triassic Yanchang Formation in Fu-Xian District, Ordos Basin, commonly known as “Zhang Jiatan shale”. First, the core is drilled into a standard core column, and the ends are cut flat, then dried at a high temperature of 100°C, and finally cooled and weighed. The basic parameters of the standard core selected for this experiment are shown in the Table 1.

We analysis the experiments about core and core section fragments in Table 1. The analysis of total rock and clay mineral composition of shale cores shows that the clay content is the highest, the mass fraction is 54.3%, followed by Quartz, the mass fraction is 24.4%, and the mass fraction of feldspar is 12.5%, the mass fraction of pyrite is 2.6%, the quality of calcite. The mass fraction is 2.1%, and the mass fraction of dolomite is 1.5%. The soil mineral is mainly composed of Yi Meng mixed layer with a mass fraction of 58.5%. The second time is illite, the mass fraction is 20.5%; the quality of chlorite. The score is 15.0%, and the kaolinite is 6.0%. The stone ratio is 20%.

Through the observation of the core, it is found that the shale rock in the Fu Xian area is dense, grayish black, black, and the fine layer of the light and dark minerals develops. It was found by environmental scanning electron microscopy (Fig. 1) that the pores of shale are dominated by micro pores, and the clay minerals are mainly mixed with imams.

EXPERIMENT

Experimental methods and processes. At present, there are few studies on shale gas mining methods in China, and there are fewer relevant experimental evaluations, most of which are limited to theoretical analysis and numerical simulation research. From the literature data, the current adsorption test methods are mainly volume method, weighing method, dynamic method, chromatography, calorimetry and so on. Different adsorption test methods have their own advantages and disadvantages. The weighing method is accurate and simple, avoiding the disadvantage where the dead volume is difficult to be measured in the volumetric method and the gas state equation is difficult to correct, and the measuring speed is fast; the disadvantage is the accuracy requirement for the balance or the spring balance.

Therefore, in order to overcome the shortcomings of various methods and to exert their advantages, this study comprehensively weighs and chromatographic methods. We use a high-precision electronic balance, a transparent window and use a high-precision pressure sensor to accurately measure the page. It can text the amount of gas adsorbed by the rock and the amount of gas displaced. We calculates the amount of CO₂ and shale gas adsorbed by combining with chromatographic analysis. The experimental process is shown in Figure 2. The experimental temperature is 30°C according to the actual parameters of the selected block, and the formation pressure is 8.259 MPa. In the experiment, the shale gas was CH₄ gas with a purity of 99.999%.

Waste pressure determination. The experiment selected No. 2 shale samples. According to the initial formation conditions, the experimental temperature was 30°C and the formation pressure was 8.259 MPa. The determination of the waste pressure is calculated by the following formula:

\[
P_{ab} = 0.3515 + 0.0010713 \times D \quad (1)
\]

In the formula: \(P_{ab}\) is the waste pressure; \(D\) is the well depth.

The depth of the selected shale sample No. 2 is 747.35 m, and the waste pressure calculated by the above formula is 1.152 MPa.
<table>
<thead>
<tr>
<th>Number</th>
<th>Hashtag</th>
<th>Diameter (cm)</th>
<th>length (cm)</th>
<th>depth (m)</th>
<th>Porosity (%)</th>
<th>Permeability (x10³ μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>XF6</td>
<td>2.436</td>
<td>5.562</td>
<td>747.35</td>
<td>4.13</td>
<td>0.0168</td>
</tr>
</tbody>
</table>

FIGURE 1
Environmental scanning electron micrograph of Experimental shale samples

FIGURE 2
Experimental procedure of replacement method
RESULTS AND DISCUSSION

Comparison of adsorption capacity of CO$_2$ and CH$_4$. The adsorption of CO$_2$ and CH$_4$ on the same shale sample was determined by the above experimental procedure. The experimental results are shown in Figure 3. It can be seen from Fig. 3 that the adsorption amount of CO$_2$ and CH$_4$ on the same shale sample increases with the increase of pressure, and the adsorption amount of CO$_2$ on the shale sample is much larger than that of CH$_4$. It means that injecting CO$_2$ to replace the CH$_4$ is theoretically feasible.

The feasibility evaluation of replacement mining. In order to evaluate the feasibility of CO$_2$ displacement and desorption of shale gas, two sets of experiments were carried out in the same core with direct depressurization and CO$_2$ displacement method in the same room, and the CO$_2$ displacement effect was compared and analyzed.

Direct depressurization experiment. At the initial formation pressure of 8.259 MPa, the CH$_4$ was fully saturated, and then it was slowly depressurized to a abandoned pressure of 1.15 MPa. The recovery degree of CH$_4$ adsorbed gas and free gas in the core under various depletion pressures was measured, and then Total recovery was obtained. The results of direct depressurization experiments are shown in Table 2.

It can be seen from Table 2 that the recovery rate of CH$_4$ in shale is 72.62% when the direct depletion mining is 1.152 MPa, and the recovery rates of adsorbed gas and free gas are 25.58% and 47.04%.

The experiment of CO$_2$ replacement method in shale gas exploration. In order to verify the feasibility of the CO$_2$ replacement method and overcome the effect of CO$_2$ injection on the replacement efficiency, an excessive amount of CO$_2$ was injected at the beginning of the experiment to ensure sufficient replacement. The experimental procedure is as follows: under the initial formation pressure of 8.259 MPa, the CH$_4$ gas is fully saturated, and then excessive CO$_2$ is injected into the system, and after being stabilized for 12 h, the pressure is gradually reduced to a waste pressure of 1.15 MPa. The experimental results of CO$_2$ replacement mining are shown in Table 3.

It can be seen from Table 3 that after the CO$_2$ is injected for replacement, the formation pressure is gradually degraded from 8.259 MPa to the abandoned pressure of 1.152 MPa, the free gas content and the total recovery are gradually increased, and the recovery ratio under the abandoned pressure is 80.29%, of which adsorbed gas and free gas are 33.19% and 47.10%, respectively.

The impact of mining methods on recovery ratio. The results of the decompression mining and replacement mining are shown in Figure 4 and 5 is shown.

![Figure 3](image)

**FIGURE 3**
The comparison chart of CO$_2$ and CH$_4$ adsorption in the same shale

**TABLE 2**

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Adsorption gas production (%)</th>
<th>Free gas production (%)</th>
<th>Recovery factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.259</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6.338</td>
<td>5.07</td>
<td>24.57</td>
<td>29.64</td>
</tr>
<tr>
<td>4.261</td>
<td>12.84</td>
<td>36.8</td>
<td>49.64</td>
</tr>
<tr>
<td>3.154</td>
<td>18.51</td>
<td>41.77</td>
<td>60.28</td>
</tr>
<tr>
<td>1.152</td>
<td>25.58</td>
<td>47.04</td>
<td>72.62</td>
</tr>
</tbody>
</table>
It can be seen from Fig. 4 and Fig. 5 that the recovery of shale gas by replacing CO$_2$ is 7.66% higher than reducing pressure directly. The recovery rate of free gas is only 0.06%. The recovery rate of adsorbed gas is increased by 7.6%, which indicates that CO$_2$ can replace CH$_4$ and effectively improve the recovery rate of shale gas. From the experimental process, after injecting some CO$_2$, the experimental cycle is significantly shortened. It can be seen that the CO$_2$ replacement method can not only improve the recovery ratio of shale, but also shorten the mining cycle. Therefore, the method that we are injecting CO$_2$ to replace shale gas is feasible and plays a role in displacement analysis.

The effect of Injection timing. According to the above procedure, the formation pressure was attenuated to 6.559 MPa and 3.718 MPa, and then CO$_2$ displacement and pressure reduction mining experiments were carried out to study the effect of different methods.
CO₂ replacement timing on oil recovery. The experimental results are shown in Tables 4 and 5.

It can be seen from Table 4 and Table 5 that when the pressure reaches 6.559 MPa, CO₂ is injected for replacement, and gradually depleted to abandoned pressure. The total recovery factor is 80.37%, and the recovery rates of adsorbed gas and free gas are respectively 33.24% and 47.13%; when the pressure reaches 3.718 MPa and then injected CO₂ for replacement, the total recovery factor under abandoned pressure is 80.16%, the recovery rate of adsorbed gas and free gas were 33.15% and 47.01% respectively.

It can be seen from Fig. 6 and Fig. 7 that CO₂ is injected under different formation pressures, and the total recovery rate of shale gas and the recovery rate of adsorbed gas are the highest when the formation pressure is 6.559 MPa, and the gas injection effect is best. The optimal injection timing is when the formation pressure is depleted to 6.559 MPa. But by observing the absolute value of the final effect from 3 timings, the impact of the injection timing on the displacement effect is not very obvious, so the CO₂ replacement can be injected at the right time in the production process, which increases the flexibility of the technology.

The optimization of injection rate. According to the optimization result of the injection timing, CO₂ is replaced when the formation pressure is depleted to 6.559 MPa, and the displacement effect of

---

**TABLE 4**
The desorption data by injecting CO₂ displacement (P=6.559 MPa)

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Adsorption gas production (%)</th>
<th>Free gas production (%)</th>
<th>Recovery factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.259</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6.559</td>
<td>4.61</td>
<td>24.46</td>
<td>29.07</td>
</tr>
<tr>
<td>5.361</td>
<td>12.12</td>
<td>30.82</td>
<td>42.94</td>
</tr>
<tr>
<td>4.081</td>
<td>19.36</td>
<td>37.43</td>
<td>56.79</td>
</tr>
<tr>
<td>2.513</td>
<td>27.00</td>
<td>43.63</td>
<td>70.63</td>
</tr>
<tr>
<td>1.151</td>
<td>33.24</td>
<td>47.13</td>
<td>80.37</td>
</tr>
</tbody>
</table>

**TABLE 5**
The desorption data by injecting CO₂ displacement (P = 3.718 MPa)

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Adsorption gas production (%)</th>
<th>Free gas production (%)</th>
<th>Recovery factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.259</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6.338</td>
<td>5.07</td>
<td>24.57</td>
<td>29.64</td>
</tr>
<tr>
<td>4.761</td>
<td>10.84</td>
<td>35.11</td>
<td>45.95</td>
</tr>
<tr>
<td>3.718</td>
<td>15.94</td>
<td>40.10</td>
<td>56.04</td>
</tr>
<tr>
<td>2.435</td>
<td>27.38</td>
<td>44.85</td>
<td>72.23</td>
</tr>
<tr>
<td>1.152</td>
<td>33.15</td>
<td>47.01</td>
<td>80.16</td>
</tr>
</tbody>
</table>

**FIGURE 6**
The impact on recovery of CO₂ injection timing
different CO₂ injection amounts is measured to optimize the optimal injection amount. The experimental results are shown in Table 6 and Figure 8. It can be seen from Table 6 and Figure 8 that with the increase of CO₂ injection amount, the shale gas recovery rate and the recovery rate of the adsorption gas increase rapidly in the early stage, and then tend to be gentle, and the optimal the injection amount of CO₂ is 0.22 times larger than the pore volume.

CONCLUSION

(1) This paper proposes a method for replacing shale gas adsorption gas by replacing CO₂. The method is essentially to inject CO₂ gas into the gas to replace the CH₄ gas that has been adsorbed on the shale to increase the shale gas recovery rate.

(2) Fu Xian shale replacement mining experiment shows that the recovery of shale gas by replacing CO₂ is 7.66% higher than reducing pressure directly, while the recovery rate of adsorbed gas increased by 7.6%. The reason for enhancing the recovery rate of gas by replacing CH₄ is that injecting CO₂ can replace CH₄ absorbed in the porous media. It plays an important role in replacement and desorption.

(3) The optimal injection timing for CO₂ replacement in Fu Xian shale injection is optimal CO₂ injection when formation pressure is depleted to 6.559 MPa. The amount is 0.22 times the pore volume.

<table>
<thead>
<tr>
<th>CO₂ injection amount (pv)</th>
<th>0</th>
<th>0.11</th>
<th>0.22</th>
<th>0.31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery factor</td>
<td>72.62</td>
<td>76.29</td>
<td>80.15</td>
<td>80.36</td>
</tr>
<tr>
<td>Absorption gas recovery factor</td>
<td>25.58</td>
<td>29.18</td>
<td>33.02</td>
<td>33.17</td>
</tr>
</tbody>
</table>

FIGURE 7
The impact on displacement desorption recovery of CO₂ injection volume

FIGURE 8
The impact of CO₂ injection volume on the desorption recovery
ACKNOWLEDGEMENTS

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THE GENETIC PARAMETERS OF WEIGHT GAIN AND FEED EFFICIENCY OF JAPANESE QUAILS (COTURNIX COTURNIX JAPONICA) UNDER TENEBRO MOLITOR L. AND CONTROL NUTRITIONAL ENVIRONMENTS

Abdullah Nuri Ozsoy*
Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Animal Sciences, 32260, Isparta, Turkey

ABSTRACT

Feeding especially those of protein sources is the main input in the total cost-price of poultry production. This fact is also valid for meat type quail populations which are subjected to the genetic breeding program. It is a subject of research that T. molitor can be a good alternative protein source. This research elucidated the effects of 10 % T. molitor addition as protein resource to quail diet on weight gains, feed intake and feed consumptions on weekly basis by comparing with control (soybean meal as main protein resource in diet). In addition, genetic parameters were estimated for weight gain and feed conversion rate characteristics. Total of 294 Japanese quails obtained from 50 full-sib families were used in this study. The overall mean weight gain was 27.7, 44.7, 60.7, 54.9 and 39.3 g at 1, 2, 3, 4 and 5 weeks of age, respectively. The overall average feed intake in the end of 1, 2, 3, 4 and 5 weeks were 50.5, 109.7, 112.0, 138.7 and 198.7 g per week, respectively, whereas the feed conversion rates for respective growth periods were 1.90, 2.52, 1.90, 2.59 and 6.04. T. molitor added 10% to the ration, resulted in a lower weight gain in the first three weeks of experiment comparing to the control group. However, this event does not apply to the later stages of growth period. The T. molitor addition into the ration led to a decrease in feed consumption after 3 weeks of age without any loss of growth performance. In this case, the feed conversion rates of birds at 4 and 5 weeks of age were lower for the T. molitor group. The heritability estimates for weight gain at 1, 2, 3, 4 and 5 weeks were 0.78, 0.55, 0.74, 0.53 and 0.21 respectively whereas those of the feed conversion rates were 0.64, 0.50, 0.69, 0.43 and 0.06. Instead of soybean meal in meat type quail diets, as an alternative dietary protein source, use of 10 % T. molitor was found to have negligible effects on genetic parameter estimates for weight gain and feed conversion rate traits.

KEYWORDS: Japanese quail, T. molitor, weight gain, feed intake, feed conversion rate, genetic parameters, genotype by environment interaction

INTRODUCTION

Japanese quail is preferred poultry animal that can be used for either meat or egg production. Quails are also considered a good model animal species due to short generation interval, their early sexual maturity, low keeping costs, very low areal requirement, very high disease resistance comparing to other poultry animals [1]. Thus quail production is done worldwide [2]. This production is made for meat in European countries and in Far-East countries for eggs. [3]. Operating costs in poultry production account for 70-80% of feed costs. Since protein is the most expensive component in the formulation of diets for quail, it has a major impact on the cost of selection programs [4]. Nowadays, the most widely used protein source in poultry feeding is soybeans due to economic reasons and readily availability of it. World-wide mass production of soybean is only made in several countries, this therefore cause price fluctuations. As a consequence of increased demand for meat and eggs push poultry breeders and scientists to seek new alternative sources of protein. One of these alternative sources could be Tenebrio molitor L. [5, 6, 7, 8, 9] because of the high levels of animal protein [5, 10] and high quality animal fats [11, 8, 5]. T. molitor may be a feed additive although there are some drawbacks for broiler. [5, 7, 12, 13] fish [14, 15, 16] and pigs [17]. However, the use of T. molitor in animal husbandry is limited depending on high cost of production, not being accepted as a feed additive in many countries, and its effects on animal and human health is not fully covered. It is hoped that this and similar studies will increase the use of T. molitor as a protein and fat feed additive in animal husbandry.

Progenies from the same female and male are regarded as full-sib. Full-sib groups are expected to have different performances under changing two
environments. If these differences are dependent on the full-sib families this indicates the occurrence of significant genotype x environment interactions [18]. Despite genotype x environment interactions were reported to be non-significant in many cases, researchers have tried to explore possible environmental effects on genotype in breeding [19, 20]. Besides, it is very important to know genetic parameter estimation in planning and implementation of breeding programs.

This study aimed to reveal and compare the effects of *T. molitor* incorporated at 10% into a broiler ration on weight gain performance, feed intake and feed conversion rate of Japanese quail. In addition, genetic parameters were also estimated by using the (co)variance components in order to determine the effects of *T. molitor* on the genetic parameters in a genetic breeding program for these properties.

**MATERIALS AND METHODS**

* Tenebrio molitor production. *Tenebrio molitor* used in the study were produced in Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Animal Breeding Laboratory. A ration containing 70% semolina, 20% wheat bran and 10% yeast was used for feeding the insects. In addition, water needs were met twice a week by supplying potatoes to the food medium. The larvae were harvested in the late larval stage. The larvae were dried at 50°C and stored at -20°C. The larvae were used as a powder in the ration preparation stage to get homogenous mixture of the ration. Raw protein, fat and fatty acid components of the larvae powder incorporated into the ration are given in Table 1.

<table>
<thead>
<tr>
<th>Components</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Crude fat</td>
<td>43.8</td>
</tr>
<tr>
<td>% Crude protein</td>
<td>46.1</td>
</tr>
</tbody>
</table>

**Fatty acid composition**

- Lauric (c12:0) 0.08
- Myristic (c14:0) 2.83
- Pentadecanoic (c15:0) 0.07
- Palmitic (c16:0) 17.58
- Palmitoleic (c16:1) 4.64
- Heptadecanoic (c17:0) 0.12
- Heptadecenoic (c17:1) 0.1
- Stearic (c18:0) 3.42
- Oleic (c18:1) 50.96
- Linoleic (c18:2) 19.10
- Linolenic (c18:3) 0.62
- Arachidic (c20:0) 0.04
- Total 99.56

**The preparation of rations.** In the study, a commercial broiler ration (BR) was obtained by Charoen Pokphand (PC) feed company. The analytical components, vitamins and amino acid composition of this ration are given in Table 2.

**TABLE 2**

<table>
<thead>
<tr>
<th>Components</th>
<th>Contents (kcal kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>22</td>
</tr>
<tr>
<td>Metabolic energy (kcal kg⁻¹)</td>
<td>3078</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>4.1</td>
</tr>
<tr>
<td>Total ash (%)</td>
<td>6.4</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>6.0</td>
</tr>
<tr>
<td>Vitamin A(IU/kg)</td>
<td>12500</td>
</tr>
<tr>
<td>Vitamin D3(IU/kg)</td>
<td>3400</td>
</tr>
<tr>
<td>Vitamin E(IU/kg)</td>
<td>78</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.36</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Two experimental rations were prepared by adding either *T. molitor* or soybean meal and fat into the commercial ration containing the prepared 22% crude protein and 3078 kcal / kg metabolic energy (ME). The control (CONT) and *T. molitor* doped ration (TML) contents are given in Table 3. All birds were allowed to access diet and water ad libitum.

<table>
<thead>
<tr>
<th>Components</th>
<th>Contents (%)</th>
</tr>
</thead>
</table>

**TABLE 1**

**Nutritional composition of *Tenebrio molitor* powder**

<table>
<thead>
<tr>
<th>Components</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Crude fat</td>
<td>43.8</td>
</tr>
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- Linolenic (c18:3) 0.62
- Arachidic (c20:0) 0.04
- Total 99.56
<table>
<thead>
<tr>
<th>Rations</th>
<th>BR¹ (%)</th>
<th>T.molitor² (%)</th>
<th>Soybean meal³ (%)</th>
<th>Vegetative fat⁴ (%)</th>
<th>Total (%)</th>
<th>Crude protein (%)</th>
<th>ME Kcal/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONT</td>
<td>90</td>
<td>-</td>
<td>8</td>
<td>2</td>
<td>100</td>
<td>~ 24</td>
<td>3142.8</td>
</tr>
<tr>
<td>TML</td>
<td>90</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>~ 24</td>
<td>3117.1</td>
</tr>
</tbody>
</table>

¹The composition of BR was given in Table 2. ²T.molitor composition: 46.1% crude protein (Table 1), 3469 ME (metabolic energy of animal fat was taken as 7920 Kcal/kg in the calculation). ³soybean meal: 46.6% crude protein, 2458 Kcal/kg ME. ⁴Vegetative fats (sunflower oil): 8800 kcal/kg ME. Data were taken from [21].

Statistical analysis. The data were subjected to ANOVA and mean separation was performed by Tukey’s test at p≤0.05. The variance components of properties were estimated by REML method in ASREML [22] software. Estimation of variance components was based on the following family model:

\[ y = Xb + Z_f f + Z_r r + Z_{f \times r} (f \times r) + e \ldots \]

\[ b = \text{Vector of fixed-effect (sex)}, \]

\[ f = \text{Vector of random family effects, } \sim \mathcal{N}(0, \sigma_f^2) \]

\[ r = \text{Vector of random ration effects, } \sim \mathcal{N}(0, \sigma_r^2) \]

\[ (f \times r) = \text{Vector of random interaction family x ration effects, } \sim \mathcal{N}(0, \sigma_{f \times r}^2) \]

\[ e = \text{Vector of random residual effects, } \sim \mathcal{N}(0, \sigma_e^2) \]

The share of family, ration and family x ration interaction in total phenotypic variation was calculated as follows:

\[ \sigma_p^2 = \sigma_f^2 + \sigma_r^2 + \sigma_{f \times r}^2 + \sigma_e^2 \]

\[ f^2 = \frac{\sigma_f^2}{\sigma_p^2}, \]

\[ r^2 = \frac{\sigma_r^2}{\sigma_p^2}, \]

\[ (f \times r)^2 = \frac{\sigma_{f \times r}^2}{\sigma_p^2} \]

Where \( \sigma_p^2, \sigma_f^2, \sigma_r^2, \sigma_{f \times r}^2, \sigma_e^2 \) donate phenotypic, family effect, rational effect and variance of error effect, respectively. \( f^2, r^2 \) and \((f \times r)^2\) refer shares of family, ration and family x ration interaction in the total variance, respectively. Heritability estimates \( h^2 \) were computed using the equation given below [23]:

\[ h^2 = 2\sigma_f^2/\sigma_p^2 \]

RESULTS AND DISCUSSION

Weight gain. Average weight gain of Japanese quails up to 5 weeks of age for two experimental groups were given in Table 4. The average weekly weight gains of 27.7, 44.7, 60.7, 54.9 and 39.3 g per week were higher than those reported by Almeida et al. [24], Razee et al. [25] and Nasiri Foomani et al. [26] but lower than those reported by Bulus et al. [27]. On the other hand, the weight gain values are quite similar to those reported by Sakunthala et al. [1]. These differences in weight gain performances reported in these studies may be induced by genetic structure, environmental conditions and treatment differences.

Feed intake and feed conversion rate. The weekly averages of feed intake and feed conversion rate of the rations were given in Table 5. As can be seen in Table 5, weekly averages of feed intake were found as 50.5, 109.7, 112.0, 138.7 and 198.7 g/week. These values were comparatively smaller than those reported for fourth and fifth weeks by Sakunthala et al. [1]; Almeida et al. [24]; Nasiri Foomani et al. [26]. On the other hand, the FCR weekly averages (1.90, 2.52, 1.90, 2.59 and 6.04) were higher than the ones reported by Bulus et al. [27] whereas smaller than the findings of Almeida et al. [24]; Nasiri Foomani et al. [26]; Varkooohi et al. [28]. However, Sakunthala et al. [1] found highly similar results. These differences can be attributed to genetic structure of their quails, growth conditions and even number of quails they used in their researches.
Despite there were no noticeable differences (p<0.01) of feed intake in the first two weeks between the rations, the birds in CONT group apparently consumed more feed than the TML group in the third, fourth and fifth weeks.

The FCR weekly averages of TML group averages were higher in the first two weeks. However this changed in favour of TML treatment in the subsequent weeks. Therefore birds have better converted TML feed into live weight in this period.

Ramos-Elorduy et al. [5] used *T. molitor* as feed for 7-21 day old broiler chicks. They tested the effect of 5% and 10% of the insects on the weight gain, feed intake and feed conversion rate of the chicks which was similar to the control group. Bovera et al. [7] also reported no extra positive effects of *T. molitor* as an alternative protein source on the performance of 30 and 62 days old broilers comparing to soybean. The results obtained in our study and the results reported in the literature are partly similar.

**Heritability estimates.** Variance components estimates obtained for weight increase and feed conversion rate properties by using the mathematical model given in Model 1 and genetic parameters calculated using these estimates were given in Table 6.

Heritability estimates for weight gain were found in the range of 0.21-0.78. Estimated heritability reported by Sakunthala et al. [1]; Nasiri Foomani et al. [26] was smaller than the ones determined in this study. The estimates of heritability range for FCR feature was 0.06 - 0.69 (Table 7)
which was partially consistent with the 0.67 value [26] and was higher than the one reported by Sakunthala et al. [1].

The share of the ration factor as a components total phenotypic variance of weight gain property was considerably low in the range of 2.64-7.2. The ratio of (family x ration) interaction in total phenotypic variance was very close to 0 except for 3th and 5th weeks (3.14-6.99). On the other hand, the share of the ration-effect was ranged between 0.0 - 17.4. It is the fact that different nature of protein sources in the rations led to a slight increase in the contribution of this element to the variance. However, this situation was not seen in the (family x ration) interaction.

CONCLUSIONS

Weight gains of the birds fed with T. molitor were found to be significantly lower than in control group (P<0.01) until the end of the 3rd week. These significant differences were disappeared at the 4th and 5th weeks. On the other hand, amounts of feed intake were found to be the same in the first two weeks but in the subsequent weeks birds consumed more in CONT treatment. This situation affected the feed conversion rate and showed that the feed conversion rates of the quails in the CONT group of the quails fed with T. molitor added ration were lower. Thus, T. molitor incorporation into BR feed as low as 10% enhanced the FCR of birds.

T. molitor addition to feed had very little contribution on the phenotypic variation of the weight gain. However, this ratio was found to be slightly higher for FCR trait. The share of interaction (family x ration) in total phenotypic variation was negligible around 0. Consequently, it can be said that the effect of T. molitor (10%) added ration on genotypic parameters will be trivial in the feeding of weight gain and feed intake breeding programs.

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ASSESSMENT OF THE SURFACE WATER QUALITY IN EASTERN MARMARA SEA

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ABSTRACT

The Sea of Marmara is polluted with domestic and industrial wastewater, drainage water from agricultural lands, shipping, and loads carried by streams. Because there are settlement areas where the industry is very concentrated in the Eastern Marmara coastal regions, stations are determined on the coasts of East Marmara. It is aimed to determine water quality in the Eastern Marmara Sea. Parameters of temperature, pH, conductivity, dissolved oxygen, oxygen saturation, turbidity, salinity, anionic detergent, phosphate, ammonium, nitrite, nitrate, boron, total coliform and fecal coliform were determined. The values were compared with the criteria values specified in the regulations and the values in other studies.

KEYWORDS:
Anionic detergent, boron, nitrate, nitrite, phosphate.

INTRODUCTION

The Sea of Marmara is an inner sea with a total area of 11 350 km2 and a volume of 3378 km3. Its topographic and hydrographic features play an important role in the dynamics and pollution of this sea. The Dardanelles strait connects it to the Aegean Sea and the Bosphorus strait to the Black Sea. The two sills of the Bosphorus have the ability to control the hydrodynamics of the Bosphorus and the Sea of Marmara. The Sea of Marmara has a maximum width of 76 km and a maximum length of 276 km and a coastal length of 927 km [1].

Due to the presence of dense residential areas and industrial zones around the Sea of Marmara, an increasing environmental and marine pollution occurs. In particular, oil tankers and other dense marine traffic are increasingly affecting existing pollution, and fish populations are adversely affected [2]. Important industrial zones located in the Marmara Sea basin discharge wastewater to this inner sea or rivers without any treatment. This leads to the pollution of the Marmara Sea. The Marmara Sea is a sea between the Mediterranean Sea and the Black Sea, and due to continuous flow, pollutants can be carried to far distances in the sea. So, even in areas where industrialization and population density are low, contamination may be the issue. The Sea of Marmara has a great volume and its duration of the water stay in this sea is long. As a result, the fact is that the polluters can remain in this environment for a long time.

Seas have been seen as a receiving environment where pollutants can be discharged for years. The Bosphorus, Izmit Gulf, Gemlik Gulf are polluted areas in the Marmara Sea. However, pollution is not limited to only these regions in the Marmara Sea. In general, sources of pollution seen in the sea can be listed as domestic and industrial waste water discharges, transport by rivers, agricultural activities, atmospheric sedimentation, ship transport, illegal discharges, oil and gas production in the sea [3, 4, 5]. The fact that the Sea of Marmara has not enough water exchange to dilute wastes and to provide natural treatment, the current and mixture movements can not provide sufficient dilution means that the biological field will be narrowed and the ecology will continue to be damaged [6].

The natural patterned bottom water of the Sea of Marmara is under the influence of three different sources in terms of organic matter. These are organic and inorganic substances originating from the Black Sea, Marmara’s own biological production and urban-industrial settlements. The presence of dense settlements and Industry around the Sea of Marmara caused advanced pollution. 10 tons of mercury, 19 000 tons of zinc, 600-4200 tons of copper and 3000 tons of cadmium are transported to the Sea of Marmara via the Bosphorus very year [7].

As the size of pollution affects the living life in the aquatic system, the natural balance is disturbed when the pollutants rise above a certain level in the environment and human health is threatened through the organisms constituting the food chain. In this study, it is aimed to determine the water quality in the stations we have identified in the east of the Sea of Marmara, as well as to determine the formation sources of pollution and to present solutions to take the necessary measures.
MATERIALS AND METHODS

The research stations are close to the settlements and industrial areas in the Eastern Marmara Sea (Figure 1). The stations coordinates were shown in the Table 1. The research materials were the water samples taken from the stations on a seasonal basis. The water samples were collected during the period of Autumn 2015 – Summer 2016. Temperature, pH, conductivity, dissolved oxygen, turbidity and salinity parameters were measured by Water Quality Meter [Water Quality Checker™ (DKK-TOA WQC 24)].

The parameters of pH, temperature, dissolved oxygen, turbidity, conductivity and salinity were measured at the sampling stations by the water quality checker. The parameters of anionic detergent [8], phosphate [9], boron [10, 11], ammonium, nitrite, nitrate [12] were determined by spectrophotometric methods in our research laboratory. Total coliform and fecal coliform were determined by the Most Probable Number (EMS) method [13]. Graphpad Prism for Windows Package Statistics software was used in the statistics analyses.

RESULT AND DISCUSSION

It was found average pH 8.39, dissolved oxygen 6.3 mg/L, temperature 15.7 °C, salinity ‰ 21.35, conductivity 3.48 S and turbidity 5.139 mg/L. The seasonal averages of anionic detergent, phosphate, ammonium, nitrite, nitrate and boron concentrations were found 0.174 mg/L, 0.015 mg/L, 0.125 mg/L, 0.189 µg.at/L, 1.748 µg.at/L ve 4.12 mg/L, respectively.

![FIGURE 1](image.png)

**TABLE 1**

<table>
<thead>
<tr>
<th>Station No</th>
<th>Station Name</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silivri</td>
<td>41°04'37&quot;N 28°14'11&quot;E</td>
</tr>
<tr>
<td>2</td>
<td>Buyuk Çekmece</td>
<td>41°01'05&quot;N 28°34'43&quot;E</td>
</tr>
<tr>
<td>3</td>
<td>Kucuk Çekmece</td>
<td>40°58'40&quot;N 28°46'25&quot;E</td>
</tr>
<tr>
<td>4</td>
<td>Uskudar</td>
<td>41°01'12&quot;N 29°00'26&quot;E</td>
</tr>
<tr>
<td>5</td>
<td>Golden Horn</td>
<td>41°01'40&quot;N 28°57'27&quot;E</td>
</tr>
<tr>
<td>6</td>
<td>Gemlik</td>
<td>40°49'51&quot;N 29°16'41&quot;E</td>
</tr>
<tr>
<td>7</td>
<td>İzmit</td>
<td>40°44'21&quot;N 29°56'25&quot;E</td>
</tr>
<tr>
<td>8</td>
<td>Tuzla</td>
<td>40°25'27&quot;N 29°09'16&quot;E</td>
</tr>
<tr>
<td>9</td>
<td>Hereke</td>
<td>40°46'56&quot;N 29°37'09&quot;E</td>
</tr>
</tbody>
</table>
The temperature, pH, dissolved oxygen, conductivity, oxygen saturation, turbidity, salinity, anionic detergent, phosphate, ammonium, nitrite, nitrate, boron, total coliform and fecal coliform mean values are shown on Figures 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15.
FIGURE 5  
Average graph of salinity values

FIGURE 6  
Average graph of conductivity values

FIGURE 7  
Average graph of turbidity values
FIGURE 8
Average graph of anionic detergent values

FIGURE 9
Average graph of phosphate values

FIGURE 10
Average graph of ammonium values
FIGURE 11
Average graph of nitrate values

FIGURE 12
Average graph of nitrite values

FIGURE 13
Average graph of boron values
Dissolved oxygen (mg/L) ≥ 6 5 4 ≤ 4 6.3
Total phosphorus (µg/L) < 14 14 - 21 22 - 30 > 30 15
Nitrite + Nitrate (µg/L) < 14 14 - 20 21 – 34 > 34 1.937

Compared with the "General Quality Criteria of Sea Water" and "Standard Values of Coastal and Sea Water Used for Recreational Purposes (0.3 mg/L)" in the framework of the Water Pollution Control Regulation, the average value of anionic detergent (0.174 mg/L) was found to be low. In addition, the pH value (8.39) was found to be appropriate to the "General Quality Criteria of Sea Water (6 – 9)". Total coliform (220 (EMS/100 mL)) and fecal coliform (20 (EMS/100 mL)) values were found to be below "Standard Values of Coastal and Sea Water Used for Recreational Purposes (Total coliform (EMS/100 mL) 1000, Fecal coliform (EMS/100 mL) 200" [14]. It is stated that about 5 - 6 mg B / L boron may be found in sea water studies. The average boron value (4.12 mg/L) is lower than these values in our study.

Compared with the "Quality Criteria According to Classes in Terms of General Chemical and Physicochemical Parameters of Coastal Waters" in...
the framework of the Above Ground Water Quality Regulation [15], it was found that first class water in terms of dissolved oxygen and nitrite + nitrate parameters and second class water quality in terms of phosphate parameter (Table 2).

When we compared phosphate and nitrite+nitrate values with "Marmara Coastal Waters Eutrophication Criteria" [16], it was determined that water quality class was oligotrophic for nitrite+nitrate and mesotrophic for phosphate (Table 3). The concentrations of phosphate, ammonium, nitrite and nitrate were higher in stations close to the settlements and in stations close to the freshwater inputs (Silivri, Golden Horn, Kucuk Cekmece, Gemlik, Izmit etc.) than in other stations.

**TABLE 3**

**Marmara Coastal Waters Eutrophication Criteria** [16].

<table>
<thead>
<tr>
<th>Water Quality Classes</th>
<th>Total phosphorus (μg/L)</th>
<th>Nitrite+Nitrate (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligotrophic</td>
<td>&lt; 14</td>
<td>&lt; 14</td>
</tr>
<tr>
<td>Mesotrophic</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>Hypertrophic</td>
<td>&gt; 30</td>
<td>&gt; 34</td>
</tr>
<tr>
<td>This study</td>
<td>15</td>
<td>1.937</td>
</tr>
</tbody>
</table>

According to “Swimming Water Quality Regulation, Swimming and Recreational Water Quality Criteria” [17], the surface active substances that react with methylene blue should be at a level that does not create permanent foam (≤ 0.3 mg/L). The average value of surface water samples (0.174 mg/L) was below the limit value in this study.

“One-way Anova” variance analysis was carried out to determine whether the amounts of pH, temperature, dissolved oxygen, conductivity, turbidity, salinity, anionic detergent, phosphate, ammonium, nitrite, nitrate and boron showed significant difference among stations. As a result of the “One-way ANOVA” test, the difference in salinity, conductivity and turbidity amounts among the stations was significant (p <0.05) and the difference of the other parameters among the stations was not significant (p> 0.05) (Table 4).

**TABLE 4**

**One-Way ANOVA test results among the stations**

<table>
<thead>
<tr>
<th>Table Analyzed (Salinity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>P value</td>
</tr>
<tr>
<td>P value summary</td>
</tr>
<tr>
<td>Are means signif. Different?</td>
</tr>
<tr>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Number of groups</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>R squared</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table Analyzed (Conductivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>P value</td>
</tr>
<tr>
<td>P value summary</td>
</tr>
<tr>
<td>Are means signif. Different?</td>
</tr>
<tr>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Number of groups</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>R squared</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table Analyzed (Turbidity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>P value</td>
</tr>
<tr>
<td>P value summary</td>
</tr>
<tr>
<td>Are means signif. Different?</td>
</tr>
<tr>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Number of groups</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>R squared</td>
</tr>
</tbody>
</table>
The salinity of the 5th station Uskudar and the 4th station Golden Horn were low. Because these stations are located in the region where the Black Sea waters with low salinity reached to the Sea of Marmara. And in parallel with this the conductivity parameters were lower than the other stations.

The turbidity of 3rd station (Kucuk Cekmecce) and 7th station (Izmit) were higher than the other stations. It can be considered that domestic and industrial wastes of Kucuk Cekmecce can be transported to the sea, it is close to the freshwater input where organic and inorganic load is high. And the 7th station is located in Izmit Gulf region and turbidity can be increased due to domestic and industrial waste load in this region. In addition, Ulva species are dominant, Gracilaria and Ceramium species are also abundant in these two stations.

The other studies conducted in the Sea of Marmara show that pollution incidents have increased in recent years and the similar values are obtained in these studies.

In the "Distribution of Land-borne discharges and Available Sea Areas in the Sea of Marmara" study, it is stated that the Sea of Marmara has been heavily polluted in recent years. It has been reported that in the rapidly growing urban settlements especially in Izmit, Gemlik and Bandirma Gulf, and living in some parts of the sea polluted with waste water, especially in a growing number of industrial establishments, life has been greatly limited and the amount of oxygen has decreased [18].

In the study on detergent pollution in the Sea of Marmara, values ranging from 1.26-52.29 μg/L in 1996 and 1.57-74.98 μg/L in 1997 were determined. In this study, the amount of detergent in deep water is found to be higher than surface water [19].

In the research conducted in Dardanelles Strait between 2001-2002, concentrations of detergents were found 62.0.05 μg/L in Gallipoli and 105.80 μg/L in Lapseki [20].

In a study carried out by Guven et al. in 2010, LAS (linear alkil benzen sulphonat) amount from Turkey coasts to the Black Sea were calculated as 12352 t/year in 2004, 7044 t/year in 2005, 22774 t/year in 2006 and 20349 t/year in 2007, respectively. Also in 2005, the highest detergent value in the Bosphorus was found to be 41.05 μg/L in Tarabya [21].

Temperature, ammonium nitrogen, nitrite, nitrate, phosphate, chlorophyll-a and silicon were measured in the study conducted in the Dardanelles Strait. Phosphate concentration dropped below 0.01 μM at some points while it reached the level of 9.09 μM at some points. The amount of phosphate has shown significant variation between sampling points. As a result, it has been determined that phosphorus is a potentially limiting nutrient element, while the total inorganic nitrogen in the working region is not limited for the phytoplankton requirement. The amount of chlorophyll-a is determined to be below 2 μg/L during the study period and the amount of nitrate is found to be highest throughout the sampling period [22].

In the study conducted in the Golden Horn, it has been seen that nutrient concentrations, water clarity and dissolved oxygen concentrations decreased considerably in parallel with the increase in unplanned settlements and industry in the Golden Horn [23].

Nitrogen parameters, physical parameters and total coliform measurements have been carried out in the Marmara Basin. In terms of nitrogen parameter, basin is 3rd and 4th class, and it is 3rd class with regard to color, dissolved oxygen, iron and total coliform parameters [24].

Anionic detergent and LAS concentration were measured in the coastal surface water of the Straits of Canakkale and Istanbul. LAS measured in the Sea of Marmara as 26.06 μg/L, in the Canakkale Strait as 24.24 μg/L, in the Istanbul Strait as 22.88 μg/L in January and in the Sea of Marmara as 42.15 μg/L, in the Canakkale Strait as 48.25 μg/L, in the Istanbul Strait as 43.43 μg/L in August [25].

In Balcioglu's research, the anionic detergent concentrations varied between 20.14 and 77.44 μg/L. Higher values are found at stations close to dense residential areas [26].

The average values of the total oxidized nitrogen concentrations in the Sea of Marmara vary between 0.45-0.7 μmol N / L and the phosphate concentrations are at the level of 0.3 μmol P / L in average. The eutrophication limit for marine environments is given as 0.2-0.4 μmol N / L for total oxidized nitrogen and 0.15 μmol P / L for phosphorus, respectively. The whole Sea of Marmara has generally an eutrophic structure [22].

In the study "Current pollution situation and solution proposals of the Sea of Marmara", since the total phosphate concentration measured in the Sea of Marmara is at the level of 0.3 μmol P / L and the eutrophication limit for marine environments is accepted as 0.15 μmol P / L for phosphorus. The mean values of the total oxidized nitrogen concentrations measured in the Sea of Marmara vary between 0.45-0.7 μmol N / L. The eutrophication limit for marine environments is 0.2-0.4 μmol N / L for total oxidized nitrogen. It is concluded that the whole Sea of Marmara have an eutrophic structure in general [6].

According to calculations, about 0.98x10^4 tons TP (total phosphate) and 1.72x10^4 tons TN (total nitrogen) per year enter from Black Sea to the Marmara Sea. 0.33x10^4 tons TP and 0.17x10^4 tons TN have entered per year from Istanbul to the Sea of Marmara since 1990 [27].

The bad water quality-eutrophic status, the low water quality-high mesotrophic status and high quality and low trophic level were detected in terms of dissolved oxygen, Chl a and trophic index in the
Gemlik Gulf (Marmara Sea) [28].

In the study of fecal coliform in the Sea of Marmara, the surface water was found to be dirtier than the lower layer water in terms of fecal coliform. Fecal coliform (FC) concentration was observed to vary between 500-4000 FC / L in surface water and 0 - 60 FC / L in bottom water [29].

The some physico-chemical parameters (pH, temperature, salinity, dissolved oxygen, turbidity, conductivity, phosphate and ammonium nitrogen) were determined at Turkey coasts. The mean pH 8.12, temperature 25.56 °C, salinity 22.27 ‰, dissolved oxygen 5.56 mg/L, turbidity 1.15 mg/L, conductivity 34977 μS/cm, phosphate 11.14 μg/L and ammonium nitrogen 0.112 mg/L were found in the sea of Marmara [30].

In the study conducted in the Gulf of Gemlik (Marmara Sea), temperature, salinity, dissolved oxygen, ammonium, phosphate and chlorophyll-a concentrations of the seawater were measured between 8.7-28.3 °C, 14.98-36.59 ppt, 2.63-17.18 mg L⁻¹, 0.02-1.93 μg-at L⁻¹, 0.1-5.1 μg-at L⁻¹ and 0.00-10.64 μg L⁻¹, respectively. Trophic Index values were between 0.91 and 4.92 and these values shown that this region has the highest quality status but the lowest trophic level [31].

In the study in the Kapıdağ Peninsula (Marmara Sea), the temperature, dissolved oxygen, salinity, ammonium-N, phosphate-P and chlorophyll-a levels were found between 7.5 - 26.0 °C, 2.77 - 10.33 mg L⁻¹, 18.06 - 35.06 ppt, 0.01 - 0.79 μg-at N L⁻¹, 0.01 - 1.48 μg-at P L⁻¹ and 0.02- 4.21 μg-at L⁻¹, respectively. The high chl-a concentrations were found in autumn-winter months. These high concentrations of chl-a have been reported to be due to river currents and anthropogenic effects [32].

**SUGGESTIONS**

The wastewater of dense residential and industrial areas located to the east of the Sea of Marmara should be provided to the sea after being treated at treatment plants. Surfactants with high biodegradability must be used to prevent detergent contamination. In detergents, measures may be taken to reduce the hardness of the water as follows: to use non-polluting substances and to reduce the phosphate load of domestic wastewater. Chemical and biological methods can be applied separately or together to remove phosphate. In these processes, phosphorus is precipitated as phosphate salts at high pH values. For advanced phosphorus treatment, shallow algae lagoons where the algae are intensively produced and harvested can also be used. Harvested algae can be considered as raw material in the production of animal feed or biogas. In terms of water management, in order to avoid any adverse developments that may arise from the boron, it is necessary to reduce industrial boron values. The wastewater should be discharged after it has been treated in the wastewater treatment plants.

**ACKNOWLEDGEMENTS**

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THE EFFECTS OF AGRICULTURAL WASTE COMPOSTS 
(BANANA PLANT WASTES-GREENHOUSE WASTES-SPENT 
MUSHROOM COMPOSTS) ON MINERAL COMPOSITION 
AND QUALITY PARAMETERS OF LETTUCE 
(LACTUCA SATIVA L.)

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ABSTRACT

Agricultural waste is generally disposed of without being utilized on agricultural land. It is estimated that such waste is of large volume with different types and amounts linked to production regions. Both environmental pollution and rich nutrient contents need to be converted into fertilizer. The aim of this research was to determine the effects of organic fertilizer prepared from the composting of banana plant waste, greenhouse waste and spent mushroom compost on the development, morphology and productivity of lettuce. The composts used consisted of different agricultural waste; banana plant waste (BPW), greenhouse plant waste (GPW), and spent mushroom compost (SMC). In these experiments, compost was used at 5 different levels for each 1 ton ha⁻¹ of pot soil and the effects on growth and mineral nutrient contents of lettuce plants were investigated during two successive seasons (autumn and spring). The results showed that the compost treatments had positive effects on lettuce growth parameters and treatments of M₃ (50%BPW+40%GPW+10%SMC) and M₄ (40% BPW+50%GPW+10%SMC) in the lettuce yield values came to the forefront in both seasons compared to the control group. M₁ (70%BPW+20% GPW+10%SMC) application was determined to be the best practice in mineral nutrition of lettuce.

KEYWORDS:
Banana plant waste, Greenhouse plant waste, Spent mushroom compost, Lettuce growth, Yield

INTRODUCTION

Lettuce is grown in large quantities throughout the world and the most important countries producing lettuce are China and the United States. In 2016, the lettuce production in Turkey was 478,442 tons and production area was 22,431 ha [1]. Lettuce (Lactuca sativa L. var. longifolia) is the major salad crop to be cultivated and commercialized internationally [2]. As an edible vegetable, lettuce is widely produced and consumed in Turkey. Lettuce is a salad that can be grown in open and covered conditions all year round, and is consumed as fresh greens [3].

Fertilization is the most important application that increases productivity in agriculture. Organic manures are used in conventional agriculture for the protection of soil properties and these materials consist of plant and animal wastes. Agricultural waste has generally been considered as a source of pollution and has not been sufficiently evaluated as a by-product of agricultural activity which could produce organic fertilizers by composting [4]. Agricultural wastes should be utilized in useful ways through composting which is the best transformation system for agricultural wastes. Composting is the method in which organic waste is decomposed by microorganisms under aerobic conditions [5, 6, 7]. During this process, micro-organisms break down and transform the organic matter and expose thermal energy. The optimum parameters for composting are as follows; C/N: 25-35:1, humidity 40-70%, temperatures below 70°C and preferably 55-65 °C and pH around 6.5-7.5 [8, 9].

The disposal of agricultural waste is inevitable because increasing urbanization and populations have increased the generation of waste throughout the world [10]. It has become an important environmental problem and solutions to these problems can be expensive. In recent years in particular, the amount of agricultural waste has escalated significantly. Doran et al. [11] determined that banana plant waste could be used in agricultural areas after being composted and in this way, the mineral content of the plant increased. Elnour et al. [12] emphasized that the application of compost obtained from banana waste resulted in a significant increase in crop production and reduced the negative effect of off-season sowing. However, it is also important to
increase the adoption of compost technology on a large scale, which leads to zero waste.

Mushroom compost cannot be used for a second time in the cultivation of mushrooms because it has lost its properties. Therefore, it is discarded randomly at the periphery of agricultural areas. However, several researchers have indicated that because of the valuable organic matter content, spent mushroom compost can be used in many different ways [13, 14, 15].

The aim of this research was to determine the effects of agricultural waste on lettuce production. Therefore, greenhouse plant waste combined with used banana plant waste and spent mushroom compost at different ratios was added to experiment soils, and the effects of these materials were determined on lettuce yield, quality and nutrient contents.

MATERIALS AND METHODS

Banana plantation waste (BPW), greenhouse tomato plant waste (GPW) and spent mushroom compost (SMC) were mixed at five different ratios based on dry material for composting with a reactor-type composting system (Table 1). Organic materials used in the experiments were ground in a hammer mill prior to the composting process so as to obtain fine grains of these materials. Then, different mixtures were prepared and added into the composting system. The mixtures to be placed into the composting reactors were formed to contain 10 kg of dry material each. The humidity of the mixtures was brought to 60-70 % level which is the optimum level for the composting process and the materials were mixed until a homogenous mixture was obtained.

The composting process was carried out in compost reactors consisting of PVC (polyvinyl chloride) material covered with glass wool against heat loss. Each reactor had a volume of 127 liters and temperature-ventilation measurements that are taken at different points were recorded inside the reactors (Figure 1). Ventilation inside the reactors was measured by radial fans.

![FIGURE 1](image1.png)

**FIGURE 1**

Properties of container composting reactor

The pre-composting experiments were performed within a 21-day period. In order to determine the optimum mixture ratio, the process temperatures and levels of dry organic materials were taken into consideration. Process temperatures were measured every 15 minutes. Management of the composting system was controlled by a PLC-based process control device [16]. The composting phase was conducted under controlled conditions and composts were kept in the maturation phase after the pre-composting process (Figure 2).

![FIGURE 2](image2.png)

**FIGURE 2**

The principle of the reactor composting system

The chemical contents of the composts obtained from different agricultural wastes at the end of the composting process are presented in Table 2. Five different composts were added to pot soil (10 kg soil pot⁻¹) taking into account humidity content, so that 1 ton is ha⁻¹ for the production of lettuce and the pot experiment was carried out in autumn (1st season) and spring (2nd season) successively. The soil used as a control in the experiment was chemically analyzed and is shown in Table 3.

<table>
<thead>
<tr>
<th>Mixture No</th>
<th>Banana Plantation Waste</th>
<th>Greenhouse Plant Waste</th>
<th>Spent Mushroom Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>M2</td>
<td>60</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>M3</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>M4</td>
<td>40</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>M5</td>
<td>75</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

**TABLE 1**

The ratios of agricultural waste in the compost mixtures (%)
RESULTS AND DISCUSSION

The effects of the different composts obtained from agricultural waste on the height, diameter, and leaf number of the lettuce are presented in Tables 4 and 5. Generally, the effects of the compost treatments on lettuce growth were found to be statistically significant except for leaf number (2nd period). The head height values of lettuce were determined to increase with compost treatments compared to the control in both seasons (p<0.001). The maximum head height, head diameter and leaf number values (except for 2nd season) were recorded in almost all the compost applications in the vegetation periods, while the minimum head height value was obtained in control treatments (Table 4). Some researchers have reported that plant growth was affected positively compared to control plants by adding organic matter and compost to soil [26, 27, 28]. Sönmez et al. [4] determined that compost added to soil increased leaf number, head height and head diameter of lettuce.

The effects of composts on marketable-total yield values of the lettuce are presented in Table 5. Vitamin C values were found to be statistically significant in the treatments only in autumn (p<0.05) and the maximum Vitamin C value in autumn was significant in the treatments only in autumn (p<0.05) were recorded in almost all the compost applications in the vegetation periods, while the minimum head height value was obtained in control treatments (Table 4). Some researchers have reported that plant growth was affected positively compared to control plants by adding organic matter and compost to soil [26, 27, 28]. Sönmez et al. [4] determined that more vitamin content was observed in control applications than in compost-added soils. The differences between treatments for marketable and total yields among compost applications in both seasons were found to be significant (p<0.001). The total and marketable yield with the compost applications increased significantly compared to the control. The maximum marketable yield values in autumn were recorded in M1 (371.5 g plant⁻¹), M2 (350.0 g plant⁻¹), M4 (353.5 g plant⁻¹) and M5 (356.3 g plant⁻¹) applications and in the spring growing season, were recorded in M4 (395.7 g plant⁻¹) application. The minimum marketable yields were recorded in the control application in both seasons. The maximum total yield values in autumn and spring seasons respectively were recorded in M4 (623.7 g plant⁻¹) and M5 (435.2 g plant⁻¹) applications. The minimum total yields were recorded in the control application in both seasons. The M3 application increased almost 4 times compared to the control in autumn and the M4 application increased 2.7 times compared to the control in spring. Xu et al. [29] determined that vegetables grown with organic manure resulted in a higher total yield than those grown with chemical fertilizers. Some researchers have reported that organic manure increased yields in vegetables [4, 26, 30, 31, 32]. ElNour et al. [12] reported that the plant weights of sorghum increased with compost obtained from

### TABLE 2

<table>
<thead>
<tr>
<th>No</th>
<th>pH</th>
<th>EC (dS m⁻¹)</th>
<th>N (mg kg⁻¹)</th>
<th>P (%)</th>
<th>K (mg kg⁻¹)</th>
<th>Mg (mg kg⁻¹)</th>
<th>Ca (mg kg⁻¹)</th>
<th>Fe (mg kg⁻¹)</th>
<th>Zn (mg kg⁻¹)</th>
<th>Mn (mg kg⁻¹)</th>
<th>Cu (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>9.13</td>
<td>12.22</td>
<td>1.76</td>
<td>1.19</td>
<td>3.05</td>
<td>0.64</td>
<td>3.61</td>
<td>3387</td>
<td>7202</td>
<td>722.5</td>
<td>139.9</td>
</tr>
<tr>
<td>M2</td>
<td>8.53</td>
<td>12.41</td>
<td>1.82</td>
<td>1.45</td>
<td>3.16</td>
<td>0.76</td>
<td>4.36</td>
<td>4665</td>
<td>6840</td>
<td>951.7</td>
<td>165.0</td>
</tr>
<tr>
<td>M3</td>
<td>8.53</td>
<td>13.76</td>
<td>1.92</td>
<td>1.67</td>
<td>3.29</td>
<td>0.76</td>
<td>5.03</td>
<td>5444</td>
<td>6185</td>
<td>1046.0</td>
<td>176.8</td>
</tr>
<tr>
<td>M4</td>
<td>8.77</td>
<td>13.58</td>
<td>1.87</td>
<td>1.49</td>
<td>3.26</td>
<td>0.64</td>
<td>4.17</td>
<td>4844</td>
<td>7446</td>
<td>958.3</td>
<td>161.1</td>
</tr>
<tr>
<td>M5</td>
<td>8.76</td>
<td>12.57</td>
<td>1.88</td>
<td>1.42</td>
<td>3.10</td>
<td>0.90</td>
<td>5.78</td>
<td>4793</td>
<td>7153</td>
<td>980.2</td>
<td>152.2</td>
</tr>
</tbody>
</table>

The pot experiments were conducted under greenhouse conditions as a randomized plot design with four replications. Normal cultivating processes were carried out during the vegetation period and the plants were harvested at the end of the experiment.

Head height (cm), head diameter (mm), leaf number (per plant), total and marketable yield and leaf color values were measured on the harvested lettuce plants. The dry weights were then determined and calculated [20, 21].

The chemical characteristics of the compost samples used in the experiments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (%)</td>
<td>0.069</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>20.2</td>
</tr>
<tr>
<td>K (mg kg⁻¹)</td>
<td>105.7</td>
</tr>
<tr>
<td>Ca (mg kg⁻¹)</td>
<td>2754</td>
</tr>
<tr>
<td>Mg (mg kg⁻¹)</td>
<td>689.7</td>
</tr>
<tr>
<td>Fe (mg kg⁻¹)</td>
<td>14.7</td>
</tr>
<tr>
<td>Zn (mg kg⁻¹)</td>
<td>1.2</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹)</td>
<td>10.8</td>
</tr>
<tr>
<td>Cu (mg kg⁻¹)</td>
<td>3.4</td>
</tr>
</tbody>
</table>

In all the compost samples, organic matter content [22], organic carbon [23], pH [24], nitrogen [25], phosphorus [26], and K, Ca, Mg, Fe, Zn, Mn and Cu [18] were determined.

All data were subjected to analysis of variance and to detect significance (p<0.05) of treatment effects, Duncan’s Multiple Range Test was used with SAS software.
banana plant waste. Rui et al. [33] stated that some organic manure (pig manure and sludge) increased yields of lettuce and are effective for crop production.

The color values (L, Hue, Chroma) were not significantly affected by the applied treatments in both growing seasons (Table 6) and all applications were included in the same group.

The effects of compost treatments on the macro element concentrations of the lettuce are presented in Table 7. While the phosphorus, potassium and magnesium concentrations of lettuce were found to be significant statistically in the autumn season, the nitrogen and phosphorus concentrations of lettuce were found to be significant in the spring season. The macro element concentrations of the lettuce with the compost treatments increased significantly compared to the control except for calcium concentration.

### TABLE 4

<table>
<thead>
<tr>
<th>Season</th>
<th>Head height cm</th>
<th>Head diameter mm</th>
<th>Leaf number per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>14.4b</td>
<td>23.3a</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>18.5a</td>
<td>24.0a</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>19.3a</td>
<td>23.0a</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>18.8a</td>
<td>24.3a</td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>19.0a</td>
<td>24.0a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.0c</td>
<td>13.3b</td>
<td></td>
</tr>
</tbody>
</table>

Sign: *** ns ns ns ns *** ***

1 Values are means (n=4). Values in a row followed by different letters indicate significant differences (p<0.05) between treatments according to a Duncan’s multiple range test.

### TABLE 5

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Vitamin C mg 100 g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Marketable yield g plant&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Total yield g plant&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autumn</td>
<td>Spring</td>
<td>Autumn</td>
</tr>
<tr>
<td>M1</td>
<td>23.3bc</td>
<td>43.6</td>
<td>371.5a</td>
</tr>
<tr>
<td>M2</td>
<td>19.8c</td>
<td>38.7</td>
<td>350.0a</td>
</tr>
<tr>
<td>M3</td>
<td>28.6ab</td>
<td>36.6</td>
<td>312.2b</td>
</tr>
<tr>
<td>M4</td>
<td>30.6ab</td>
<td>45.7</td>
<td>353.5a</td>
</tr>
<tr>
<td>M5</td>
<td>29.9ab</td>
<td>40.4</td>
<td>356.3a</td>
</tr>
<tr>
<td>Control</td>
<td>32.0a</td>
<td>35.2</td>
<td>145.0c</td>
</tr>
</tbody>
</table>

Sign: *** ns ns ns ns ns ns

1 Values are means (n=4). Values in a row followed by different letters indicate significant differences (p<0.05) between treatments according to a Duncan’s multiple range test.

### TABLE 6

<table>
<thead>
<tr>
<th>Season</th>
<th>L</th>
<th>Hue</th>
<th>Chroma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autumn</td>
<td>Spring</td>
<td>Autumn</td>
</tr>
<tr>
<td>M1</td>
<td>53.82</td>
<td>44.95</td>
<td>118.58</td>
</tr>
<tr>
<td>M2</td>
<td>53.61</td>
<td>44.96</td>
<td>119.56</td>
</tr>
<tr>
<td>M3</td>
<td>53.66</td>
<td>48.01</td>
<td>119.60</td>
</tr>
<tr>
<td>M4</td>
<td>49.65</td>
<td>44.88</td>
<td>120.88</td>
</tr>
<tr>
<td>M5</td>
<td>52.71</td>
<td>46.37</td>
<td>119.86</td>
</tr>
<tr>
<td>Control</td>
<td>52.85</td>
<td>46.44</td>
<td>119.17</td>
</tr>
</tbody>
</table>

Sign: ns ns ns ns ns ns

1 Values are means (n=4). Values in a row followed by different letters indicate significant differences (p<0.05) between treatments according to a Duncan’s multiple range test.

2 Significance levels: ***: p<0.001 ns: not significant
TABLE 7
Effects of compost treatments on macronutrient concentrations of lettuce 1

<table>
<thead>
<tr>
<th>Season</th>
<th>Autumn</th>
<th>Spring</th>
<th>Autumn</th>
<th>Spring</th>
<th>Autumn</th>
<th>Spring</th>
<th>Autumn</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>2.37</td>
<td>3.32a</td>
<td>0.38a</td>
<td>0.36a</td>
<td>6.30a</td>
<td>6.00</td>
<td>0.86</td>
<td>0.81</td>
</tr>
<tr>
<td>P (%)</td>
<td>2.42</td>
<td>3.43a</td>
<td>0.34ab</td>
<td>0.28b</td>
<td>6.58a</td>
<td>6.43</td>
<td>0.77</td>
<td>0.84</td>
</tr>
<tr>
<td>K (%)</td>
<td>2.43</td>
<td>3.24a</td>
<td>0.23c</td>
<td>0.35a</td>
<td>6.14a</td>
<td>5.72</td>
<td>0.59</td>
<td>0.79</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>2.20</td>
<td>3.13a</td>
<td>0.28bc</td>
<td>0.31ab</td>
<td>5.18ab</td>
<td>5.77</td>
<td>0.61</td>
<td>0.75</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>2.11</td>
<td>3.38a</td>
<td>0.35ab</td>
<td>0.31ab</td>
<td>7.04a</td>
<td>5.30</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>Control</td>
<td>1.98</td>
<td>2.46b</td>
<td>0.25c</td>
<td>0.22c</td>
<td>3.70b</td>
<td>4.28</td>
<td>0.49</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Sign. b: ns, **, **, **, * ns, ns, * ns

1 Values are means (n=4). Values in a row followed by different letters indicate significant differences (p<0.05) between treatments according to a Duncan’s multiple range test.

2 Significance levels: *: p<0.5 **: p<0.01 ns: not significant

The nitrogen concentration of lettuce increased with all compost treatments and the minimum value was found in the control treatments in the spring season. However, the nitrogen concentration of lettuce in autumn did not vary significantly. The maximum phosphorus concentration of lettuce was obtained from M1 (0.38%) treatment in autumn, and from M1 (0.36%) and M3 (0.35%) in spring. The phosphorus concentration of lettuce increased with M1 treatment in both seasons. The minimum phosphorus concentration of lettuce was obtained from the control treatment in both periods. The potassium concentration of lettuce in autumn were obtained from M1 (6.30%), M2 (6.58%), M3 (6.14%) and M5 (7.04%) treatments and the minimum value was determined in the control (3.70%) treatment. However, the potassium concentration of lettuce did not vary significantly in both seasons. The calcium concentration of lettuce did not vary significantly in both seasons. The highest magnesium content of lettuce in autumn was obtained from M1 (0.42%) treatment and the minimum value was determined from the control (0.26%) treatment. Nitrogen and organic matter treatments have been previously shown to increase the nutrient concentrations of plants [4, 34, 35, 36]. Gezgin et al. [37] determined that humic acid materials at different doses increased the nutrient contents of lettuce. Vidigal et al. [38] reported that N, P and K contents of leaf increased with rising compost rates except for Ca content. Rodrigues and Casali [39] reported that the treatments of organic manure increased the yield and nutrient content in lettuces. Malinowska [40] reported that the compost applied affected the macronutrient concentration in ryegrass and their total amount in the biomass.

The effects of composts on the micro element concentrations of the lettuce are presented in Table 8. The iron concentrations in both seasons were not found to be statistically significant. Furthermore, the manganese concentrations in autumn and the copper concentration in spring were not found to be significant. The zinc contents of lettuce were found to be significant in both seasons.

The maximum zinc content was obtained from the control application in both seasons. The highest manganese concentration of lettuce in spring was determined from M1 (103.04 mg kg\(^{-1}\)) treatments. The highest copper concentration of lettuce in autumn was determined from M1 (6.99 mg kg\(^{-1}\)). The metal concentrations in lettuce increased in the spring season in particular. It is estimated that the decomposition of organic matter further decomposed micro elements in the spring season. Abdel-Sabour and El-Seoud [41] determined that the metal concentrations depend on the amount of compost applied to the soil and the treatment period. Sönmez et al. [4] reported that composts obtained from different agricultural plant wastes increases the plant growth and nutrient contents. Özgüven and Katkat [42] reported that the amount of available zinc increased on the topsoil because of the high level of organic matter. Sönmez and Kaplan [43] determined that the metal

TABLE 8
Effects of different composts on micronutrient concentrations of lettuce 1

<table>
<thead>
<tr>
<th>Season</th>
<th>Fe (mg kg(^{-1}))</th>
<th>Mn (mg kg(^{-1}))</th>
<th>Zn (mg kg(^{-1}))</th>
<th>Cu (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>M1</td>
<td>109.38</td>
<td>233.45</td>
<td>76.94</td>
<td>103.04a</td>
</tr>
<tr>
<td>M2</td>
<td>82.35</td>
<td>444.62</td>
<td>64.69</td>
<td>94.07ab</td>
</tr>
<tr>
<td>M3</td>
<td>81.10</td>
<td>357.92</td>
<td>59.09</td>
<td>85.51ab</td>
</tr>
<tr>
<td>M4</td>
<td>91.28</td>
<td>361.15</td>
<td>49.94</td>
<td>86.62b</td>
</tr>
<tr>
<td>M5</td>
<td>82.30</td>
<td>169.15</td>
<td>48.62</td>
<td>77.69b</td>
</tr>
<tr>
<td>Control</td>
<td>72.18</td>
<td>340.15</td>
<td>48.77</td>
<td>74.13b</td>
</tr>
</tbody>
</table>

Sign. b: ns, **, **, **, * ns, ns, * ns

1 Values are means (n=4). Values in a row followed by different letters indicate significant differences (p<0.05) between treatments according to a Duncan’s multiple range test.

2 Significance levels: *: p<0.5 ***: p<0.001 ns: not significant
concentrations of carnations increased with compost treatments.

CONCLUSION

This study was conducted to determine the effects of agricultural waste on plant growth, yield and nutrient content of lettuce. Composts obtained from banana plant waste, greenhouse plant waste and spent mushroom compost were observed to provide significant increases in many parameters in plant development. While all composts are effective in general, the M3 (50%BPW+40%GPW+10% SMC) and M4 (40%BPW+50%GPW+10%SMC) treatments were prominent in the development and productivity parameters of lettuce compared to the control group. M1 (70%BPW+20%GPW+10% SMC) treatment was determined as the best treatment for plant nutrient content. Agricultural waste is an important problem in farming areas and the best method of re-use is composting in terms of the environment and health. Thus, it is possible to get rid of both waste and soil properties and to increase productivity.

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RESPONSE OF SOWING TIME ON THE SEED YIELD TRAITS, YIELD AND QUALITY OF SAFFLOWER GENOTYPES IN SOUTHEASTERN ANATOLIA OF TURKEY

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ABSTRACT

This study was conducted to determine the suitable sowing date for two cultivar of safflower during 2013/14 and 2014/15 growing seasons at the research field of Dicle University, Anatolia, Turkey. The current research included four sowing dates viz., i) 20 October ii) 20 November, iii) 20 February, iv) 30 March; two genotypes of i) Remzibey, and ii) Dincer and fitted them in randomized complete block design with three replications. The results revealed that the sowing dates remarkably influenced the yield traits of plant height, the number of branches, the number of capitulum and seed yield of safflower. The genotype Dincer performed better than the genotype Remzibey regarding yield traits and yield. The highest seed yield was obtained from the genotype Dincer (1891.3 kg ha\(^{-1}\)) with the second sowing time of 20 November and the lowest yield (517.8 kg ha\(^{-1}\)) was obtained from Remzibey with the fourth sowing time of 30 March Regarding the quality properties, it was observed that, the sowing time was significantly influenced the oil content, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gama-linolenic acid, eicosadienoic acid and O/L. Oil content, meristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linoleic acid, gamma-linolenic acid, eicosadienoic acid, nervonic acid, , unsaturated fatty acid (UFA), SFA/UFA and O/L also varied significantly between the genotypes and the genotype Dincer performed better than Remzibey Yield traits, seed yield and, as well as quality traits of safflower were also influenced by the interaction of sowing time and genotypes in this study.

KEYWORDS:
Safflower (Carthamus tinctorius L.), sowing date, yield traits, yield, quality.

INTRODUCTION

Safflower (Carthamus tinctorius L.) is a multipurpose oil seed crop which can tolerate drought, heat, cold and saline stresses. It is an annual oilseed crop and its seed oil contents are between 20 to 40% [1]. Seeds contain ‘Saffranin’ which can be used for medicine production and food reservation. The byproduct, which remains after producing oil from the seeds, is very nutritional as an animal and birds feedings [2]. The seeds are composed of 15-20% protein, and the food value of the protein derived from the seeds is high [3]. Safflower is also grown as a vegetable crop, cut flower, fodder crop, medicinal plant, a dye crop for the textile industry, and safflower oil is also used in the manufacture of high-quality paint [4]. Although, vegetable oil is a principal source of fats in the diet, the present trend in oilseed breeding is to reduce the concentration of saturated fatty acids [5]. Safflower oil naturally contains the lowest levels of total saturated fatty acids among cultivated oil seed crops [6].

Safflower has been grown commercially for its edible oil and natural dye sources in the world [7]. It is an important and low production cost and lesser water and nutrient needs of safflower appeal to farmers as an alternative plant in fallow areas [1]. Oilseed 8crops growing have always been an important subject in Turkey agriculture. Due to rapid population growth and limited amount of oilseeds production in Turkey, vegetable oil need of our country continues to increase. Despite the efforts of the government to increase oilseeds production, Turkey continues to import vegetable oil (50%) due to meet up her internal demand of oil seeds and products [8]. The demand for oil production in Turkey is increasing day by day [9] and, winter oilseed crops including safflower have a potential to meet much of Turkey’s oil requirements. The alternative oil seed crops should be introduced in present production system for overcoming the shortage in oil production [1]. In order to reduce deficiency in oil production oilseed crop production areas and oil yield should be increased or alternative oil crops should be introduced [10].

Winter oilseed crops like safflower have a potential to solve the problems. The main purpose of
growing safflower crop in many countries around the world is for producing high quality of oil reliable for human consumption [2]. However, the yield of safflower is very low in Turkey as compared to other safflower producing countries. There are many reasons for lower yield of safflower in Turkey, and suitable (high yielding) genotypes along with their optimum sowing are the most important agronomic principles among them. A suitable combination of genotype and sowing date is the most important factor in acquiring economic yield. Different sowing dates caused flowering and seed development to occur during periods of widely different temperatures, radiation, and day length [11]. Sowing time is a major agronomic factor affecting both seed and oil yield in safflower [7,1]. Sowing date has an expressing influence and determining the appropriate sowing date is one of the most critical factors for optimizing safflower productivity [12]. If the sowing time is not well defined, germination can be irregular or may not occur, so sowing time significantly increases safflower yield [13]. Therefore, determining optimum sowing time and selecting suitable the cultivar for growing regions are necessary to obtain safflower with high yield and quality. Considering the above facts, this study was undertaken to determine the optimum sowing date for safflower genotypes under the arid conditions of Southwestern Anatolia of Turkey.

MATERIALS AND METHODS

Location and duration. To determine the suitable sowing date for safflower genotypes, two experiments were carried out at the Research Field of Dicle University, Turkey in the growing seasons of 2013/14 and 2014/15.

Soil properties. The soil samples were taken at 0-30 cm depth before the sowing the crop for both years for determination of chemical and physical properties of the soil. The soil contains 71.6% clay, 1.25% organic matter, 1.63 kg da⁻¹ phosphorus, potassium high level, 13.02% alcalin, 0.01-0.02% salt and pH 7.73.

Weather information. Weather data on maximum, minimum, and average temperature, and rainfall during experimental period were given at the Turkish state meteorological station Turkey. During experiment temperature fluctuated from 11.3 to 18°C. The average temperature was around 14.5°C. The average rainfall 42.07 mm. Weather data are presented in Fig. 1.

Experimental treatments and design. Two factorial experiment consisting factor A: four sowing times viz. i) 20 October ii) 20 November, iii) 20 February iv) 30 March, and factor B: two safflower genotypes viz. i) Remzibey and ii) Dincer was conducted in both years. The experiments were laid out in a randomized complete blocks design (RCBD) with replications.

TABLE 1
Sowing and harvesting dates for both season 2013/14 and 2014/15

<table>
<thead>
<tr>
<th>Sowing Time</th>
<th>2013-14</th>
<th>Harvesting Date</th>
<th>2013-14</th>
<th>2014-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>20.10.2013</td>
<td>I.</td>
<td>25.06.2014</td>
<td>20.06.2015</td>
</tr>
<tr>
<td>II.</td>
<td>20.11.2013</td>
<td>II.</td>
<td>05.07.2014</td>
<td>10.07.2015</td>
</tr>
</tbody>
</table>

FIGURE 1
Climatic parameters in two experimental years
TABLE 2

Results of variance of analysis for mean values of yield parameters tested

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Seed yield</th>
<th>1000 seed weight (g)</th>
<th>Number of capitulum</th>
<th>Plant height (cm)</th>
<th>Number of seed per table</th>
<th>Number of branches per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years (Y)</td>
<td>1</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Sowing time (ST)</td>
<td>3</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Varieties (V)</td>
<td>1</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>ST x V</td>
<td>3</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>10.97</td>
<td>3.26</td>
<td>10.11</td>
<td>5.5</td>
<td>14.58</td>
<td>11.89</td>
</tr>
</tbody>
</table>

ns: not significant *Significant at P < 0.05 **Significant at P < 0.01

Collection of plant materials and their properties. The genotypes were collection from commercial cultivars. Dincer (thornless, oil content 25-28%, protein content 14%, early majority group), Remzibey (Thorn, oil content 35-38%, early majority group).

Experimentation. Each plot included 6 rows, row distance 0.7 m and each row was 5 meters length and row space was 10 cm. The crop was sown according to the treatment specification in both years. Fertilizers were applied according to the recommended doses of 24 kg N ha⁻¹ and 24 kg P ha⁻¹ (20-20-0) (Fertilization was done before sowing of the crop and at the stem elongation stage (50 kg N ha⁻¹)). Ammonium nitrate (33%N) was used as the source of nitrogen. After emergence, manual thinning was used to obtain normal density. Weeds were controlled by manual weeding before stem elongation. Irrigation and other intercultural operations were done as necessary. The crop was harvested at optimum time of maturity, tagged and carried out at the field laboratory for taking data. The sowing time and harvesting date showed in Table 1.

Data collection. At harvesting time, yield contributing characteristics such as plant height, number branches per plant, number of tables, number of seeds per table, number of capitulum, 1000-seed weight, and seed yield were determined. Oil content, protein content and Fatty acid content: Protein content (%) was measured by Kjeldahl method [39]. Oil content (%) was measured by Soxhlet instrument with n-hexane (60°C) as organic solvent. Fatty acid compositions of seeds were measured with a gas chromatography and the analyses were performed at the central laboratories of of Tubitak – Mam (Marmara Research Center) according to the method described by [40].

Statistics analysis. Data for each experiment was analysed by JMP 5.0 software program for comparison of Data was analyzed by JMP package programT Mean tested by Tukey at the 1% level.

RESULTS AND DISCUSSION

Yield contributing traits and yield. The results indicated that the yield differences measured for sowing dates and cultivars were associated to the changes in the number of capitulum, seed number and 1000-seed weight. Statistically significant differences among cultivars were observed for seed yield, 1000 seed weight, plant height and number of capitulum, plant height and number of branches per plant in different sowing dates (Table 2). The highest seed number was found from Dincer variety with 25.73% (Table 6). The safflower yield is determined by the prevailing weather environment throughout its life cycle and the imposed cultural practices [14]. The variations in yield values can be due to environmental conditions or to the genetic potential for seed yield of examined cultivars. Arslan [15] found that seed yield of safflower plant was directly influenced by head diameter, head number per plant and seed number per head. Weiss [16] observed that three important selection criteria affected on seed yield such as number of head per plant, number of seed per head, and 1000-seed weight. Several studies in safflower revealed that seed yield and oil content were affected by sowing dates and cultivars. The late sowing in safflower showed reduction in in seed yield and yield attributes such as head per plant and seed per head [17].

The present results highlighted the practical importance of sowing date with appropriate cultivars in seed yield formation in safflower. accordingly, the highest seed yield was obtained from Dincer with 1891.3 (kg ha⁻¹) at (II. sowing time) and the lowest value was obtained from Remzibey (517.8 kg ha⁻¹) with IV. sowing date. According to the two-year averages, the highest number of capitulum was obtained from the genotype Remzibey with 20.85 at II. Sowing time and Dincer with 20.35 at (I. Sowing time), while the lowest value was obtained from IV. Sowing time. The highest plant heights were obtained from I and II. Sowing time, while the lowest values was obtained from Remzibey and Dincer with IV. sowing time (52.73 and 59.75, respectively) (Table 5). The highest seed
TABLE 3

Results of variance of analysis for mean values of quality parameters tested

<table>
<thead>
<tr>
<th>Variance</th>
<th>DF</th>
<th>Oil content</th>
<th>Protein content</th>
<th>Miristoleic</th>
<th>Palmitoleic</th>
<th>Stearic</th>
<th>Oleic</th>
<th>Linoleic acid</th>
<th>Alpha-Linolenic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years (Y)</td>
<td>1</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Sowing Time (ST)</td>
<td>3</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Varieties (V)</td>
<td>1</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>ST x V</td>
<td>3</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Error (CV (%))</td>
<td>16</td>
<td>8.5</td>
<td>7.9</td>
<td>6.93</td>
<td>2.5</td>
<td>6.3</td>
<td>4.04</td>
<td>8.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variance</th>
<th>Gamma-Linolenic acid</th>
<th>Arachidonic acid</th>
<th>Eicosadieneic acid</th>
<th>Lignoseric acid</th>
<th>Nervonic acid</th>
<th>Saturated Fatty Acids (SFA)</th>
<th>Unsaturated Fatty Acid (UFA)</th>
<th>SFA/UFA</th>
<th>Oleic/Linolenic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years (Y)</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Sowing Time (ST)</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Varieties (V)</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>ST x V</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Error (CV (%))</td>
<td>2.5</td>
<td>3.9</td>
<td>1.9</td>
<td>5.3</td>
<td>2.14</td>
<td>2.2</td>
<td>0.33</td>
<td>2.6</td>
<td>13.7</td>
</tr>
</tbody>
</table>

ns: not significant *Significant at P < 0.05 **Significant at P < 0.01

TABLE 4

Mean values of seed yield and yield attributes for safflower cultivars

<table>
<thead>
<tr>
<th>Sowing Time</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Year average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remzibey</td>
<td>Dincer</td>
<td>means</td>
</tr>
<tr>
<td>I</td>
<td>350.0c</td>
<td>800.0b</td>
<td>675.0b</td>
</tr>
<tr>
<td>II</td>
<td>1277.3b</td>
<td>1852.0a</td>
<td>1564.6a</td>
</tr>
<tr>
<td>III</td>
<td>1228.0b</td>
<td>1640.0a</td>
<td>1434.0a</td>
</tr>
<tr>
<td>IV</td>
<td>600.0c</td>
<td>665.3c</td>
<td>632.6b</td>
</tr>
<tr>
<td>Means</td>
<td>913.8</td>
<td>1239.3</td>
<td>1076.5</td>
</tr>
</tbody>
</table>

1000 seed weight

| I           | 31.5     | 33.3    | 32.4    | 36.4     | 42.5     | 39.5    | 34       | 37.9    |
| II          | 32.2     | 33.9    | 33.1    | 38.5     | 40.2     | 39.3    | 35.3     | 37      |
| III         | 32       | 32.7    | 32.1    | 38.6     | 41.7     | 40.1    | 35.3     | 37.2    |
| IV          | 32       | 35.7    | 33.8    | 37       | 40.3     | 38.6    | 34.5     | 38      |
| Means       | 31.9b    | 33.9a   | 32.8    | 37.6b    | 41.2a    | 39.3    | 34.8b    | 37.5a   |

LSD<sub>0.05</sub> 1.12 1.6 2.6
LSD<sub>0.01</sub> ns ns
LSD<sub>mean</sub> ns ns

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test, ns: not significant

number was obtained from Dincer with 25.73. The lowest branch number was obtained from IV. Sowing time in the 2013/14 growing season (4.9). According to (Table 4) the highest 1000 seed weight was obtained from Dincer with 37.5g, while the lowest value was obtained from Remzibey with 34.8g. Ozel et al. [9] observed that, the effects of different sowing dates on flower yield of safflower in the Turkey, and they observed significant difference among the sowing dates. Juknevicius and Pekarskas [18] recorded that the maximum seed yield was obtained at the first sowing date on Why there is a point observed that Sowing of safflower on early time achieved the maximum seed yield [19, 20].

The declination of yield in late sowing dates is due to reduction in one or more of its components such as heads number/plant, seeds number/head and 1000-seed weight and also to the reduction in length of growth period through acceleration of maturation time [21]. The reasons of yield increment are due to existence more number of primary
branches and longer growing season at first sowing date, resulted in higher dry matter accumulation, better translocation of photosynthetic products to flower, more pollination rate and then more heads formation [22]. The decrease in seed yield could be attributable to higher air temperatures at the time of flowering when plantings were delayed, and thus pollination and fertilization events were generally obstructed [5]. Different sowing dates caused flowering and grain filling to occur at different temperature, radiation, and day length [11]. Uher [23] was investigated the effect of sowing dates on different cultivars of safflower, and suggested that later sowing decreased numbers of head in plant. Juknevicius and Pekarskas [18] reported that seed weight and seed viability of safflower decreased by late sowing. The amount of loss in yield and 1000-seed weight was more in late-maturing cultivar in comparison with early maturing [24].

**Seed quality properties.** Agricultural implementations such as sowing date and different environmental factors are major factors which affect plant development. Oil content is one of the main components, which play a crucial role in safflower seed quality. Statistically significant differences among cultivars were observed for unsaturated and saturated fatty acids in different sowing dates (Table 3). The highest According of (Table 7) The averages show that sowing time did not affect protein content, the highest oil content was obtained from Dincer with III. Sowing time (28.07%), while the lowest value was obtained from Remzibey with IV. sowing time (23.59%). The mirisitic acid and palmitic acid rate of Dincer variety higher than Remzibey in Table 8. The highest palmitoleic acid rate from Dincer variety with 0.079%, while the highest stearic acid from Remzibey with 2.38% (Table 9). According of Table 11, The highest Alpha-Linolenic and Gamma-Linoleic acids rate were obtained from Remzibey variety (respectively, 0.202% and 0.475%). According to the two-year averages, the highest SFA/UFA was obtained from Remzibey with 9.72%, and the highest O/L rate was obtained from Remzibey (0.542%) with II. sowing date, while the lowest rate from Dincer variety. The oil content of safflower influence by genotype, climatic conditions, geographical location, growing dates and growing conditions. The environments played an important role in change of fatty acid composition and temperature is the main factor affecting fatty acid composition [25]. Sowing date can affect oil percentage and fatty acid composition at the time of seed development [5]. The minimum and maximum values for oleic acid and linoleic acid presented in Table 10. The oleic acid rates of the Remzibey was highest with 30.92% from II. sowing time, and the Dincer variety was lowest with 12.45% from I. sowing time. The other hand, the highest linoleic acid rate was ranged from 75.26 to 76.55% with Dincer variety with early sowing time (I, II. and III. sowing time). The ratio of oleic and linoleic acids in seed oil is mainly dependent on climatic conditions, especially humidity and temperature, during seed development stage Geigel et al., [7] revealed that during flower initiation to seed maturity stage, oil percentage and the four major fatty acids in safflower grains were influenced by sowing dates, and the availability of water, resulted in high oil percentage.

Fatty acid composition of plant oils plays an important role in determining the quality properties. The results of analysis of variance demonstrated that differences among treatments were significant for quality properties (Table 3). The effects of sowing time, cultivars, and their interactions had significant effects on the oil content, protein contents and fatty acids of safflower seed. Sowing time was found to insignificantly affect protein content, meristic acid, palmitoleic acid, arachidonic acid, lignoceric acid, nervonic acid, saturated fatty acid (SFA), unsaturated fatty acid (UFA) and SFA/UFA (Table 3). The effects of the year were significant on oil content, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gama-linolenic acid and eicosadienoic acid. According to the results obtained from this study, the variety was found to insignificantly affect protein content, arachidonic acid, lignoceric acid, nervonic acid and saturated fatty, whereas other quality treats was found significantly. According to sowing time x variety interaction for oil content was observed insignificant for oil content, protein content, meristic acid, palmiti acid, palmitoleic acid, stearic acid, alpha-linolenic acid, arachidonic acid, lignoceric acid, nervonic acid, saturated fatty acid (SFA), unsaturated fatty acid (UFA) and SFA/UFA. While the effects of the interaction was significant on oleic acid (O), linoleic acid (L), gamma-linoleic acid, eicosadienoic acid, and O/L (Table 3). A lot of factors such as climatic factors, variety, and location etc. influenced the amount of oil and seed quality in various crops [26, 27, 28, 29,30]. According of (Table 12, 13, 14 and 15), The highest Eicosa-dienoic acid, Nervonic acid, Unsaturated fatty acid (UFA) and saturated fatty acid (SFA) / Unsaturated fatty acid (UFA) were obtained from Remzibey variety (respectively, 0.339%, 0.187%, 89.28% and 9.72%).

Sowing date can be a major factor that affects oil content at the time of seed development [5]. Ada [31] observed that the most suitable sowing time for high oil rates is autumn. It is likely that increased temperature during the seed filling could be a major cause for reduction in oil content due to late sowing [32]. The Rise in temperature condition during seed development stage was a main reason in the decrease in oil content and thus increases protein content due to late sowing [32]. Golkar et al. [33] observed that the protein contents of the seeds in-
creased with late sowing [11]. Juknevičius and Pekarskas [18] observed that the maximum of oil and crude protein of safflower was found in the first sowing date. The genotype and sowing time where it is planted are important factors affecting fatty acid composition of safflower seed [34]. That climatic conditions, particularly temperature during growing season and development stages of seed, changed the composition of fatty acids was indicated by [35] and [36]. According to Gecgel et al. [7], spring sowings increased oil and oleic acid rates, but reduced linoleic acid rates. Sowing time is a significant factor in oil production and fatty acid composition [37]. The findings of Naghab et al. [38] do not support our results because those authors suggested that autumn sowing is the most suitable sowing time for high oleic acid production; according to the averages of all of the years of our study, oleic acid rates were not affected by sowing time.

### TABLE 5
Mean values of seed yield and yield attributes for safflower cultivars tested

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>Number of capitulum</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Year average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
</tr>
<tr>
<td>I</td>
<td>15.20cd 22.66ab 18.93b 14.8 18.03 16.41 15.00bc 20.35a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>24.53a 21.60abc 23.06a 17.16 15.16 16.16 20.85a 18.38ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>17.53bcd 17.06bcd 17.30bc 16.4 16.16 16.28 16.96abc 16.61abc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>15.86cd 13.53d 14.70c 12.23 13.93 13.08 14.05c 13.73c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>18.28 18.71 18.49 15.14 15.82 15.48 16.71 17.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD&lt;sub&gt;v&lt;/sub&gt;</td>
<td>ns</td>
<td></td>
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</tr>
<tr>
<td>LSD&lt;sub&gt;ST&lt;/sub&gt;</td>
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<td>6.52</td>
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</table>

<table>
<thead>
<tr>
<th>Plant height</th>
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<tr>
<td>IV</td>
</tr>
<tr>
<td>Means</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;v&lt;/sub&gt;</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;ST&lt;/sub&gt;</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;Means&lt;/sub&gt;</td>
</tr>
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</table>

### TABLE 6
Mean values of seed yield and yield attributes for safflower cultivars

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>Seed number</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Year average</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
</tr>
<tr>
<td>I</td>
<td>24.40 24.16 24.28a 23.26 26.80 25.03 22.83 25.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>26.83 28.70 27.76a 16.10 25.33 20.71 21.46 27.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>19.43 23.36 21.40b 20.53 24.8 22.66 19.98 24.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>22.62b 26.02a 24.57 20.44b 25.45a 22.94 21.53b 25.73a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD&lt;sub&gt;v&lt;/sub&gt;</td>
<td>2.24</td>
<td></td>
<td></td>
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<tr>
<td>LSD&lt;sub&gt;ST&lt;/sub&gt;</td>
<td>4.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD&lt;sub&gt;Means&lt;/sub&gt;</td>
<td>22.33</td>
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</table>

<table>
<thead>
<tr>
<th>Branch number</th>
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<tbody>
<tr>
<td>I</td>
</tr>
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<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>Means</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;v&lt;/sub&gt;</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;ST&lt;/sub&gt;</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;Means&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant.
### TABLE 7
Oil and protein content of safflower varieties

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>2013-14&lt;sup&gt;a&lt;/sup&gt;</th>
<th>2014-15</th>
<th>Years average&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remzibej</td>
<td>Dincer</td>
<td>Means</td>
</tr>
<tr>
<td>II</td>
<td>26.30b</td>
<td>27.00b</td>
<td>26.65a</td>
</tr>
<tr>
<td>III</td>
<td>24.47d</td>
<td>29.03a</td>
<td>26.75a</td>
</tr>
<tr>
<td>IV</td>
<td>23.33e</td>
<td>25.16d</td>
<td>24.24b</td>
</tr>
<tr>
<td>Means</td>
<td>25.04b</td>
<td>26.95a</td>
<td>25.99</td>
</tr>
<tr>
<td>LSDmeans</td>
<td>0.78</td>
<td>ns</td>
<td>0.64</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

### TABLE 8
Fatty acid composition of safflower varieties

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Years average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remzibej</td>
<td>Dincer</td>
<td>Means</td>
</tr>
<tr>
<td>I</td>
<td>0.083</td>
<td>0.103</td>
<td>0.093</td>
</tr>
<tr>
<td>II</td>
<td>0.086</td>
<td>0.110</td>
<td>0.098</td>
</tr>
<tr>
<td>III</td>
<td>0.096</td>
<td>0.111</td>
<td>0.103</td>
</tr>
<tr>
<td>IV</td>
<td>0.096</td>
<td>0.120</td>
<td>0.108</td>
</tr>
<tr>
<td>Means</td>
<td>0.090</td>
<td>0.110</td>
<td>0.100</td>
</tr>
<tr>
<td>LSDmeans</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

### TABLE 9
Fatty acid composition of safflower varieties

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Years average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remzibej</td>
<td>Dincer</td>
<td>Means</td>
</tr>
<tr>
<td>I</td>
<td>0.040</td>
<td>0.073</td>
<td>0.059</td>
</tr>
<tr>
<td>II</td>
<td>0.060</td>
<td>0.080</td>
<td>0.070</td>
</tr>
<tr>
<td>III</td>
<td>0.070</td>
<td>0.086</td>
<td>0.078</td>
</tr>
<tr>
<td>IV</td>
<td>0.040</td>
<td>0.076</td>
<td>0.058</td>
</tr>
<tr>
<td>Means</td>
<td>0.054</td>
<td>0.078</td>
<td>0.066</td>
</tr>
<tr>
<td>LSDmeans</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant
### TABLE 10

#### Fatty acid composition of safflower varieties

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Years average$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remziy</td>
<td>Dincer</td>
<td>Means</td>
</tr>
<tr>
<td>I</td>
<td>24.43bc</td>
<td>11.80e</td>
<td>18.12d</td>
</tr>
<tr>
<td>II</td>
<td>31.26a</td>
<td>13.14de</td>
<td>22.20a</td>
</tr>
<tr>
<td>III</td>
<td>22.81c</td>
<td>13.58de</td>
<td>18.19b</td>
</tr>
<tr>
<td>IV</td>
<td>26.17b</td>
<td>16.18d</td>
<td>21.17a</td>
</tr>
<tr>
<td>Means</td>
<td>26.16a</td>
<td>13.67b</td>
<td>19.91b</td>
</tr>
<tr>
<td>LSD$_{means}$</td>
<td>3.28</td>
<td>3.86</td>
<td>4.12</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

### TABLE 11

#### Fatty acid composition of safflower varieties

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Years average$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remziy</td>
<td>Dincer</td>
<td>Means</td>
</tr>
<tr>
<td>I</td>
<td>0.193</td>
<td>0.160</td>
<td>0.176</td>
</tr>
<tr>
<td>II</td>
<td>0.210</td>
<td>0.170</td>
<td>0.190</td>
</tr>
<tr>
<td>III</td>
<td>0.196</td>
<td>0.166</td>
<td>0.181</td>
</tr>
<tr>
<td>IV</td>
<td>0.213</td>
<td>0.180</td>
<td>0.196</td>
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<tr>
<td>Means</td>
<td>0.406</td>
<td>0.169</td>
<td>0.287</td>
</tr>
<tr>
<td>LSD$_{means}$</td>
<td>-</td>
<td>-</td>
<td>0.038</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

### TABLE 12

#### Fatty acid composition of safflower varieties

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>2013-14</th>
<th>2014-15</th>
<th>Years average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remziy</td>
<td>Dincer</td>
<td>means</td>
</tr>
<tr>
<td>I</td>
<td>0.133</td>
<td>0.120</td>
<td>0.126</td>
</tr>
<tr>
<td>II</td>
<td>0.183</td>
<td>0.143</td>
<td>0.163</td>
</tr>
<tr>
<td>III</td>
<td>0.127</td>
<td>0.203</td>
<td>0.165</td>
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<tr>
<td>IV</td>
<td>0.110</td>
<td>0.150</td>
<td>0.130</td>
</tr>
<tr>
<td>Means</td>
<td>0.138</td>
<td>0.154</td>
<td>0.146</td>
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<tr>
<td>LSD$_{means}$</td>
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<td>-</td>
<td>-</td>
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</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

---

$^a$Means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test.
### TABLE 13
**Fatty acid composition of safflower varieties**

<table>
<thead>
<tr>
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<th></th>
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<th></th>
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<tbody>
<tr>
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<td>2014-15</td>
<td>Years average</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
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<td></td>
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</tr>
<tr>
<td>I</td>
<td>0.150 0.143 0.146</td>
<td>0.148 0.143 0.145</td>
<td>0.149 0.143</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.157 0.147 0.152</td>
<td>0.155 0.151 0.153</td>
<td>0.156 0.149</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0.160 0.150 0.155</td>
<td>0.167 0.155 0.161</td>
<td>0.163 0.153</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0.157 0.167 0.162</td>
<td>0.153 0.163 0.158</td>
<td>0.155 0.165</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Means</strong></td>
<td>0.156 0.151 0.153</td>
<td>0.155 0.153 0.154</td>
<td>0.155 0.152</td>
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In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

### TABLE 14
**Fatty acid composition of safflower varieties**

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<td>2014-15</td>
<td>Years average</td>
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</tr>
<tr>
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<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>9.03</td>
<td>9.13</td>
<td>9.08</td>
<td>8.98</td>
<td>9.08</td>
<td>9.03</td>
<td>9.00</td>
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<td>-</td>
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</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant

### TABLE 15
**Fatty acid composition of safflower varieties**

<table>
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<tr>
<th>Sowing time</th>
<th>SFA/UFA</th>
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</thead>
<tbody>
<tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td>Remzibey Dincer means</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>9.84</td>
<td>9.54</td>
<td>9.69</td>
<td>9.81</td>
<td>9.54</td>
<td>9.67</td>
<td>9.82</td>
<td>9.54</td>
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<td><strong>LSD&lt;sub&gt;means&lt;/sub&gt;</strong></td>
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<td>-</td>
<td>0.26</td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

In each column, means followed by the same letter within columns are not significantly different (P < 0.01) according to Tukey test. ns: not significant
CONCLUSION

In the light of these findings, it can be concluded that, the yield and quality of the safflower is influenced by sowing time conditions and cultivar characteristics. Therefore, early sowing time may be recommended conditions of study. For this reason, planting dates (20 November) for Southestern Anatolia can be recommended in order to obtain high yield and quality from safflower.

REFERENCES


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e-mail: fozturk@sirnak.edu.tr
APPLICATION OF ELECTRICAL CONDUCTIVITY TEST TO DETERMINE VIGOR OF COMMON VETCH

*Vicia sativa* L.

Negar Ebrahim Pour Mokhtari¹, Mustafa Kızılşınmık²

¹Gaziantep University, Islahiye Vocational School, Organic Farming Department, Gaziantep, Turkey
²Department of Field Crops, Sütçü İmam University, Kahramanmaraş, Turkey

**ABSTRACT**

The seeds of 12 different vetch (*Vicia sativa* L.) cultivars harvested during 2010, 2011, 2012, 2013 and 2014 were used in this study. Initial vigor of the seed lots ranged 95 – 100%. As seed vigor tests, normal germination percentage, seedling emergence percentage, mean germination time, mean emergence time and electrical conductivity (for 24 hours) were used and potential use of these tests for emergence estimation of the vetch species and their efficiency in classification of vetch seed lots were investigated. Present findings revealed that EC (Electrical conductivity) test could be used for emergence estimation of the species and classification of vicia seed lots. EC values of seed lots varied between 17.14 - 29.45 μS cm⁻¹g⁻¹ and all of them showed significant differences in term of EC lying in different statistical groups. It was concluded based on the findings of present studies that electrical conductivity values could be used for rapid emergence estimation of seed lots ($r = -0.597$, $p < 0.05$).

**KEYWORDS:**
Electrical conductivity test, Vigor, *Vicia sativa* L.

**INTRODUCTION**

A Seed trait such as vigor is a crucial and intrinsic part of measuring the potential success rate in the process chain of seedling production [1]. Furthermore, seed vigor during storage and later on in different field conditions is responsible for both seed growers and producers [2]. Generally, the consensus has been that the application of electrical conductivity test provides a progressive and non-destructive approach in the determination of seed vigor [1]. It is important to understand why determination of seed vigor is a significant factor in seedling production. Firstly, it assists in the identification of distinct physiological characteristics among seeds within a seed lot. Secondly, it helps to prevent potential seed germination failure in the field by identifying possible faults that may arise during storage or transportation or when the seed is exposed to harsh environmental conditions [3]. There are number of methods besides electrical conductivity that measure some degree of the seed weakening or seed decay. Accelerated aging test, cold test, seedling vigor classification, and triazolium are the main methods used for measuring seed vigor. However, most of the seed vigor testing techniques are expensive and tedious. Furthermore, results obtained tend to vary due to lack of standardisation [4]. Ferguson [5] emphasise that seed vigor tests must be consistent and accurate to promote efficient elimination of poor quality seeds. Additionally, Hampton and Tekron [6] has underscored the need for an objective, systematic, efficient and economic method of determining seed vigor. Electrical conductivity test forms the basis of this study and falls within the scope of argument. The test is dependent on the permeability of the cell membrane thus guaranteeing reliability and enhanced decision making [1]. Electrical conductivity provides better results compared to the other methods of seed vigor test [7], therefore, this study employs the same technique of electrical conductivity in determining the seed vigor. The technique behind electrical conductivity is simple; deionised water is used to soak different seed lots, followed by measurement of EC are measured after a 24 hours. The approach facilitates repeatability. The integrity of the cellular membrane affects the rate of leaching; therefore, low electrical conductivity means higher seed vigor. One of the most significant advantage offered by the electrical conductivity test is the ability to classify seed lots depending on their utilisation potential [8] and helps in drawing a distinction between laboratory and field tests. Consequently, the results obtained from these two environments must be analysed carefully. Observations made on the rate of electrolyte leakage, particularly, the commercial seed lots indicates that the amount of dead tissues on the cotyledons is directly linked to increased electrolyte leakage. However, this does not inhibit germination of seeds under laboratory conditions [9].

The study aimed to confirm the seed vigor of 12 different vetch cultivars of different ages to determine their vigor by conducting an EC test and comparing the EC results to ascertain vigor by evaluating and comparing germination percentage, seedling
emergence percentage, mean germination time, mean emergency time and electrical conductivity (for 24 hours). Efficiency of these tests in estimation of emergence rates of Vicia sativa L. cultivars and classification of vicia seed lots were investigated.

**MATERIALS AND METHODS**

**Seed material.** Twelve seed lots of Common Vetch (Vicia sativa L.) produced by various seed companies, were obtained from East Mediterranean Agricultural Research Institute at Adana and different seed companies at Kahramanmaras, Turkey. Some lots obtained from the research institute were in plastic packets without report of germination percentages. But had records about their physical purity, name of the cultivar and lot number. The seed packets were stored at 5°C until the experiments were completed. The experiments were conducted at the seed laboratory of the Faculty of Agriculture, Sutcu Imam University, Kahramanmaras, Turkey.

**Seed moisture content (SMC) and seed weight (SW).** Mean seed weight (1000 seed weight) was determined from a sample of 400 seeds (four replicates of 100 seeds). Seed moisture content (mc) was determined by oven dry method following low constant temperature oven method. [10] on two replicates of the seeds held at 103°C for 17 h, and expressed on a fresh weight basis.

**Germination tests.** Four replicates of 50 seeds per lot were placed in petri dishes on germination paper (Whatman No: 5, 9 cm) moistened with 3 ml distilled water. The petri dishes were then placed in polyethylene bags to prevent water loss during the test and held at 20 ± 1°C. At the final count, (10 days after the commencement of the test) the seedlings were classified as either normal or abnormal [10].

**Field emergence percentage (FEP).** The field experiments were conducted at the Research Farm of the Faculty of Agriculture; Sutcu Imam University, Turkey. Sowing 50 seeds on a sandy-loam soil at a depth of 3 cm. Number of emerged seedlings in each plot were counted every day until the stable seedling establishment.

**Electrical Conductivity test.** Seed EC test was performed with 50 seeds per replicate (four replicates). After determining seed weight, it was imbibed in 250 ml of deionized water, in Erlen Mayer flasks, which were sealed with plastic foil and kept in a germinator for 24 h at 20 ± 2°C. After that, seed-water mixture was gently stirred for 15 s and EC was measured in the solution above the submerged seeds using a standard conductivity meter, previously calibrated with 0.1% and 0.01% potassium chloride (KCl). EC readings in μS cm⁻¹ were divided with seed weight, to express the final EC value in μS cm⁻¹g⁻¹ seed.

**Statistical analysis.** All experimental data was subjected to one way ANOVA and regression analysis to perform statistical analysis using IBM SPSS 24 comparison among means was made at 5% level of probability. All data taken in percentages were subjected to arcsine transformation before carrying out the statistical analysis.

**RESULTS AND DISCUSSION**

The mean germination times of vetch seed lots ranged 2.07 - 3.18 days (Table 1). The shortest mean germination time was observed in seed lot 9, the longest mean germination time was observed in seed lot 5 and the seed lots No. 2, 3, 4, 6, 7, 8, 9 and 11 were statistically similar and placed in the same statistical group. The seed lots No. 1 (IC-11 IV-B) and 5 (IC-11 II-B) took the maximum mean germination time, were statistically similar and were placed in the same statistical group (Table 1).

**Initial vigor and moisture of the seeds.** The Seeds of 12 different vetch cultivars harvested during 2010, 2011, 2012, 2013, and 2014 showed initial vigor of 95 – 100%, such that 6 of them showed 100%, 5 of them showed 99% and 1 showed 95% germination percentage. As can be inferred from the initial seed vigor, all seed lots used in the present experiments had higher vigor values compared to the value specified for commercially available cultivars namely IC-11 IV-B, TA-17 II-B, IC-12 IV-B, TA-10 II-B, IC-11 II-B, TA-11 II-B, Ozveren, Cuku-rova, Selcuuk-99, Ege Beyazi and Cumhuriyet 89. Initial moistures of the seed lots varied between 5.69 – 9.28%. The variations in germination percentage and seed moisture of vetch seed lots are provided in Table 1.

**Normal germination percentage (NGP) and mean germination time (MGT).** Normal germination percentages of the seed lots varied between 68 – 98%. Although the seed lots showed statistically different germination percentage and were placed in different statistical groups, normal germination percentages of the seed lots varied within narrow ranges (Table 1). Mean germination times of vetch seed lots varied between 2.07 - 3.18 days. The minimum germination time was observed in seed lot 9 and the longest seed germination time was observed on seeds obtained from lot No. 5. The seed lots No. 2, 3, 4, 6, 7, 8, 9 and 11 were statistically similar and placed in the same statistical group. The seed lots No. 1 and 5 with the longest germination times and were placed in the same statistical group (Table 1).
Electrical Conductivity Test. Electrical conductivity is a biochemical seed vigor test and the tests were carried out for 24 hours. The results are shown in Table 1. The statistical results about electrical conductivity of each seed lots were statistically different and placed in different statistical electrical conductivity groups. The lowest electrical conductivity values were obtained from seed lot No. 9. The maximum electrical conductivity value was observed in seed lot No. 11 (Table 1).

Relationships among measures of seed quality. There were several significant correlations between various measurements of seed quality for all 12 seed lots (Table 2). These results emphasis existence of a highly significant negative correlation (r = -0.77; p<0.01) was observed between 1000 seed weight and EC and a positive linear correlation (r = 0.68; p<0.05) was observed between thousand seed weight and GP. Thousand seed weight did not have significant correlations with FEP, MET, MGT, NGP and SMC (seed moisture content) (p>0.05). SMC also did not show significant correlations with GP, NGP, MET, FEP and EC (p>0.05). There was a highly significant negative correlation (90.3%) between GP (germination percentage) and EC (r = -0.90; p<0.01). GP did not show significant correlations with FEP, MET, MGT and NGP (p>0.05). There was a highly significant negative correlation (80.7%) between NGP and MET (r = -0.80; p<0.01) and there was a significant linear correlation (58.9%) observed between NGP and FEP (r = 0.58; p<0.05). NGP did not show significant correlations with EC and MGT (p>0.05). MGT did not show significant correlations with MET, FEP and EC; also MET did not show significant correlations with FEP and EC (p>0.05). There was a significant negative correlation (r = -0.59; p<0.05) between FEP and EC. The results of correlation analyses are summarized in Table 2. As can be seen from the table, some combinations yielded significant correlations. Field emergence (%) and EC tests had the maximum percentages with maximum significance and, thus were placed among the combinations allowing reaching an outcome in the minimum time. Present findings revealed a significant negative correlation (r = -0.59; p<0.05) between FEP and EC.

### Table 1

<table>
<thead>
<tr>
<th>Seed Lot Number</th>
<th>Variety</th>
<th>Year of Production</th>
<th>1000 Seed Weight (g)</th>
<th>Seed Moisture Content (%)</th>
<th>Germination (%)</th>
<th>MGT (d)</th>
<th>MET (d)</th>
<th>Field Emergence (%)</th>
<th>EC</th>
<th>T N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 IC-11 IV-B</td>
<td>2010</td>
<td>64.47</td>
<td>7.24</td>
<td>99</td>
<td>70 g</td>
<td>2.88 ab</td>
<td>22.70 a</td>
<td>55.00</td>
<td>19.71 b</td>
<td>7.79 a</td>
</tr>
<tr>
<td>2 TA-17 II-B</td>
<td>2010</td>
<td>52.71</td>
<td>5.69</td>
<td>100</td>
<td>68 g</td>
<td>2.26 c</td>
<td>21.11 c</td>
<td>55.00</td>
<td>19.23 b</td>
<td>7.78 a</td>
</tr>
<tr>
<td>3 IC-12 IV-B</td>
<td>2011</td>
<td>64.19</td>
<td>8.13</td>
<td>99</td>
<td>71 fg</td>
<td>2.34 cd</td>
<td>20.79 c</td>
<td>60.00</td>
<td>20.1 bcd</td>
<td>7.76 a</td>
</tr>
<tr>
<td>4 TA-10 II-B</td>
<td>2011</td>
<td>63.17</td>
<td>7.84</td>
<td>100</td>
<td>76 f</td>
<td>2.28 cd</td>
<td>16.28 c</td>
<td>76.00</td>
<td>19.81 bcd</td>
<td>7.74 a</td>
</tr>
<tr>
<td>5 IC-11 II-B</td>
<td>2010</td>
<td>66.15</td>
<td>7.72</td>
<td>99</td>
<td>84 de</td>
<td>3.18 a</td>
<td>16.57 a</td>
<td>74.00</td>
<td>19.26 bcd</td>
<td>7.72 a</td>
</tr>
<tr>
<td>6 TA-11 II-B</td>
<td>2010</td>
<td>65.87</td>
<td>7.62</td>
<td>100</td>
<td>82 e</td>
<td>2.41 cd</td>
<td>14.21 c</td>
<td>54.00</td>
<td>20.71 b</td>
<td>7.71 a</td>
</tr>
<tr>
<td>7 ÖZVEREN</td>
<td>2012</td>
<td>72.41</td>
<td>7.49</td>
<td>99</td>
<td>96 ab</td>
<td>2.49 c</td>
<td>15.61 c</td>
<td>76.00</td>
<td>19.93 b</td>
<td>7.69 a</td>
</tr>
<tr>
<td>8 ÇUKUROVA</td>
<td>2014</td>
<td>57.12</td>
<td>8.08</td>
<td>99</td>
<td>91 bc</td>
<td>2.44 cd</td>
<td>15.18 c</td>
<td>73.00</td>
<td>18.98 b</td>
<td>7.67 a</td>
</tr>
<tr>
<td>9 ÖZVEREN</td>
<td>2014</td>
<td>63.60</td>
<td>9.16</td>
<td>100</td>
<td>90 c</td>
<td>2.07 d</td>
<td>16.62 d</td>
<td>71.00</td>
<td>17.14 d</td>
<td>7.65 a</td>
</tr>
<tr>
<td>10 SELCUK-99</td>
<td>2014</td>
<td>49.87</td>
<td>6.96</td>
<td>100</td>
<td>89 cd</td>
<td>2.58 bc</td>
<td>16.68 b</td>
<td>61.00</td>
<td>20.39 b</td>
<td>7.63 a</td>
</tr>
<tr>
<td>11 EGE BEYAZI</td>
<td>2013</td>
<td>32.387</td>
<td>9.28</td>
<td>95</td>
<td>86 cde</td>
<td>2.44 cd</td>
<td>17.88 c</td>
<td>50</td>
<td>29.45 a</td>
<td>7.60 a</td>
</tr>
<tr>
<td>12 CUMHURIYET</td>
<td>2014</td>
<td>65.45</td>
<td>8.71</td>
<td>100</td>
<td>98 a</td>
<td>2.6 bc</td>
<td>14.31 b</td>
<td>82</td>
<td>17.31 cd</td>
<td>7.59 a</td>
</tr>
</tbody>
</table>

All values shown in a single column by different alphabetic letters are significantly different using Duncan multiple range test (p<0.05) different

### Table 2

Correlation coefficients between laboratory measures of seed quality and seedling emergence in modules of 12 seed lots of *Vicia sativa* L.

<table>
<thead>
<tr>
<th>R</th>
<th>1000 seed weight</th>
<th>SMC</th>
<th>GP</th>
<th>NGP</th>
<th>MGT</th>
<th>MET</th>
<th>FEP</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 seed weight</td>
<td>1</td>
<td>-0.124</td>
<td>0.680*</td>
<td>0.069</td>
<td>0.163</td>
<td>-0.180</td>
<td>0.565</td>
<td>-0.777**</td>
</tr>
<tr>
<td>SMC</td>
<td>1</td>
<td>0.447</td>
<td>0.503</td>
<td>-0.147</td>
<td>-0.389</td>
<td>0.257</td>
<td>0.259</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>1</td>
<td>-0.050</td>
<td>-0.122</td>
<td>-0.163</td>
<td>0.405</td>
<td>-0.903**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGP</td>
<td>1</td>
<td>0.033</td>
<td>0.807**</td>
<td>0.589</td>
<td>-0.073</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGT</td>
<td>1</td>
<td>0.071</td>
<td>0.091</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>1</td>
<td>-0.575</td>
<td>0.124</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEP</td>
<td>1</td>
<td>0.124</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: significant at p<0.05, **: significant at p<0.01,
Electrical conductivity was found as an effective method in classification of seed vigor of Vicia seed lots. The seed lots were placed in different statistical groups with regard to their electrical conductivities (Table 1). It was also found that electrical conductivity values could be used to estimate emergence ratios of seed lots ($r=-0.597$, $P<0.05$) (Table 2). Electrical conductivity values of the present seed lots ranged 17.14-29.45 μScm$^{-1}$g$^{-1}$. EC is a simple and repeatable electro-biochemical test that can be used to estimate emergences [11]. EC has been worked out in several legumes including peas and is used as a vigor test approved by ISTA. In this way, efficient use of a standard test was ensured for accredited laboratories. A proper test procedure should also be identified for the other legume species. In electrical conductivity tests carried out on some legume species, electrical conductivity values was reported as between 31.7-88.1 μScm$^{-1}$g$^{-1}$ for soybean [12], as between 96.4 -153.7 μScm$^{-1}$g$^{-1}$ for mung beans [13], as between 79.5-135.5 μScm$^{-1}$g$^{-1}$ for beans [14], as between 17-40 μScm$^{-1}$g$^{-1}$ for kidneybeans [15]. The findings for kidney-beans were the closest ones to present values. During EC tests, water-soaking durations may also vary among the species. It was indicated in a previous chickpea studies that 2 hours soaking duration could be used for classification of chick pea seed lots [16]. Prolonged soaking durations increase electrical conductivity values, but does not change order or classification of seed lots. Therefore, 24 hours soaking durations of the present study did not result in any handicaps for vetches. Samarah [17] reported electrical conductivities of seed lots of Vicia sativa harvested at different growth periods as between 54 - 427 μScm$^{-1}$g$^{-1}$. Present differences were mainly coming from the differences in seed growth and seed cultivar. In present study, the efficiency of EC in identifying especially genotype-induced power differences was tried to be tested. Therefore, different cultivars were used to identify differences in quality attributes of seed lots with EC. Use of EC for genotypic differentiation has not been much studied, but Panobianco and Vieira [18] indicated that EC could be used in genotypic differentiation in soybeans. Right at this point, there is need to know whether the differences originated from the physiological age of the seeds or from the genetic differences. EC has also been used to estimate storage [19], emergence under stress and optimum conditions of growth [20]. In this study, potential use of EC for emergence estimation of Vicia species was tested. Present correlation analyses revealed that EC could be used in emergence estimation of seed lots. Similar findings were also reported for different legume species [12, 13, 15]. Present findings also indicated that electrical conductivity test results were closely related to the physical and chemical properties of the seeds. In case the seed lots were not rinsed through during sampling, the residues over them may increase their electrical conductivity.

Although fungicide treatments do not influence electrical conductivity of seed lots [21], coloring materials and adhesives used on the seeds (chemical treatment) indicate that these seeds may have been influenced through electrical conductivity values. Therefore, Mavi and Demir [22] based on electrical conductivity test results for cucurbits showed that the subjected lots to electrical conductivity tests could bear the same characteristics.

**CONCLUSION**

The results showed the most significant relationship between field emergence (field emergence (%) and EC test in the field and EC provided rapid estimates of seed germination. In addition, it should be ensured before carrying out the EC test that the seeds are of uniform age or harvested at the same time that would help to determine seed vigor easily. Large differences in seed vigor and quality with in a lot could give erroneous results and reduce effectiveness of the test. If the viability percentage among the seeds of a single lot varies and these differences could lead to erroneous results. The seed viability in vicia species are genotype-related and correlated with the seed vigor. Therefore, care should be taken during carrying out of an EC based seed viability test.

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NANOPORCE STRUCTURE CHARACTERISTICS OF THE LOWER CAMBRIAN ORGANIC-RICH SHALE IN SOUTHERN CHINA USING N2 ADSORPTION AND GEOCHEMICAL EXPERIMENTS

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ABSTRACT

In this paper, the pore structure of the Lower Cambrian organic-rich shale in southern China was systematically studied. The results show that the TOC content of the shale is between 0.45% and 8.50%, with an average of 3.97%. The main pore types include intergranular pores, organic matter pores, clay pores and micro-cracks. The effective pore space of the shale is mainly composed of nanopores with a pore diameter below 6.5 nm, and the organic matter pores are the main contributors to the specific surface and pore volume. The slit-shaped pore is the most predominant type of pore in the shale. Organic matter and epigenetic quartz generally co-exist closely. When the TOC content of shale exceeds 4%, the main pore type inside is organic matter pores.

KEYWORDS:
Organic-rich shale, Lower Cambrian, pore structure, organic matter, epigenetic quartz

INTRODUCTION

Shale gas has become an important supplement to conventional oil and gas energy due to its low pollution and low greenhouse effect [1]. Shale gas is a clean, efficient energy source that is mainly stored in organic-rich shale and its interlayers. Its main state of occurrence include free state, adsorbed state and dissolved state [2]. The formation of shale gas includes biological genesis and thermal genesis, and some are mixed genesis [3-4]. The composition of shale gas is mainly composed of methane (90%) and a small amount of hydrocarbons, CO2, nitrogen and H2S, which are present in pores and cracks in free or adsorbed state [5].

In recent years, research on microscopic pore structure of shale has become a hot spot in the field of unconventional oil and gas exploration and development worldwide [2, 6-10]. With the deepening of research work, researchers have gradually realized that the study of microscopic pores in shale is challenging. The pore characteristics of the shale are controlled by the combined action of inorganic mineral components and organic matter systems. The shale has a wide distribution of pore size and its heterogeneity is strong. Therefore, the effective quantitative analysis of the pore structure is difficult.

In this paper, the pore structure characteristics of organic-rich shale in southern China were systematically studied by low temperature liquid nitrogen adsorption experiment, geochemical experiment and scanning electron microscopy. The research has certain reference value for shale reservoir evaluation.

MATERIALS AND METHODS

The study area is located in the northeastern Guizhou province of the southern China. The shale samples were collected from the Lower Cambrian Niutitang Formation and the Bianmachong Formation in the TM1 and TX1 wells in the area. There are 22 samples in total, and the buried depth is in the range of 1 400 m-1 800 m. There are 15 samples in the TM1 well, all of which are collected from the Niutitang Formation, numbered TM-1 to TM-15 respectively. There are 7 samples in TX1 well, and 4 samples were collected from the Bianmachong Formation, numbered TX-1 to TX-4, and 3 samples were collected from the Niutitang Formation, numbered TX-5 to TX-7. The shale samples of the Niutitang Formation are collected from the main hydrocarbon-bearing interval and belong to the shallow-water continental shelf deposits. The shale samples of the Bianmachong Formation are mainly tidal flat and neritic shelf deposits.

The TOC content test instrument is a CS-200 carbon sulfur analyzer, and the experimental basis is GB/T19145-2003. The X-ray diffraction test instrument is a PanalyticalX’PertPRO MPD X-ray diffractometer with the test standard SY/T6201-1996. The X-ray diffraction (XRD) instrument is a PanalyticalX’ PertPRO MPD X-ray diffractometer with a
A field emission scanning electron microscope was used to observe the micro-structure of the shale. The test instruments for porosity and permeability are KXD-II porometer and permeameter (QT-2), respectively. The test standard is SY/T5336-1996 [11]. The low-pressure N2 adsorption instrument is the “Quantachrome-nova 2000” Surface Area Analyzer and Pore Size Analyzer with a test standard of SY/T 6154-1995 [12].

The TOC content of the shale samples is between 0.45% and 8.50% with an average of 3.97%. The distribution of Ro is 2.22%-2.85%, and the average value is 2.49%. The measured shale samples have higher maturity and are in the stage of over-maturity evolution. The average TOC content of the Niutitang Formation shale is 4.62%, while the average TOC content of the Shimaochong Formation shale is only 1.06%.

In terms of mineral composition, the quartz content in the sample is 27.5%-68.1%, the average value is 45.7%; the clay mineral content is 8.7%-54.9%, and the average value is 26.9%. Clay mineral types include illite, imiline and chlorite, without kaolinite. The correlation between TOC content and various mineral components was analyzed. The correlation between TOC content and quartz and clay mineral components is very good (Figure 1a-b); at the same time, there is also a very good negative correlation between quartz and clay mineral components (Figure 1c).

According to the pore origin, the pores in the organic-rich shale can be divided into intergranular pores, organic matter pores, clay pores and micro-cracks [13]. The intergranular pores are the original pores preserved by the detrital mineral particles (feldspar, quartz, calcite, cuttings and primary clay minerals) in the sedimentary environment [14]. The pore size of the intergranular pores is 1-10 um, which is less associated with other types of pores (Figure 2a). Organic pores are the most abundant pore types, and their pore diameters are mainly distributed at 8-850 nm (Figure 2b). The diameter of the clay pore is generally 10-200 nm, and its shape is often an irregular polygon (Figure 2c). Micro-cracks have a jagged or curved shape (Figure 2d), which is mainly controlled by the difference in mineral brittleness. The length of micro-cracks is generally 1-20 um.

Relationship between organic and mineral components

**FIGURE 1**

According to the classification of the International Union of Pure and Applied Chemistry (IUPAC) [15-17], the adsorption isotherm of this shale sample belongs to type IV. The pore types of shale samples belong to H2, H3 and H4 types [18]. The statistical results show that the "ink bottle" type pore (H2 type) accounts for 27.3%; the parallel plate type pore (H3 type) accounts for 13.6%; and the slit-shaped pore (H4 type) accounts for 59.1%. It can be seen that the slit-shaped pore is the most predominant type of pore in the shale.

The porosity of the shale sample ranges from 0.62% to 3.34%, the average value is 1.73%; the permeability ranges from 0.000 6 mD to 0.011 0 mD, and the average value is 0.003 1 mD. There is no significant correlation between the porosity and permeability of the shale, indicating a poor connectivity between the pores. This is related to the large number of "ink bottle" pores distributed in the shale.

**N2 ADSORPTION ISOTHERMS AND PORE STRUCTURE CHARACTERISTICS**

The average pore size of the shale sample has a good negative logarithm correlation with specific surface area and pore volume, indicating that the specific surface area and pore volume increase with the decrease of shale pore size (Figure 3a-b). However, there is a good positive logarithm correlation between specific surface area and pore volume, indicating that the larger the total pore volume of the shale, the larger the specific surface area (Figure 3c). The difference in pore structure parameters with different average pore size is very obvious. As the...
average shale pore size increases from 4 nm to 15 nm, the pore size increases by 3.75 times, and its specific surface area decreases from 25 m²/g to 2.5 m²/g, which reduced reduced by 10 times (Figure 3a). Correspondingly, in this process, the pore volume decreased from 0.016 cm³/g to 0.004 5 cm³/g, which was reduced by nearly 3.6 times (Figure 3b). The Niutitang shale has a smaller average pore size, larger specific surface area and pore volume than the Bianmachong shale (Figure 3).

The gas-bearing properties of the Lower Cambrian Niutitang Formation shale in this area are much larger than the Bianmachong shale. The average porosity (1.64%) of the shale samples in the Niutitang Formation is slightly smaller than the Bianmachong shale (2.29%); however, the average permeability of the shale sample in the Niutitang Formation (0.003 4 mD) is significantly higher than the Bianmachong shale (0.001 2 mD). This indicates that the connectivity between the pores in the Niutitang shale is better, while the interior of the shale in the Bianmachong Formation contains a higher proportion of isolated pores, resulting in "high porosity and low permeability". These features can also be seen from the argon ion scanning electron microscope image. The shale reservoir mainly develops organic matter pores (OMP) and clay pores (CP) (Figure 4). The pores in the shale of the Niutitang Formation are mostly in the form of "pore clusters", and usually contain a large number of parallel plate-like pores.
and slit-shaped pores with good connectivity (Figure 4a). However, the distribution of organic matter pores and clay pores in the Bianmachong Formation shale is scattered and isolated (Figure 4b), which is an important reason for the relatively low permeability of the shale. Overall, the good connectivity between pores has an extremely important impact on the gas-bearing properties of ancient shale reservoirs.

It can be seen from the boundary line between the Niutitang Formation and the Bianmachong Formation shale in Figure 3a-b that the main effective pore space of the Lower Cambrian shale is mainly composed of nanopores with a pore diameter of less than 6.5 nm. These nanoscale pores are the main pore types of the "potential shale" of the Niutitang Formation in southern China.

**RELATIONSHIP BETWEEN ORGANIC AND MINERAL COMPONENTS AND PORE STRUCTURE PARAMETERS**

The shale specific surface area and pore volume have a good negative exponential correlation with clay mineral content. With the increase of clay mineral content, the specific surface area and pore volume both decrease (Figure 5a-b). This suggests that clay mineral pores are not the main type of effective pore space in the shale. Clay minerals are a plastic mineral that is prone to deformation (Figure 6a). The pores distributed in the clay are easily filled or closed under the action of underground fluids and stresses. Therefore, the clay mineral composition has a negative influence on the specific surface and pore volume of the shale (Figure 5a-b).

**FIGURE 4**

FE-SEM images of the shale in the target layer

Notes: Argon ion photomicrograph. (a) CY-1 well, 1 424.5 m. Both organic matter pores (OMP) and clay pores (CP) are well developed. The pores appear as "pore aggregation" and contain a large number of parallel plate-like pores and slit-type pores. The connectivity of the pores is good; (b) TX1 well, 1 707.5 m. Organic matter pores (OMP) and clay pores (CP) were observed in the sample, and the distribution of pores is relatively isolated.

**FIGURE 5**

Relationship between organic and mineral components and pore structure parameters
There is a good positive exponential correlation between specific surface area and quartz content (Figure 5c), and there is a certain positive exponential correlation between pore volume and quartz content (Figure 5d). This indicates that pores in quartz minerals are also important contributors to specific surface area and pore volume [19, 20]. It is often observed from scanning electron microscopy that the pores in the epigenetic quartz are developed, and the sapropel amorphous body (SAB) has a close symbiotic relationship with the epigenetic quartz (Figure 6b). Therefore, there is usually good connectivity between the pores in the quartz and the organic matter pores. Quartz has high strength and rigidity, it is not easily deformed in the ground, and the pores can be well preserved. At the same time, as a brittle mineral, the quartz particles are prone to rupture due to stress concentration under a high stress environment, which can also increase the contribution of the specific surface area and pore volume of the shale.

For TOC, it has a very good positive exponential correlation with specific surface area and pore volume (Figure 5e-f). When the TOC content is less than 4%, the organic matter in the shale is mostly distributed in the form of dispersion. However, when the TOC content is greater than 4%, it is often observed from scanning electron microscopy that the organic matter in the shale is mostly distributed in the form of blocks or strips, and the pores are mainly distributed along the organic matter (Figure 6c). A large amount of organic matter can be interconnected to form a continuous network structure that blocks clay and silty particles from each other [21]. The large number of continuously distributed organic matter pores have self-similarity or homogeneity, resulting in a small change in pore structure parameters. Organic matter pores are the main contributors to the specific surface area and pore volume of the Lower Cambrian shale in this area.

CONCLUSIONS

1. TOC content of the study organic-rich shale is between 0.45% and 8.50%, with an average of 3.97%. The main pore types include intergranular pores, organic matter pores, clay pores and micro-cracks.

2. The effective pore space of the shale is mainly composed of nanopores with a pore diameter below 6.5 nm, and the organic matter pores are the main contributors to the specific surface area and pore volume.

3. The TOC content of the shale is positively correlated with the quartz content and negatively correlated with the clay mineral. The slit-shaped pore is the most predominant type of pore in the shale.

4. Organic matter and epigenetic quartz generally co-exist closely. When the TOC content of shale exceeds 4%, the main pore type inside is organic matter pores (OMP).

ACKNOWLEDGEMENTS

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PARTICLE SIZE DISTRIBUTION OF MONO-, TETRA-, AND OCTA-CHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN AMBIENT AIR DURING HAZE EPISODES

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ABSTRACT

There are few studies on the levels and particle size distributions of lower chlorinated dibenzo-p-dioxins and dibenzofurans (such as mono-CDD/Fs; dibenzo-p-dioxins and dibenzofurans labelled DD/Fs) in the atmosphere, while lots of information on tetra- to octa-chlorinated homologues has been found. In this study, the distribution characteristics of mono-, tetra-, and octa-chlorinated dibenzo-p-dioxins and dibenzofurans in different sizes of urban airborne particles during haze episodes were investigated. Tetra-, and octa-chlorinated DD/DFs were mainly in the particles and the concentrations both increased as the particle size decreased, which was not the case for the monochlorinated DD/Fs. It is necessary to consider the lower chlorinated homologues to improve our understanding of the environmental behaviour during haze episodes.

KEYWORDS:
PCDD/Fs, persistent organic pollutant, particle size, distribution, air

INTRODUCTION

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are the classic unintentionally produced persistent organic pollutants (UP-POPs) with properties of toxicity, persistence and bioaccumulation. It usually induces hormone-dependent cancers and undesirable reproductive effects in humans. It is difficult to degrade and can be easily accumulated and magnified in organisms, which further make it harmful for human health and the environment. Dioxins were originate mainly from the chemical and industrial thermal processes [1]. It is therefore the studies on the environment media were necessary and important for taking measures to control and prevent these exposure risks.

Up to date, most of previous studies have focused on tetra- to octa-chlorinated dibenzo-p-dioxins and dibenzofurans (abbreviations used later are tetra = Te; octa = O; dibenzo-p-dioxins and dibenzofurans=DD/Fs) in the multimedia, especially for the seventeen 2,3,7,8-PCDD/Fs [2-4]. Not much attention have been drawn to the lower chlorinated DD/Fs, might due to the absence of assigned toxic equivalent factors for the mono- to tri-chlorinated congeners (abbreviations used later are mono = Mo; tri = Tr) [5]. But the lower chlorinated congeners, such as 2-MoCDF and 3-MoCDF, presented embryotoxicity and mutagenicity [6, 7]. As described previously [8-10], Te- to OCDD/Fs (labelled Cl4=DD/Fs) presented obvious distribution characteristics in the ambient air that Cl4=DD/Fs were mainly in the particles and the concentration increased as the particle size decreased, and it was not the case for the Mo- to Tr-CD/DFs (labelled Cl1=DD/Fs) [11]. It was found that MoCDFs usually dominant in the ambient air. Lower chlorinated DD/Fs, such as TeCDFs, were usually worked as the raw materials for the formation of the higher chlorinated DD/Fs, such as OCDD/Fs [12, 13]. Additionally, with the increase of number of chlorine, the gas-particle partitioning behaviours, particle size distributions and adsorption behaviours would present difference [14-16]. However, to the best of our knowledge, there are no systematical studies on the particle size distribution of Mo-, Te- and OCDD/Fs in the ambient air and no comparisons of these distributions.

In this study, we examined the Mo-, Te- and OCDD/F concentrations in different sized airborne particles in an area and evaluated as a preliminary step toward further comprehensively understanding the particle size distributions of airborne PCDD/Fs and improve our ability to control these risks during haze episodes.

MATERIALS AND METHODS

Sample Collection. Particle size distributions of Mo-, Te- and OCDD/Fs in the ambient air were the primary aim of this study. To solve it, therefore, a continuous sampling activity covering about two months at a research station (115.51°E, 38.87°N) were designed and carried out. The site was away
from the main roads and obvious sources to guarantee the collected data stability and comparability. More concretely, KS-303 PM$_{10,2.5,1.0}$ sampler (Kálmán System, Budapest, Hungary) were used to collect the samples with the sampling volume ca. 1500 m$^3$. Five air samples were collected and each sample included the gas phase and different sized particle (aerodynamic diameters ($d_{ae}$) of $>$10 µm, 2.5–10 µm, 1.0–2.5 µm, and $<$1.0 µm) phase samples. Each sample was wrapped separately in aluminum foil and stored in a sealed polyethylene bag. Then, the samples were duly transported to the laboratory and stored at $-20\,^\circ\text{C}$ until analysis. Additionally, days when PM$_{1.0}$ concentrations were $>100\,\mu\text{g m}^{-3}$ and visibility was $<10\,\text{km}$ were classified as haze days [17]. More details of as-obtained samples during haze episodes were described in the Table 1.

Sample analysis. Analysis of Mo-, Te- and OCDD/Fs were performed as previously described [18, 19]. Briefly, each sample was spiked with $^{13}$C$_{12}$-labeled PCDD/Fs and extracted with a mixture solvent of dichloromethane and hexane (1:1 v/v) by Soxhlet extraction for 24 h. The extract was split into two aliquots, one that was analyzed for the Te- and OCDD/Fs, and another that was analysed for the MoCDD/Fs. Each aliquot was cleaned up, concentrated and transferred into 10 µL of nonane in a minivial. The $^{13}$C$_{12}$-labeled injection standards were added before the instrumental analysis.

An Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5 MS column (60 m $\times$ 0.25 mm $\times$ 0.25 µm, Agilent Technologies, Santa Clara, CA, USA) that was coupled to an Autospec Ultima high resolution mass spectrometer (Waters, Milford, MA, USA) with an electron impact (EI) ion source were used to analyse the mono-, tetra- and octa-chlorinated DD/Fs. The mass spectrometer had a solution of at least 10,000 and operated in selected ion monitoring mode. The condition parameters of instrumental analysis were accordance with the previous literature [19, 20].

Quality assurance/quality control. A breakthrough test, adding a half PUF cartridge in series after the first PUF, was performed, and no breakthrough was found after a 24 h sampling period. All samples were spiked with $^{13}$C$_{12}$-labeled compounds before extraction and at the end of the sample preparation procedure to allow the performance of the analytical method to be monitored. A signal/noise ratio $>3$ was used to calculate the limits of detection (LOD) in this study. The concentrations of PCDD/Fs below the LOD were assigned values equal to half of the LOD. Field blank and laboratory blank samples were included in each batch of analyzed samples. No obvious contamination was found in the blank samples. The recoveries for the mono-, tetra- and octa-chlorinated DD/Fs were 67–89%, 70–108% and 70–128%, respectively. Additionally, the levels of significance were set at the 95% confidence interval ($p = 0.05$). SPSS 22.0, Origin 8.5, and Excel 2013 were used for data analysis.

RESULTS AND DISCUSSION

Concentration and profile. As presented in the Figure 1, the concentrations of PCDD/F in the ambient air during haze episodes were analysed and compared. The MoCDF concentrations were obviously higher than other PCDD/F congeners (paired t test, $p <0.05$). For MoCDF congeners, 3-MoCDF concentrations were dominant, being 5.76–17.7 pg m$^{-3}$. The 2,3,7,8-TeCDF was lowest, ranged from 54.1 to 99.3 fg m$^{-3}$. The OCDF concentrations were in the middle level. Although the toxicity was not big than the TeCDD, the toxic equivalent quantity concentration should not be ignored.

PCDD/F congeners in the gas and particles. There were obvious differences in the gas/particle partitioning behaviours of the different PCDD/F congeners, as depicted in the Figure 2. A large fraction of each MoCDF congener was found in the gas phase. TeCDF and OCDF in the particle partition fractions were both higher than the MoCDFs. On the whole, the polychlorinated dibenzofurans (PCDFs) in the particle partition fraction increased as the chlorine number increased. Compared to the DFs, a higher fraction increased in the particles as the chlorine number of PCDD/Fs increased, which might be due to DDs have lower vapour pressures than the corresponding dibenzofuran congeners.

| TABLE 1 | Specific sampling information for the collected air samples. |
|---------|------------------|------------------|------------------|------------------|
|         | H1               | H2               | H3               | H4               |
| Mean T (°C)   | 15               | 14               | 10               | 12               |
| Humidity      | 43%              | 35%              | 40%              | 55%              |
| Window rate (m s$^{-1}$) | 0.5              | 0.6              | 0.8              | 1.1              |
| Atm (Hpa)     | 1009             | 1011             | 1015             | 1021             |
| Visibility (Km) | 3.8             | 4.8              | 2.2              | 4.5              |
| Sampling Volume (m$^3$) | 2300             | 2308             | 2312             | 2311             |
| PM$_{1.0}$ (µg m$^{-3}$) | 187              | 174              | 271              | 185              |
| Particles (µg m$^{-3}$) | 313              | 270              | 466              | 279              |
FIGURE 1
Concentrations of the 10 Mo-, Te- and OCDD/F congeners in the ambient air samples.

FIGURE 2
PCDD/F congener distributions in the gas and particles

FIGURE 3
Proportions of PCDD/Fs with different sizes of particles in the ambient air.
The particle size distributions of the Mo-, Te- and OCDD/Fs were investigated and shown in the Figure 3. The 2,3,7,8-TeCDD/F concentrations increased as the particle size decreased and over 90% were found in the \( d_{50} < 2.5 \) \( \mu \text{m} \) particles. The similar distribution tendencies were similar to OCDD/Fs and higher distribution fractions were found, but not to the MoCDFs. The MoCDF fraction in the \( d_{50} < 1.0 \) \( \mu \text{m} \) was slightly higher than other sized particles, and over nearly 65.0% was found in the \( >1.0 \) \( \mu \text{m} \) particles. For MoCDDs, it was shown that the fraction was higher in the \( >1.0 \) \( \mu \text{m} \) particles, being up to 81.1%.

Another usual approach for studying the distribution of pollutants in the different size fractions is to use normalized histograms representing \( dC/d\log D_p \) versus \( D_p \), where \( dC \) is the concentration of PCDD/Fs in each size fraction; and \( D_p \) is the aerodynamic diameter [21, 22]. Figure 4 shows that the MoDFs were prone to distribute in the coarse particles \( (d_{50} < 1.0 \) \( \mu \text{m} \) ), especially dominant in the 1.0–2.5 \( \mu \text{m} \) particles. In the \( >1.0 \) \( \mu \text{m} \) particle fraction, the distribution tendency decreased as the particle size increased, which is similar with MoCDDs. It is might due to the physicochemical properties of themselves and particles. But for TeCDFs and OCDD/Fs, the distribution tendency decreased as the particle size increased.

CONCLUSIONS

Mo-, Te- and OCDD/F distributions in different sized particles \( (d_{50} > 10 \) \( \mu \text{m} \), 2.5–10 \( \mu \text{m} \), 1.0–2.5 \( \mu \text{m} \), and \( <1.0 \) \( \mu \text{m} \) ) during haze episodes were preliminarily investigated. The MoCDD/Fs were tended to distribute in the \( >1.0 \) \( \mu \text{m} \) particles (over 60%), and the fractions were equal in each above sized particle. It was not the case for the Te- and OCDD/Fs that the concentrations increased as the particle size decreased. It is necessary and useful for further comprehensively understanding the particle size distributions of airborne PCDD/Fs and improve our ability to control these risks during haze episodes.

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ANTIFUNGAL POTENTIAL OF ESSENTIAL OILS OF
SALVIA OFFICINALIS AND SALVIA TOMENTOSA PLANTS
ON SIX DIFFERENT ISOLATES OF
ASCOCYHTA RABIEI (PASS.) LABR.

Melih Yilar*, Yusuf Bayar
Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Kirsehir, Turkey

ABSTRACT

This study was conducted to determine the antifungal activity of the essential oils of the Salvia officinalis and Salvia tomentosa plants on Ascochyta rabiei (Chickpea blight) which is an important chickpea disease. As a result of the study, S. officinalis and S. tomentosa plant essential oils were applied to the isolates of A. rabiei at the doses of 0 (Control), 1, 2, 4, 8, 10 µL petri-1. Essential oils are impregnated on the filter papers stuck to the petri dish with a micropipette at the application doses and the lids were immediately covered with a parafilm and left to incubate at the temperature of 23 °C for 15 days. At the end of the 15-day incubation, the measurements of mycelium diameters of isolates in petri dishes were carried out with electronic calipers. As a result, S. officinalis essential oil inhibited mycelium growth of Isolate 2 and Isolate 3 by 100% at the dose of 10 µL petri-1. S. tomentosa essential oil inhibited the mycelium growth of Isolate 3, Isolate 4, Isolate 5, Isolate 6 by 100%. A difference was found in the responses of Anthracnose isolates to plant essential oils and to application doses. S. tomentosa was found to be more effective on the isolates. The findings indicate that both of these essential oils can be used as an alternative to synthetic fungicides in controlling the A. rabiei pathogen.

KEYWORDS:
Antifungal activity, Ascochyta rabiei, essential oil, Salvia

INTRODUCTION

Being a protein source for people around the world, edible grain legumes meet the vegetable protein need of human nutrition by 22% and of animal nutrition by 38%. Turkey is one of the gene centers of legumes [1]. Chickpea, which is in the group of edible grain legumes, is the first plant to be cultivated [2] and it is one of the important plant protein sources rich in amino acids such as leucine, histidine, isoleucine, lysine, phenylalanine, trionin, valine, which are of importance in human nutrition [3]. In Turkey in 2016, the cultivation area of chickpeas was 360 ha and the production was 455 thousand tons [4]; however, this production amount is not at the desired level, which may be due to the fact that the producers do not apply appropriate cultivating techniques and the problems, such as, failure to combat the disease, pests and weeds and resistant varieties are not as widespread as they should be.

The grain yield of chickpea is significantly affected by abiotic factors (drought and salinity, etc.). In addition, there are more than 50 plant pathogens known to affect the chickpea yield adversely [5]. Among these pathogens, Ascochyta rabiei (Chickpea blight) is widely seen in the production areas in Turkey as well as in the world [6] and causes significant yield losses. In addition to causing infection in all aboveground parts of the plant, this fungus causes circular necrotic stains on leaflets and cones. Under favorable environmental conditions and in the presence of susceptible hosts, this pathogen causes up to 100% product losses in chickpea production areas [7, 8].

One of the methods that producers use extensively in controlling Ascochyta rabiei pathogen is chemical spraying. Researchers have turned to alternative fighting methods due to the fact that the chemical drugs used in disease control have detrimental effects on the environment and human health and that the pathogen gains resistance to these drugs that were used. One of these methods is the use of herbal medicines. For this reason, researchers have begun and still continue to carry out studies on the use of herbal essential oils and extracts, which are environmentally and human-friendly, in controlling diseases, pests and weeds.

This study was conducted to investigate the effectiveness of Salvia officinalis and Salvia tomentosa plant essential oils against 6 different isolates of Ascochyta rabiei pathogen causing significant loss in chickpea production areas.
MATERIALS AND METHODS

Obtaining Plant Material and Essential Oil. The plant material, *Salvia officinalis* and *Salvia tomentosa*, was collected from Tokat province in 2014-2015 by taking the aboveground parts. The essential oils were obtained from the plant materials dried in the shade at room temperature using the hydro-distillation method with the help of Clevenger apparatus. The obtained essential oils were stored in the refrigerator at 4 °C until use.

Development of Fungus Cultures. The *Ascochyta rabiei* isolates used in the experiment were obtained from stock cultures in the Phytochemical Laboratory of the Department of Plant Protection, Agricultural Faculty of Ahi Evran University. The *A. rabiei* isolates obtained from stock cultures were transferred to the PDAs and were incubated at 22±1 °C for 12 hours in the dark period and for 12 hours in the light period. The mediums developed in 7 days were used in studies.

Determination of Antifungal Effect of the Essential Oil in In-vitro Conditions. To determine antifungal activity, about 10 ml of PDA medium was prepared in 60 mm diameter petri dishes for the development of disease agents. 5 mm-diameter mycelium discs were transferred to the middle of the petri dishes that paper was pasted. *M. spicata* essential oil in the concentrations of 0 (Control), 1, 2, 4, 8 and 10 μl petri-1 was added onto the paper pasted with a micropipette. The petri dishes were covered with a parafilm and incubated for 15 days at a temperature of 23±1 °C for 12 hours in the dark and for 12 hours in the light period.

At the end of this period, the development of fungal mycelium (colony) was measured by electronic calliper. The applications were conducted 4 times with 2 repetitions. The percent inhibition of different doses of essential oil was calculated by comparing the mycelium development in the essential oil-containing petri with that of the control petri [9].

\[ \text{MGI}=100\times(\text{dc}-\text{dt})/\text{dc} \]

MGI: Mycelium Growth Inhibition rate (%)
dc: Mycelium development in the control petri
dt: Mycelium development in the petri containing essential oil

Statistical Analysis. The significance levels of the differences between the treatments were determined by analysis of variance (ANOVA) and the means were compared using the DUNCAN test. The statistical analyzes were conducted using SPSS software program (Ver.15.0, SPSS). The data obtained in the experiment were analyzed by Probit analysis and LC₅₀, LC₉₀ values were calculated using the SPSS 15 analysis program.

RESULTS AND DISCUSSION

The infections caused by plant pathogenic fungi cause loss of yield in agricultural products, both in the field and after the harvest. In addition, they lead to significant damage due to the fact that they are also the infection sources in the following years. Determination of environmental and human health effects of chemical medication, which is one of the methods used to prevent these losses, and resistance problems caused by in disease agents made it difficult for producers to work on controlling diseases. For this reason, researchers have begun to work on natural compounds that may be alternative to synthetic pesticides. It has been reported in various studies that plant extracts and essential oils have a significant effect on plant pathogens [10-14].

It is revealed by this study that the effectiveness of *Salvia officinalis* and *Salvia tomentosa* essential oils against the isolates of *A. rabiei* pathogen which is an important chickpea disease. The antifungal activity performed by the essential oils

### Table 1

The antifungal activity of *Salvia officinalis* and *Salvia tomentosa* essential oils on the mycelium growth of *A. rabiei* isolates

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Doses μl petri-1</th>
<th>Mycelium growth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>isolate 1</td>
<td>isolate 2</td>
</tr>
<tr>
<td>Control</td>
<td>44.42±0.36</td>
<td>33.22±0.64</td>
</tr>
<tr>
<td>1</td>
<td>37.63±2.48</td>
<td>29.13±1.64</td>
</tr>
<tr>
<td>2</td>
<td>32.02±5.62</td>
<td>24.01±2.20</td>
</tr>
<tr>
<td>4</td>
<td>29.12±3.90</td>
<td>21.41±1.65</td>
</tr>
<tr>
<td>8</td>
<td>17.59±0.98</td>
<td>17.42±1.28</td>
</tr>
<tr>
<td>10</td>
<td>12.52±1.03</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>Salvia officinalis</em></td>
<td>Control</td>
<td>45.42±0.51</td>
</tr>
<tr>
<td>1</td>
<td>33.89±0.54</td>
<td>38.22±0.52</td>
</tr>
<tr>
<td>2</td>
<td>29.24±0.62</td>
<td>34.33±0.52</td>
</tr>
<tr>
<td>4</td>
<td>27.22±0.59</td>
<td>32.58±0.51</td>
</tr>
<tr>
<td>8</td>
<td>20.18±0.60</td>
<td>26.54±0.62</td>
</tr>
<tr>
<td>10</td>
<td>18.62±0.55</td>
<td>15.77±0.48</td>
</tr>
</tbody>
</table>

* Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)
on the isolates is shown in Table 1, 2 and 3 and in Figure 1 and Figure 2.

*Salvia officinalis* essential oil had different levels of effect on *A. rabiei* isolates. This effect varied depending on the increasing doses of *S. officinalis* essential oil and on the isolates. Isolate 2 and Isolate 3 are the most sensitive, while Isolate 5 and Isolate 6 were the isolates that showed the highest resistance at the highest dose of the essential oil. *S. officinalis* essential oil inhibited mycelium growth of Isolate 2 and Isolate 3 by 100% compared to the control (Table 1; Figure 1).

*S. tomentosa* essential oil was found more effective than *S. officinalis* on *A. rabiei* isolates. When compared to the control, *S. tomentosa* essential oil inhibited the mycelium growth of Isolate 1 and Isolate 2 by 59.00% and 63.85%, respectively, while it inhibited the mycelium growth of other *A. rabiei* isolates (Isolate 3,4,5,6) by 100% (Table 1, Figure 2).

In dose-response trials, the values LC50 and LC90 values *S. officinalis* essential oil on the isolates of *A. rabiei* (Isolate 1, 2, 3, 4, 5, 6) was found 6.57, 5.78, 5.48, 5.70, 7.85, 7.57; 13.11, 10.75, 10.19, 11.23, 15.48, 14.85, respectively (Table 2).

In the dose-response trials in the current study, the LC50 and LC90 values of *S. tomentosa* essential oil on *A. rabiei* isolates were found 1.15, 8.45, 6.03, 5.40, 4.60, 4.39; 16.77, 15.98, 9.89, 10.52, 8.81, 9.90, respectively (Table 3).

![FIGURE 1](image1.png)

**FIGURE 1**
The inhibition rates of *Salvia officinalis* essential oil on *A. rabiei* isolates (%)

![FIGURE 2](image2.png)

**FIGURE 2**
The inhibition rates of *Salvia tomentosa* essential oil on *A. rabiei* isolates (%)
### TABLE 2

The dose-response results of *S. officinalis* essential oil on *A. rabiei*

<table>
<thead>
<tr>
<th>Antracnose isolates</th>
<th>LC₅₀ (µg/mL)</th>
<th>LC₉₀ (µg/mL)</th>
<th>Slope + Standard error</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>isolate 1</td>
<td>6.57 (4.82-9.45)</td>
<td>13.11 (10.01-21.46)</td>
<td>0.20±0.016</td>
<td>18.21</td>
</tr>
<tr>
<td>isolate 2</td>
<td>5.78 (2.87-12.11)</td>
<td>10.75 (7.38-31.70)</td>
<td>0.26±0.018</td>
<td>61.83</td>
</tr>
<tr>
<td>isolate 3</td>
<td>5.48 (2.73-10.60)</td>
<td>10.19 (7.08-26.59)</td>
<td>0.27±0.019</td>
<td>59.57</td>
</tr>
<tr>
<td>isolate 4</td>
<td>5.70 (4.17-7.75)</td>
<td>11.23 (8.83-16.74)</td>
<td>0.32±0.017</td>
<td>18.28</td>
</tr>
<tr>
<td>isolate 5</td>
<td>7.85 (5.72-12.75)</td>
<td>15.48 (11.31-29.86)</td>
<td>0.68±0.016</td>
<td>18.84</td>
</tr>
<tr>
<td>isolate 6</td>
<td>7.57 (5.69-11.27)</td>
<td>14.85 (11.19-25.45)</td>
<td>0.18±0.016</td>
<td>16.22</td>
</tr>
</tbody>
</table>

### TABLE 3

The dose-response results of *S. tomentosa* essential oil on *A. rabiei*

<table>
<thead>
<tr>
<th>Antracnose isolates</th>
<th>LC₅₀ (µg/mL)</th>
<th>LC₉₀ (µg/mL)</th>
<th>Slope + Standard error</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>isolate 1</td>
<td>1.15 (3.80-31.7)</td>
<td>16.77(10.52-124.30)</td>
<td>0.13±0.015</td>
<td>32.73</td>
</tr>
<tr>
<td>isolate 2</td>
<td>8.45(6.36-13.28)</td>
<td>15.98(11.84-29.19)</td>
<td>0.17±0.016</td>
<td>16.62</td>
</tr>
<tr>
<td>isolate 3</td>
<td>6.03(4.00-9.07)</td>
<td>9.89(7.51-17.48)</td>
<td>0.33±0.21</td>
<td>44.31</td>
</tr>
<tr>
<td>isolate 4</td>
<td>5.40(2.02-13.01)</td>
<td>10.52(7.01-39.63)</td>
<td>0.25±0.018</td>
<td>68.69</td>
</tr>
<tr>
<td>isolate 5</td>
<td>4.60(2.35-7.77)</td>
<td>8.81(6.30-18.14)</td>
<td>0.30±0.20</td>
<td>48.26</td>
</tr>
<tr>
<td>isolate 6</td>
<td>4.39(0.39-12.65)</td>
<td>9.90(6.30-56.49)</td>
<td>0.23±0.018</td>
<td>70.66</td>
</tr>
</tbody>
</table>

The difference that occurs in both sage species arises from the differences in the proportions and the compounds of the essential oil compositions that the species contain. The main component of the *S. tomentosa* species collected from Tokat province was determined β-Thujene, while the main component of *S. officinalis* essential oil was determined 3-Thujanone [15]. Salvia species are rich in terms of biological activity. In the studies carried out on biological activity, it was reported by researchers that Salvia species have antibacterial [16], insecticidal [17], antioxidant [18], antiviral [19], antimicrobial [20], antifungal [21] and herbicidal effects [22]. Salvia species contain phenolic compounds and flavonoids having phytotoxic characteristic in their constituents [23].

In similar studies, researchers investigated the activities of different plant essential oils and extracts on *A. rabiei*. Zerroug et al. [24] reported that *A. rabiei* never developed mycelium at doses of 1.5, 3, 6 mg/ml of *Saccocalyx satureioides* Coss. Et. Dut. plant essential oil. In similar studies, plant extracts were investigated for their activity on *A. rabiei* mycelium development. In controlling *A. rabiei*, the usability of the n-hexane extract of *Datura Metel* [25], the water, ethanol and n-hexane extracts of the leaf, fruit, root and shoot shell of *Syzygium cumulus* [26], the organic solvents of allelopathic trees [27], the organic solvent extracts of *Tagetes erectus* plant [28] and Chenopodium album extracts [29] has been revealed.

### CONCLUSION

The researches that carried out on the potential of the secondary metabolites, such as, plant essential oils and extracts to be an alternative to pesticides in fighting against the pathogens, diseases, pests and weeds in economically important plants continue to be the primary studies. This study revealed that *S. officinalis* and *S. tomentosa* essential oils have a high level of antifungal effect on the *A. rabiei* isolates. It was determined that this effect differs according to isolates and sage species. Today, these studies have become more valuable owing to the fact that the fungicides used for disease control create resistance problem in plant pathogens and in addition to that their detrimental effects on the environment and people have been revealed. In the current study, it has been suggested that *S. officinalis* and *S. tomentosa* essential oils could be used in controlling the disease.

### REFERENCES


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MICROSCOPIC PORE STRUCTURES AND MOVABLE FLUID DEPOSIT CHARACTERISTICS OF TIGHT SANDSTONE RESERVOIR IN CHANG-8 OIL RESERVOIR, HUJIANSHAN OILFIELD, ORDOS BASIN

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ABSTRACT

The author investigated and outlined the complex geological characteristics of Chang-8 oil reservoir in Hujianshan Oilfield, Ordos Basin, including its significant anisotropy, complex microscopic pore structures, low movable fluid saturation, and intensive fluid distribution characters, etc. To answer these questions, the author carried out experiments by means of normal physical characteristics, casting thin-section, SEM analysis, conventional mercury injection and NMR, etc. Observations were made particularly on the microscopic pore structures of Chang-8 oil reservoir in Hujianshan Oilfield, and its influence to the saturation level of movable fluids. The study suggested that the reservoir spaces of Chang-8 were predominately primary intergranular pores and feldspar vugular pores. These pore structures were categorized into four groups of reservoir type that represent varied reserving and filtration capacities, i.e. Types I (the highest), II, III and IV (the lowest), according to their varied capillary pressure curves and corresponding parameters. Each had a NMR T2 spectrum similar to its corresponding distribution pattern of mercury saturation at pore throat radius, suggesting a positive correlation between the T2 value and the pore throat diameter. Pore throat diameter was thus highlighted as a major factor affecting that determined the distribution of movable fluids, along with an intensive positive correlation between permeability and movable fluid saturation that provided an intuitive parameter representing the filtration capacity of pore structures.

KEYWORDS:
NMR, saturation of movable fluids, microscopic pore structures, Chang-8 tight reservoir, Hujianshan oilfield

INTRODUCTION

The tight sandstone reservoir, comparing to the regular sandstone reservoir, is featured with smaller size of power throat (nanometer/micrometer order), complex pore structure, and intensive macro anisotropy [1-2], etc. Pore structure, among all these features, is particularly highlighted for its influence to the reserving and fluid infiltration characters of the reservoir that directly determine the reserving and filtration capacity of the reservoir, as well as the oil production capacity, displacement efficiency and oil recovery of the oil field [3-6]. Microscopic pore structures, types and evolution of pores and reserving property parameters of the tight sandstone reservoir have been under intensive study by researchers around the world [7-10]. A study on characteristics of pore structure evolution in tight reservoirs is thus carried out, so as to provide an approach to properly assess the exploring potential of the reservoir, and to make proper choices on exploring techniques.

NMR T2 spectrum has been extensively used as a typical experimental technique for researchers on reserving characters of movable fluids. It is proven to be a reliable approach to gather movable fluid parameters for studies on microscopic pore structures [11-12]. The downfield NMR T2 spectrum, which is proven to be directly correlated to the pore structure, presents a fairly faithful pattern of the sample’s pore distributions [13-14]. Liu Tangyan [15] and Fang Tao [16] assumed a linear correlation between T2 and the pore distribution pattern, and thus derived a NM capillary pressure curve from the NMR T2 spectrum. Wang Weimin et al. [17] believed that volume fractions of movable fluids attained by NMR were of great value in assessing the development of microfractures, clay minerals and secondary pores. Gao et al. [18] believed that the NMR T2 spectrum of the ultra-low permeability reservoir can be categorized into 5 types, with a reducing permeability accompanying an increasing development of throats of radius < 1µm. Li Haibo et al. [19] combined the techniques of NMR and waterflood to quantitatively observed the oil phase distributions of the reservoir before and after waterflooding. Zheng Ke et al. [20] analyzed 3 typical low-permeability reservoirs by means of NMR, suggesting that with a higher permeability comes the stronger correlation between movable
fluid parameters and permeability. As to Chang-8 oil reservoir in Hujianshan Oilfield, studies on influential factors to the movable fluids from the perspective of microscopic pore structures are rarely seen. To deeply investigate the pore structure characters of this reservoir type, experimental techniques like casting thin-section, scanning electron microscope, high-pressure mercury injection, NMR and actual sandstone waterflooding model, etc., so as to reveal the influence of reserving characters and pore structures of movable fluids to movable fluid saturation in tight sandstone reservoirs, which hopefully will provide viable data and references to developers of such reservoirs.

**EXPERIMENTAL**

Relaxation time as a crucial physical quantity in NMR is jointly determined by the physical properties and fluid characteristics of the rock. With the same pore fluids, relaxation time is solely determined by the former [21]. Due to the relaxation mechanism of NMR and the measuring principle of rock physical characteristics [22-24], different pore throats in the rock presents varied relaxation time that are manifested in the T2 spectrum as differed spectra peaks, representing the features of different throats. In the rock core saturated with water, the pore water exists in two reserving states, with one part in movable state, and another part in bound state (mainly capillary water and pellicular water). The T2 relaxation time of the bound water is relatively short, and that of the movable water is relatively longer. Thus the T2 spectrum can be used to analyze the existence of fluids in pores, which enables quantitative measurement of fluid saturation of the movable and bound water. NMR T2 measurement in this study is done by the MesoMR23-60H-I device provided by the Shaanxi Key Laboratory of Advanced Stimulation Technology for Oil & Gas Reservoirs, at a steady temperature of 20°C. The mercury injection experiment is done with AutoPoreIV9510 automatic mercury injection apparatus from MicromeriticsR, with a maximum working pressure of 414 MPa, and a throat radius measuring coverage of 0.003~1,000 μm.

**Experimental Samples.** The differences in physical properties and microscopic pore structures among the 4 testing blocks are due to differed pore combinations, along with the varied types, contents and occurrences of interstitial materials. All testing samples were taken from the Chang-8 oil reservoir, Hujianshan Oilfield in mid-west Yishan clinoform (Fig.1) in a delta front facies at the depth of 2,197.6m~2,185.2m. The rocks are mainly lithic feldspar sandstone with a porosity of 5.9%~15.7% (mainly intergranular pores and vugular pores; Table 1), and a permeability of 0.05×10^{-3} μm²~1.69×10^{-3} μm².

It is learnt, from rock cores and casting thin-sections taken from over 30 cored wells in Chang-8 oil reservoir, that the dominant lithological combination of the observed zone are lithic feldspar sandstone and feldspar lithic sandstone (Fig. 2), with an average porosity of 9.6%, and an average permeability of 0.51×10^{-3} μm². According to the national petroleum/natural gas industry standard SY/5 6285-2011, Chang-8 oil reservoir consists dominantly of tight sandstone reservoirs of ultra-low porosity and ultra-low permeability, mainly fine sandstone mingled with small volume of argilla, with a maximum granular size of 0.2 mm~1.7 mm (0.41 mm in average). The rock granules are moderately/well sorted, mainly in the shape of minor prismatic psephicity, cemented predominantly in porous type, plus porous-film and enlarging-porous types, with moderate structural maturity. Rock support is mainly of the type of granular support in dot-line contacts, plus local line contacts.

![FIGURE 1](Location of Hujianshan Oilfield in the Ordos Basin)
TABLE 1

<table>
<thead>
<tr>
<th>Well ID</th>
<th>Depth/m</th>
<th>Sedimentary microfacies</th>
<th>Porosity/ %</th>
<th>Permeability/ x10-3 μm²</th>
<th>Pore Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>2585.2</td>
<td>Underwater distributary channel</td>
<td>15.7</td>
<td>1.69</td>
<td>Intergranular pore</td>
</tr>
<tr>
<td>H23</td>
<td>2375.5</td>
<td>Underwater distributary channel</td>
<td>11.3</td>
<td>0.55</td>
<td>Intergranular pore, feldspar dissolved pores</td>
</tr>
<tr>
<td>A296</td>
<td>2197.6</td>
<td>Underwater distributary channel</td>
<td>11.9</td>
<td>0.5</td>
<td>Feldspar dissolved, pores, intercrystalline pore</td>
</tr>
<tr>
<td>H26</td>
<td>2199.9</td>
<td>Underwater distributary channel</td>
<td>5.9</td>
<td>0.05</td>
<td>Pores, intercrystalline pore, micropores</td>
</tr>
</tbody>
</table>

TABLE 2

Statistics of pore types in Chang-8 Oil Reservoir, Hujianshan Oilfield

<table>
<thead>
<tr>
<th>Layer</th>
<th>Intergranular pore</th>
<th>Intergranular dissolution pore</th>
<th>Feldspar dissolved pores</th>
<th>Lithic fragments dissolved pores</th>
<th>Pores, intercrystalline pore</th>
<th>Micro fissure</th>
<th>Surface porosity/ %</th>
<th>Average pore radius/μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang 8I</td>
<td>0.73</td>
<td>0.11</td>
<td>0.84</td>
<td>0.1</td>
<td>0.1</td>
<td>0.03</td>
<td>1.93</td>
<td>27.63</td>
</tr>
<tr>
<td>Chang 8II</td>
<td>4.34</td>
<td>0.19</td>
<td>0.63</td>
<td>0.27</td>
<td>0.01</td>
<td>0.08</td>
<td>4.14</td>
<td>68</td>
</tr>
<tr>
<td>Chang 8</td>
<td>1.89</td>
<td>0.81</td>
<td>0.2</td>
<td>0.11</td>
<td>0.03</td>
<td>0.04</td>
<td>3.08</td>
<td>52.36</td>
</tr>
</tbody>
</table>

FIGURE 2

Major Pore Types of Sandstone Reservoir in Chang-8 Oil Reservoir, Hujianshan Oilfield
(a: intergranular pores, Well H23#, 2,375.5 m; b: intergranular pores, H26#, 2,199.9 m; c: feldspar vugular pores, Well H68#, 2,378.6 m; d: kaolinite intracrystalline pores, H67#, 2,426.7 m; e: illite intracrystalline pore, H68, 2,378.6 m; f: microfissure, H23#, 2,375.5 m)

RESULTS AND DISCUSSION

According to information gathered with scanning electron microscope and casting thin-section techniques, major pore types of the target stratum of the observed zone are compaction, cementation and corrosion.

No development is observed for the modified intergranular pores (Fig. 2-a & 2-b), vugular pores (Fig. 2-c), intracrystalline pore (Fig. 2-d & 2-e) and fissure (Fig. 2-f). Observations suggest a rather low total surface porosity of 3.08%, including 1.89% for primary intergranular pores, and 1.01% for vugular pores (Table 2). The pores are mainly stuffed with authigenic cement and matrix, with moderate/poor pore coordination, and moderate connectivity (Fig. 2). The reservoir’s reserving and infiltration capabilities are directly determined by its distribution character of pores and interstitial material.

Pore Structure Types and Features. Pore structure is a microscopic physical property that directly represents the reserving quality of the reservoir, as the feature of pore development influences the permeability rule of petroleum and natural gas [25]. Presently there are numerous study methods
and categorizing approaches concern reservoir pore structures [26]. In this study, 41 pieces of mercury injection samples were taken, and the pore structures in the observed zone were categorized into 4 groups, i.e. Types I, II, III and IV, according to the mercury injection parameters and corresponding capillary pressure curves (Table 3 & Fig. 3).

Type I structure is mainly consisted of micro-medium-sized pore throats. Its capillary pressure curve tilts toward the lower left corner of the chart, with a relatively steady section below S_{Hg-50}, suggesting a low displacement pressure (0.01 MPa ~ 1.04 MPa) (Fig. 3). The pore throat radius ranges mainly from 0.36 μm to 3.18 μm, with a relatively small skewness (-0.55~0.24), with a sorting coefficient of 1.1~2.5 (1.7 in average). The maximum mercury saturation ranges between 78.7% and 93.9% (87.5% in average) (Table 3). The pores are mainly residual intergranular types and feldspar vugular ones. The clay minerals are mainly chlorite and kaolinite. Type I structure provides the best reserving and infiltration capacities among all reservoir types. It is usually seen in thick sand bodies of the underwater distributary channel.

Type II structure is mainly consisted of micro-fine-sized pore throats. Its capillary pressure curve is similar to that of Type I, yet with a relatively higher displacement pressure (0.33 MPa ~ 2.91 MPa) than Type I (Fig. 3). The pore throat radius ranges mainly from 0.17 μm to 1.86 μm, with a skewness (-0.69~0.16) in relatively normal distribution, with a sorting coefficient of 1.28~2.89 (2.00 in average). The maximum mercury saturation ranges between 69.9% and 89.5% (78.4% in average) (Table 3). Intensified mechanical compaction and cementation were observed, with increased loss of primary intergranular pores, along with increased development of secondary vugular pores, granular mold pores and intergranular pores. Type II structure provides fairly good reserving and infiltration capacities among all reservoir types. It is usually seen in underwater distributary channels or sides of the riverway connected to the central channel.

Type III structure is mainly consisted of micro-sized pore throats. Its capillary pressure curve tilts toward the upper right corner of the chart, with a relatively drastic change below S_{Hg-50}, suggesting a displacement pressure (2.30 MPa ~ 5.32 MPa) higher than the previous two types (Fig. 3). The pore throat radius ranges mainly from 0.03μm to 0.87μm, with a relatively small skewness (-0.82~0.04), with a sorting coefficient of 1.8~3.6 (2.3 in average). The maximum mercury saturation ranges between 59.8% and 87.3% (73.2% in average) (Table 3), a level relatively lower than the previous two types. More intensified mechanical compaction and cementation were observed, with significant loss of primary intergranular pores, along with relatively more developed vugular and intracrystalline pores. The surface porosity of intergranular pores is lower than that of vugular pores. Type III presents a relatively thinner single sand body than Type I and Type II, with significant structural anisotropy among sand strata and the sand body pore structures, suggesting the deteriorating reserving and infiltration properties. Type III performs in a mediocre manner in terms of reserving and infiltration capacities. It is mainly seen at top/bottom and sides of the underwater distributary channel, and in thin sand bodies of the water way.

### Table 3

<table>
<thead>
<tr>
<th>Classification parameters</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity/%</td>
<td>10.8~18.2</td>
<td>9.4~14.1</td>
<td>6.5~12.4</td>
<td>6.2~10.4</td>
</tr>
<tr>
<td>Permeability/×10⁻³μm²</td>
<td>0.26~1.67</td>
<td>0.07~1.53</td>
<td>0.03~1.0</td>
<td>0.01~0.87</td>
</tr>
<tr>
<td>Displacement pressure/MPa</td>
<td>0.1~1.04</td>
<td>0.33~2.91</td>
<td>2.3~5.32</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Pore throat radius/µm</td>
<td>0.36~3.18</td>
<td>0.17~1.86</td>
<td>0.03~0.82</td>
<td>0.01~0.87</td>
</tr>
<tr>
<td>Sorting coefficient</td>
<td>1.1~2.5</td>
<td>1.28~2.89</td>
<td>1.8~3.0</td>
<td>1.9~3.6</td>
</tr>
<tr>
<td>Maximum Mercury Saturation/%</td>
<td>78.7~93.9</td>
<td>69.9~89.5</td>
<td>59.8~87.3</td>
<td>54.3~80.2</td>
</tr>
<tr>
<td>Skewness coefficient</td>
<td>-0.55~0.24</td>
<td>-0.69~0.16</td>
<td>-0.82~0.02</td>
<td>-1.01~0.04</td>
</tr>
<tr>
<td>Pore Structure</td>
<td>Intergranular pore</td>
<td>Intergranular pore, feldspar dissolved pores</td>
<td>Feldspar dissolved, pores intercrystalline pore</td>
<td>Pores intercrystalline pore, micropores</td>
</tr>
<tr>
<td>Number of samples</td>
<td>8</td>
<td>15</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>
Type IV structure mainly consists of absorption-micro throats. Its capillary pressure curve tilts towards the upper right corner of the chart with barely any leveling-out, and steady increase can be seen below SHg-50. Type IV provides the highest displacement pressure (>4 MPa) (Fig. 3). Its throat radius is distributed predominately below 0.2 µm, with a fine skewness (-1.01 ~ 0.04), a sorting coefficient of 1.9 ~3.6 (2.7 in average). The maximum mercury saturation ranges between 54.3% and 80.2%, 63.5% in average (Table 3). Relatively intensive compaction and carbonate cementation are observed. Clay minerals include predominately illite, plus high content of mica and matrix. Almost all intergranular pores were lost. Development of matrical micropores and intracrystalline micropores were observed, with moderate development feldspar vugular pores observed. Type IV represents an ineffective reservoir in terms of reserving and filtration capacities. It is mainly seen at sides of the distributary channel connecting the interdistributary bay, or in micro-facies like interdistributary bays.

Conclusively, Type I pore structure of the Chang-8 oil reservoir presents the best reserving and filtration capacities, with yet a lower extensiveness of development. Type II and Type III are more extensively distributed. Type IV is proven to be unavailable.

Movable Fluid Saturation Study by NMR. According to the categorized pore structures of the target strata, 4 pieces of sandstone samples of corresponding structural types were taken for NMR tests. Qualitative and quantitative assessments were made on the reservoir’s reserving capacity and microscopic pore structures, on basis of the features of movable fluid saturation and T2 values. Experiments suggested two types of NMR T2 spectra of Chang-8 oil reservoir, i.e. the single-peak type and the type with a higher left peak and a lower right peak (Fig. 4).

Detailed analyses were carried out on the 4 typical NMR samples. As shown in Table 4 and Fig. 4, the H2# sample belongs to the Type I reservoir, with a movable fluid saturation of 60.82%. The H2# sample presents a single-peak T2 spectrum, with its main peak located 10ms right to the T2 relaxation time, which suggests a relatively concentrated pore size distribution in the rock core, with intergranular pores significantly more developed than vugular pores and intracrystalline pores. It also provides a larger throat radius and excellent connectivity among throats. The H23# sample belongs to the Type II reservoir, with a movable fluid saturation of 44.37%. The H23# sample presents a dual-peak T2 spectrum, with the left peak higher than the right peak. The main peak is located 1~4ms within the T2 relaxation time, and the minor peak 40~100 ms within the relaxation time, suggesting a relatively scattered distribution of pore radius. Intergranular pores and vugular pores are dominant in number. Type II pores are relatively smaller than Type Is in terms of throat number and radius, yet with a relatively fair connectivity among throats. The A296# sample belongs to the Type III reservoir, with a movable fluid saturation of 23.9%. The A296# sample presents a single-peak T2 spectrum, with the left peak higher than the right peak. The main peak is located 1~4ms within the T2 relaxation time, and the minor peak 40~100 ms within the relaxation time, suggesting a relatively scattered distribution of pore radius. Intergranular pores and vugular pores are dominant in number. Type II pores are relatively smaller than Type Is in terms of throat number and radius, yet with a relatively fair connectivity among throats. The A296# sample belongs to the Type III reservoir, with a movable fluid saturation of 23.9%. The A296# sample presents a single-peak T2 spectrum, with the main peak located within 2~10ms of the T2 relaxation time, suggesting a relatively concentrated distribution of pore radius. Significant increase in intracrystalline pores is observed, yet no intergranular pore is observed. Decreased throat number and radius are observed, suggesting poor throat
connectivity. The H26# sample belongs to the Type IV reservoir, with a movable fluid saturation of 12.69%. The H26# sample presents a single-peak T2 spectrum, with the main peak located within 0.7~3ms of the T2 relaxation time, suggesting a relatively unified pore type. Significant decrease is observed in radius and number of effective throats, suggesting a poor throat connectivity. Significantly improved sorting of pore structures were observed, mainly intracrystalline pores and micropores.

It can be learnt from Fig. 3 and Fig. 4 that movable fluids mainly exist in major pores connected by effective throats; while movable fluids barely exist in throats and micropores, which provide the reserving space mainly for the bound fluid. The T2 value of the movable fluid is directly correlated to the throat radius in mercury injection, and its T2 spectrum is similar to the distribution of mercury saturation of its corresponding throat radius.

Analyses were carried out, comparing the T2 spectrum of movable fluids of samples taken from 4 wells with their corresponding distribution of throat radius (Fig. 4), under the natural circumstance with a lower limit of 0.1µm for movable throat radius [27]. It is learnt that in the Type I reservoir, infiltration is mainly contributed by fine-medium-sized throats, mainly 0.1µm~2µm in size; among them the throats sized over 0.1µm accounts for 72.6% of the effective movable throat space, and the throats sized 0.32µm~1.81µm provide over 90% of the infiltration capacity. In the Type III reservoir, infiltration is also contributed mainly by micro-sized throats, mainly 0.3µm~7µm in size; among them the throats sized over 0.1µm accounts for 53.6% of the effective movable throat space, and the throats sized 0.21µm~0.98µm provide over 90% of the infiltration capacity. In the Type III reservoir, infiltration is also contributed mainly by micro-sized throats, mainly around 0.01µm in size; among them the throats sized over 0.1µm accounts for 33.7% of the effective movable throat space, and the throats sized 0.04µm~0.2µm provide over 90% of the infiltration capacity. It is clear that movable fluid saturation mainly distributes in effectively connected throat spaces. The radius and distribution of effective throat are major control factors determining the saturation level and distribution features of the movable fluids.

The contribution curve of throat size to permeability (Fig. 4) suggests that the difference and peak value of mercury saturation is always lagged behind the contribution and peak value of permeability; while throat radius that contributes the most of the permeability does not contribute the most of the corresponding mercury injection volume. It can be deduced that, while permeability is contributed by a series of larger-sized throats, those contributing a larger share of permeability takes a relatively smaller size. Besides, with an increasing permeability among the samples, the throat radius corresponding to the peak contribution to permeability increases significantly, while the total contribution of all throats below the contribution peak shrinks gradually, suggesting that an increased permeability comes with an increased number of larger throats. Clearly it is a small number of larger-sized throats that contribute a major share of permeability of the ultralow/superlow-permeability sandstone reservoir.

**Influence of Pore Structure Parameters to Movable Fluid Saturation.** By comparing parameters of microscopic pore structures of the Chang-8 oil reservoir of Hujianshan oilfield with its NMR movable fluid saturation readings (Table 5), clear positive correlations can be found between the movable fluid saturation, and the median radius, the sorting coefficient, and the maximum mercury saturation (each with a correlation coefficient R2 of 0.2401, 0.6183 and 0.6468), along with a negative correlation with the displacement pressure, with an R2 of 0.5033. Thus one can conclude that, for a given reservoir, a higher movable fluid saturation requires a higher sorting coefficient, a larger throat radius, as well as more throats in number and larger connective spaces between effective throats. Besides, movable fluid saturation is positively correlated with physical properties, e.g. R²=0.8299 between saturation and permeability. The above facts suggest that permeability is mainly contributed by throats within the pore network, and throat radius is proven to be a critical parameter determining the movable fluid saturation of the target reservoir. Throat size can be used to directly represent the NMR T2 value (Fig. 4).

**TABLE 4**

**Statistics of Characteristic Parameters of Microscopic Pore Structures with Different Movable Fluid Distributions**

<table>
<thead>
<tr>
<th>Well ID</th>
<th>Depth/m</th>
<th>Porosity/%</th>
<th>Permeability/×10⁻³µm²</th>
<th>Displacement pressure/MPa</th>
<th>Sorting coefficient</th>
<th>Median radius/µm</th>
<th>Maximum Mercury Saturation/%</th>
<th>Movable fluid saturation/%</th>
<th>Movable fluid porosity/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>2585.2</td>
<td>15.7</td>
<td>1.69</td>
<td>0.4</td>
<td>2.1</td>
<td>0.23</td>
<td>90.52</td>
<td>60.82</td>
<td>9.43</td>
</tr>
<tr>
<td>H23</td>
<td>2375.5</td>
<td>11.3</td>
<td>0.55</td>
<td>0.33</td>
<td>2.2</td>
<td>0.12</td>
<td>90.64</td>
<td>44.37</td>
<td>4.63</td>
</tr>
<tr>
<td>A296</td>
<td>2197.6</td>
<td>11.9</td>
<td>0.5</td>
<td>0.4</td>
<td>2</td>
<td>0.26</td>
<td>89.3</td>
<td>23.9</td>
<td>2.53</td>
</tr>
<tr>
<td>H26</td>
<td>2199.9</td>
<td>5.9</td>
<td>0.05</td>
<td>3.58</td>
<td>1.5</td>
<td>0.02</td>
<td>83.4</td>
<td>12.69</td>
<td>0.69</td>
</tr>
</tbody>
</table>
Movable Fluid Distribution Characteristics and Corresponding Pore Structure Features of Chang-8 Oil Reservoir, Huijianshan Oilfield, Ordos Basin

(A-Cumulative mercury saturation; B-Ejection saturation; C-Permeability contribution value; D-Cumulative permeability contribution value; E-Pore-throat mercury saturation. a: NMR T2 spectra of the 4 samples; b: Type I, pore throat features of well H2#; c: Type II, pore throat features of well H23#; d: Type III, pore throat features of well A296; e: Type IV, pore throat features of well H26#)

<table>
<thead>
<tr>
<th>High pressure mercury intrusion experiments parameters</th>
<th>Correlation with movable fluid saturation R²</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Displacement pressure/MPa</td>
<td>0.5033</td>
<td>Linear</td>
</tr>
<tr>
<td>Median radius/µm</td>
<td>0.2401</td>
<td>Linear</td>
</tr>
<tr>
<td>Sorting coefficient</td>
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<td>Linear</td>
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<tr>
<td>Maximum Mercury Saturation %</td>
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<td>Linear</td>
</tr>
<tr>
<td>Permeability/x10⁻³µm²</td>
<td>0.8299</td>
<td>Linear</td>
</tr>
</tbody>
</table>
CONCLUSION

1) The Chang-8 oil reservoir in Hujianshan Oilfield is a tight lithologic deposit, mainly composed of pore cementation, typically featured with poor physical properties, suggesting a tight sandstone reservoir with ultralow porosity and superlow permeability. The reserving space is composed predominantly of intergranular pores, plus feldspar vugular pores in the second place.

2) The target stratum is categorized into 4 types in terms of pore structure on basis of varied capillary pressure curves and their corresponding characteristic parameters. Each type of pore structure represents a varied pattern of pores, with the infiltration and reserving capacities differed in a decreased order. It is found that Type II and Type III are the most extensively developed pore structures in the Chang-8 oil reservoir.

3) The Chang-8 oil reservoir in Hujianshan Oilfield is generally at a low level in terms of movable fluid saturation. The T2 spectra of Chang-8 are shown in both the single-peak and the dual-peak (higher left peak vs. lower right peak) patterns. The T2 value of the movable fluid changes at the same pace with the mercury injection throat radius, suggesting throat radius as a critical parameter that represents the reservoir’s movable fluid saturation. The pattern of the T2 spectrum is similar to the distribution of mercury saturation upon the corresponding throat radius. A relatively good correlation is observed between permeability and movable fluid saturation, suggesting permeability as an intuitive parameter that represents the infiltration capability of the pore structure.

ACKNOWLEDGEMENTS

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INTRODUCTION

Planktonic organisms are well-known as indicators of trophic state of aquatic environments, as they can quickly react to the changes of environmental conditions [1]. Zooplankton are represented mainly by Rotifera, Cladocera and Copepoda groups in freshwater ecosystems [2] and it is the second level of the food chain providing the energy flow between low and high levels [3]. Zooplankton is also the main food resource of invertebrates, fish larvae, fishes, and occasionally of aquatic birds [4, 5].

Despite significant ecological importance of zooplankton communities, they have usually been studied for diurnal/vertical migration aspect in strafited deep lakes [6, 7, 8, 9, 10]. The gap in the knowledge is even more dramatic for their ecological requirements in newly created reservoirs [11, 12], which have recently become very crucial for irrigation and drinking water supply all over the world, especially in temperate and sub-tropical regions including Turkey where the studies have mostly focussed on the determination of water quality, or the flora and fauna of several aquatic groups (e.g., phytoplankton, zooplankton, fish). Reservoirs have largely been responsible for water course changes, sediment transport, water quality and temperature variations, and introductions of non-native species, hence are considered unstable environments that suffer from eutrophication within a short time after they are established [13]. In this regard, an understanding of the ecological status of these artificial water bodies by determining dynamics of important biotic communities is highly significant [12]. Notably, the zooplankton community of a reservoir plays very important role, as they are major resource of energy and nutrient flow in the food web [14] and affected greatly by water level fluctuations and related environmental variations within a reservoir system. The aim of the present study was therefore to reveal ecological requirements of the zooplankton communities and indicator zooplankton species of trophic status in varying species richness in some reservoirs of Black Sea Region (Kocaeli Province, Turkey) that represented a gradient of environmental characters.

ABSTRACT

In the present study, eleven man-made reservoirs were visited between 17 and 20 June 2009 and 18 and 21 July 2010 to determine ecological requirements of zooplankton communities by major environmental variables (water temperature, dissolved oxygen concentration, pH, conductivity). The water temperature, dissolved oxygen concentration, pH, conductivity values during the study among the sites ranged between 15.6-28.3 °C; 7.94-13.4 mg L⁻¹; 8.07-8.66; and 219-464 (μS cm⁻¹), respectively. Major limnological variables showed significant differences both temporally (between years) (except for DO and EC) and spatially (i.e. sampling sites) (p < 0.05). A total of 62 taxa of zooplankton represented by 41 Rotifera, 11 Cladocera, and 10 Copepoda was identified. The most common species were rotifers Keratella cochlearis (Gosse) (11 sampling sites), Asplanchna priodonta (Gosse) and Synchaeta oblonga Ehrenberg, Polyarthra vulgaris (Carlin) (10 sampling sites), cladocerans Bosmina longirostris (Müller) and Chydorus sphaericus (Müller) (11 sampling sites) and copepod Acanthocyclops venustus (Norman & Scott) (6 sampling sites). Predominant and also most of the other identified species in the present study were known as eutrophication indicators. Species richness (as total number of species) of the sampling sites changed between 9 (Ütük reservoir) and 27 (.LUD\R÷OXUHVHUYRLU). Abundances of main taxonomic zooplankton groups were similar but varied temporally and spatially. Sampling sites were determined as β-mesosaprobic. Statistically, cladoceran C. sphaericus was determined as the indicator species throughout the study, which is common and resistant to the wide range conditions. The results of the present study are generally indicative of unpolluted waters. All of the identified zooplankton species are the first report for the studied water bodies.

KEYWORDS:
Rotifera, Cladocera, Copepoda, Water quality, Turkey
MATERIALS AND METHODS

Study area. Kocaeli Province is located in north-west Anatolia (30°21’ E, 40°31’ N) and is one of the most populated and industrialised regions of Turkey (Figure 1). There are many reservoirs established for mainly irrigation purposes and flood control but most importantly for drinking water supply. For instance, one of these reservoirs, Yuvacik Reservoir as the biggest one provides drinking water for approximately 1.6 million people living in Kocaeli Province. Most of other reservoirs in the province have similar sizes (from 0.2 to 1.6 km²) and water depths (~10 m) and are usually utilized for irrigation. The Mediterranean climate dominates in the region with warm and rainy winters, hot and dry summers, and a relatively high mean annual precipitation (768–1153 mm). Different size of agricultural activities and human-induced pollution are highly common in the province, which makes the water polluted and its budget low for natural and artificial water bodies.

FIGURE 1
The sampling area and reservoirs (1) Denizli; (2) Sevindikli; (3) Sipahiler; (4) Süverler; (5) Davuldere; (6) Ütük; (7) Çayırköy; (8) Arklar; (9) Ketenciler; (10) Kirazoğlu; (11) Yuvacik.

<table>
<thead>
<tr>
<th>Sampling stations</th>
<th>Locality</th>
<th>Coordinates</th>
<th>Sampling dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Denizli</td>
<td>40°53.55’ N</td>
<td>20.06.2009-21.05.2010</td>
</tr>
<tr>
<td>2</td>
<td>Sevindikli</td>
<td>40°53.20’ N</td>
<td>20.06.2009-21.05.2010</td>
</tr>
<tr>
<td>3</td>
<td>Sipahiler</td>
<td>40°52.15’ N</td>
<td>20.06.2009-21.05.2010</td>
</tr>
<tr>
<td>4</td>
<td>Süverler (Çağırgan)</td>
<td>40°54.23’ N</td>
<td>17.06.2009-18.05.2010</td>
</tr>
<tr>
<td>5</td>
<td>Davuldere</td>
<td>40°54.27’ N</td>
<td>17.06.2009-18.05.2010</td>
</tr>
<tr>
<td>6</td>
<td>Ütük</td>
<td>40°57.05’ N</td>
<td>17.06.2009-19.05.2010</td>
</tr>
<tr>
<td>7</td>
<td>Çayırköy</td>
<td>40°48.19’ N</td>
<td>17.06.2009-19.05.2010</td>
</tr>
<tr>
<td>8</td>
<td>Arklar</td>
<td>40°56.49’ N</td>
<td>17.06.2009-19.05.2010</td>
</tr>
<tr>
<td>9</td>
<td>Ketenciler</td>
<td>40°46.33’ N</td>
<td>18.06.2009-20.05.2010</td>
</tr>
<tr>
<td>10</td>
<td>Kirazoğlu</td>
<td>40°40.28’ N</td>
<td>18.06.2009-20.05.2010</td>
</tr>
<tr>
<td>11</td>
<td>Yuvacik</td>
<td>29°58.10’ E</td>
<td>19.06.2009-20.05.2010</td>
</tr>
</tbody>
</table>
Sample collection. The study was carried out in eleven different water resources between 17 and 20 June 2009 and 18 and 21 July 2010 (Table 1). Main physicochemical variables (water temperature, electrical conductivity, pH, and dissolved oxygen) were measured for all sampling stations in situ using the YSI 6820 Multi-parameter Instrument. Measured environmental variables were evaluated according to the [15] and Regulation of Water Pollution and Control (RWPC) issued by the Ministry of Environment and Forestry [16]. Zooplankton samples were collected at different points of the studied reservoirs with a 55μm pore sized plankton net, vertically. In each lake, all samples were representively taken at approximately 10 m depth before the dam and outflow, and center of the reservoir. After the samples taken in their bottles, the zooplankton samples were preserved with 4% formaldehyde solution. Zooplankton species were identified under a binocular microscope. Identification of the organisms were performed according to the following references: [17], [18] and [19]. Species richness was determined as the total number of species. Zooplankton abundance were calculated as individual per liter (ind. L⁻¹).

Statistical analyses. Species richness was given as a total number of species. To determine the saprobic index (S) of each study site by the sampling times following equation was used after [20]. The individual valences of the rotifers required on the saprobic index formula were used according to the [21].

\[
S = \frac{\sum (s \cdot h)}{a} 
\]

where:
- \( S \): the valence of each rotifer
- \( s \): the valence of each rotifer
- \( h \): the relative frequency
- \( a \): the accumulation of putrescible organic matter and its decomposition, varied between 60 ind. L⁻¹ (Davuldere reservoir in 2009) and 505 ind. L⁻¹ (Ketenciler reservoir in 2010).

The data were controlled for normal distribution (Shapiro-Wilk test) and for homogeneity. Non-parametric test (Kruskal-Wallis) was applied to determine main limnological (temperature, dissolved oxygen, pH, and conductivity) variables and taxonomic groups of zooplankton by stations because the data were not distributed normally. Also, analysis of variance (one way ANOVA) was applied to state significant differences of main limnological variables and taxonomic groups of zooplankton between study sites (11 sampling stations), and the Tamhane’s as the post-hoc test was used for the multiple comparisons of significant differences in one-way ANOVA. However, the differences of environmental conditions and taxonomic groups of zooplankton between two sampling years (2009/2010) was determined by using non-parametric Mann Whitney U test. These statistical analyses were performed using SPSS 19.0. To classify 11 reservoirs and identify indicator species in two different sampling dates, two-way indicator species analysis (TWINSPAN) was used as a divisive technique [22]. 0, 2, 5, 10 and 20% were set as pseudospecies cut levels and relative abundance of zooplankton were used in TWINSPAN, which was performed by the WinTWINS package program. Taxa that were represented ≤ 3 replicates in the dataset were excluded from the analyses.

RESULTS

The measured physicochemical variables during the study were presented in Table 2. Water temperature was determined between 15.6-28.3 °C (Table 2). Dissolved oxygen concentration of the study area was found in acceptable values (≥ 5 mg L⁻¹) for the aquatic life in general (DO = 7.94-13.4 mg L⁻¹, Table 2) [23]. pH ranged between 8.07-8.66 (Table 2), therefore it was in alkaline side with the suitable values for the accepted water quality criteria of aquatic environments by [15]. Conductivity ranged between 219-464 μS cm⁻¹ and positively correlated with the water temperature (Table 2). Also, the water quality classes of the studied sites according to the Regulation of Water Pollution and Control (RWPC) issued by the Ministry of Environment and Forestry [16] were given in Table 2. Accordingly, the water quality classes were determined as first class, except for Kirazoğlu, Davuldere and Ketenciler reservoirs (Table 2). Four main limnological variables (i.e. temperature, dissolved oxygen, pH and electrical conductivity) showed usually significant differences by sampling stations and dates (Table 3).

All of the studied sites were dominated by rotifer group during the two years sampling period (Figure 2). Total zooplankton abundance along the study period varied between 60 ind. L⁻¹ (Davuldere reservoir in 2010) and 505 ind. L⁻¹ (Ketenciler reservoir in 2009). Copepoda may not able to proceed up to 40 % of total zooplankton. Cladocera were not below 10 % of total zooplankton abundance without some exceptions, whereas rotifers were predominated all studied sites in all sampling periods generally (Figure 2).

Saprobic index, which was used to determine the accumulation of putrescible organic matter and its decomposition, varied between 1.43 (Sipahiler reservoir in 2009) and 2.22 (Ütük reservoir in 2010). This index allow to dependable identification of decomposition degrees via evaluate the BOD levels [24]. In terms of saprobic index values most of the studied sites in the present study were β-mesosaprobic. Saprobity values were significantly different both temporally and spatially (p < 0.01; t-test).
TABLE 2
Some physicochemical variables of the sampling stations; Water temperature (T), Dissolved Oxygen (DO), pH, Electrical Conductivity (EC), S (Saprobic index).

<table>
<thead>
<tr>
<th>Locality</th>
<th>No of sampling station</th>
<th>Years</th>
<th>T (°C)</th>
<th>DO (mg L⁻¹)</th>
<th>pH</th>
<th>EC (μS cm⁻¹)</th>
<th>Water Quality Classes*</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ütük Reservoir</td>
<td>1</td>
<td>June</td>
<td>26.1</td>
<td>8.98</td>
<td>8.17</td>
<td>319</td>
<td>I</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May</td>
<td>21.4</td>
<td>9.82</td>
<td>8.28</td>
<td>300</td>
<td>I</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>26.0</td>
<td>7.94</td>
<td>8.19</td>
<td>435</td>
<td>II</td>
<td>1.76</td>
</tr>
<tr>
<td>Kirazoğlu Reservoir</td>
<td>2</td>
<td>May</td>
<td>20.8</td>
<td>8.65</td>
<td>8.45</td>
<td>409</td>
<td>II</td>
<td>1.76</td>
</tr>
<tr>
<td>Denizli Reservoir</td>
<td>3</td>
<td>June</td>
<td>26.5</td>
<td>8.60</td>
<td>8.22</td>
<td>350</td>
<td>I</td>
<td>2.13</td>
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<tr>
<td></td>
<td></td>
<td>May</td>
<td>21.2</td>
<td>8.47</td>
<td>8.32</td>
<td>361</td>
<td>I</td>
<td>1.63</td>
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<td>Davulder Reservoir</td>
<td>4</td>
<td>June</td>
<td>26.3</td>
<td>8.14</td>
<td>8.11</td>
<td>402</td>
<td>II</td>
<td>1.80</td>
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<tr>
<td></td>
<td></td>
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<td>21.0</td>
<td>9.77</td>
<td>8.23</td>
<td>371</td>
<td>I</td>
<td>1.63</td>
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<tr>
<td>Ketenciler Reservoir</td>
<td>5</td>
<td>June</td>
<td>26.1</td>
<td>8.32</td>
<td>8.18</td>
<td>464</td>
<td>II</td>
<td>1.55</td>
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<td>21.1</td>
<td>8.78</td>
<td>8.42</td>
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<td>II</td>
<td>1.78</td>
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<tr>
<td>Yuvacik Reservoir</td>
<td>6</td>
<td>June</td>
<td>15.6</td>
<td>8.64</td>
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<td>I</td>
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<td>18.5</td>
<td>8.60</td>
<td>8.66</td>
<td>219</td>
<td>I</td>
<td>1.76</td>
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<td>Arklar Reservoir</td>
<td>7</td>
<td>June</td>
<td>25.2</td>
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<td>8.14</td>
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<td>380</td>
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<td>1.98</td>
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<td>24.8</td>
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<td>1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May</td>
<td>20.0</td>
<td>8.83</td>
<td>8.41</td>
<td>232</td>
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<td>1.62</td>
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<td>Çağırın (Süverler)</td>
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<td>June</td>
<td>28.3</td>
<td>10.09</td>
<td>8.26</td>
<td>326</td>
<td>I</td>
<td>1.58</td>
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<td>May</td>
<td>21.4</td>
<td>10.51</td>
<td>8.32</td>
<td>313</td>
<td>I</td>
<td>1.85</td>
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<td>June</td>
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<td>8.67</td>
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<td>324</td>
<td>I</td>
<td>1.43</td>
</tr>
<tr>
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<td>8.39</td>
<td>8.23</td>
<td>346</td>
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<td>1.68</td>
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<td>13.40</td>
<td>8.51</td>
<td>275</td>
<td>I</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>22.6</td>
<td>12.30</td>
<td>8.60</td>
<td>381</td>
<td>I</td>
<td>1.69</td>
</tr>
<tr>
<td>* Anonymous, 2004</td>
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</table>

TABLE 3
Statistical results of main limnological variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>By sampling locations (11 sites)</th>
<th>By sampling dates (2 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kruskal-Wallis</td>
<td>Mann Whithney U</td>
</tr>
<tr>
<td>Temperature</td>
<td>$\chi^2 (11) = 25.695; p = 0.007$</td>
<td>$U = 108.00, p = 0.000$</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>$\chi^2 (11) = 56.160; p &lt; 0.001$</td>
<td>$U = 550.00, p = 0.270$</td>
</tr>
<tr>
<td>pH</td>
<td>$\chi^2 (11) = 47.451; p &lt; 0.001$</td>
<td>$U = 298.50, p = 0.000$</td>
</tr>
<tr>
<td>Electrical Conductivity</td>
<td>$\chi^2 (11) = 61.459; p &lt; 0.001$</td>
<td>$U = 639.00, p = 0.919$</td>
</tr>
</tbody>
</table>

FIGURE 2
Temporal distribution of taxonomic zooplankton groups during the study periods: a-(ind. L⁻¹), b- (N %). (I: 2009; II: 2010, explanation of numbers (1-11) were given in Figure 1 and Table 1).
Both abundances of taxonomic zooplankton groups (rotifer, cladocera, copepod) and total zooplankton during the study period varied significantly amongst the sampling sites (Kruskal-Wallis; \( p < 0.05 \)) (Table 4). Also, according to the descriptive statistics (Tamhane’s) all spatial faunal data differed significantly from each other (One-way ANOVA, \( p < 0.05 \)). When zooplankton abundances analysed temporally, each group showed significant differences, except for copepods between years (Mann Whitney U, \( p < 0.05 \)) (Table 4).

The TWINSPAN divided the sampling stations with only some exceptions into two groups by the sampling dates, which denotes 2009 samples in left side and 2010 samples in right side of the figure (Figure 3). The indicator taxa for the 2009 samplings included *Chydorus sphaericus*, while rotifers *B. calyciflorus, Polyarthra vulgaris, Sychaeta oblonga* were the dominant species in 2009 (Figure 3). Also, rotifer *Trichocerca inermis* was the indicator species in Ütük (2009, 2010), Süverler (2009, 2010), Ketciciler (2009) and Denizli (2010) reservoirs, whereas copepod *Thermocyclops crassus* was the indicator species in other reservoirs for 2009 samplings and for Arkılar in 2010 (Figure 3). In 2010 only 7 stations could be sampled and rotifer *Cephalodella gibba* was determined as indicator species in Ketciciler, Sipahi, Yuvacık, Çayırköy, Kirazögli, Davulder and Sevindikli reservoirs (Figure 3).

During the study period, a total of 62 zooplankton species was determined (Table 5). The species belong to the Rotifera (41 taxa), Cladocera (11 taxa), and Copepoda (10 taxa) (Table 5). The availability of the identified species by the stations was shown in Table 6. The most common species for the study sites were rotifers *K. cochlearis* (11 sampling sites), *A. priodonta, P. vulgaris and S. oblonga* (10 sampling sites), cladocerans *B. longirostris and C. sphaericus* (11 sampling sites) and copepod *A. venustus* (6 sampling sites) (Table 5). Species richness of the sampling sites changed between 9 (Ütük) and 27 (Kirazögli) (Table 6).

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Statistical results of zooplankton taxonomic groups.</strong></td>
</tr>
<tr>
<td><strong>By sampling locations (11 sites)</strong></td>
</tr>
<tr>
<td>Abundance (ind. L(^{-1}))</td>
</tr>
<tr>
<td>Rotifer</td>
</tr>
<tr>
<td>Cladocera</td>
</tr>
<tr>
<td>Copepoda</td>
</tr>
<tr>
<td>Total Zooplankton</td>
</tr>
<tr>
<td>Ordo</td>
</tr>
<tr>
<td>----------------------</td>
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<tr>
<td>Adinetida</td>
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**TABLE 5**

Hierarchical classification of species.

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**TABLE 6**

Taxonomic classification of species.

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Taxonomic classification of species.

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**TABLE 8**

Taxonomic classification of species.
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. crassus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Species richness</strong></td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Total species richness</strong></td>
<td>9</td>
<td>27</td>
<td>18</td>
<td>15</td>
<td>18</td>
<td>19</td>
<td>11</td>
<td>17</td>
<td>11</td>
<td>22</td>
<td>16</td>
</tr>
</tbody>
</table>

*as total number of species.
DISCUSSION

In the present study, zooplankton fauna of 11 reservoirs were sampled in Kocaeli Province for the first time. Most reservoirs under study (Ütük, Kirazlı, Davuldere, Ketençiler, Arklar, Sevindikli, Çağırca, Sipahiiler, Çayrıköy) are being used for irrigation, and Yuvacık reservoir is the main drinking water source of the Kocaeli Province.

[25] stated that rotifers are the dominant group of the zooplankton in freshwaters. Our results are in agreement with the literature on this statement, as a total of 62 zooplankton species was determined in the present study, which were represented by 41 Rotifera, 11 Cladocera and 10 Copepoda species, respectively (Table 5 and 6). In many previous studies conducted in different areas (Table 7 and 8), Rotifera were also dominant zooplankton group (as the total number of species). Most of the species during the study (e.g. A. priodonsta, B. angularis, B. calyciflorus, B. quadridentatus, C. gibba, F. terminalis, K. cochlearis, L. (M.) closterocerca, L. patella, P. doli- choptera, P. vulgaris, T. patina, E. dilatata, A. rec- tangular, B. longirostris, C. sphaericus) are cosmopolitans, which can be found in aquatic macro vegetation [26, 27, 28, 29, 30, 31, 32, 33, 34].

Most members of the plankton can be used as indicators of the trophic status of reservoirs [2, 35, 36]. Further, [21] and [25] reported that rotifer quantity is the best biological indicators of the water quality. However, structure of rotifer fauna or presence of some indicator species is not itself enough to consider a water body as eutrophic, although density of the rotifer fauna is the characteristic of the eutrophic waters [37]. For this to be happened, data on abundances of rotifer species along with species richness (i.e. species diversity) should be collected in a longer sampling period [e.g. 38, 39, 40]. According to [41] cladoceran biomass contribute highly to the total zooplankton biomass in lakes, where the trophic states were low, and also their dominancy decrease with the increasing eutrophication. Furthermore, cyclopoid copepods are considered as characteristics of eutrophic environments [1].

Most frequent taxa in the present study (rotifers A. priodonta, B. angularis, B. calyciflorus, B. quadridentatus, C. cochlearis, L. patella, P. vulgaris, Trichocerca spp.; cladocerans A. rectangulara, B. longirostris, C. sphaericus and D. brachyuram, and also cyclopoid copepods) are commonly found in temperate regions, and they are known as indicators of eutrophication [42, 43, 44, 45, 27, 2, 35, 28, 18, 30, 31, 19, 25, 36, 46, 47, 48]. In the present study, rotifers dominated in all sampling times and sites with more than 50 % of total zooplankton (Figure 2). Although rotifer fauna seemed to indicate that studied water bodies in Kocaeli province can be classified as eutrophic, their saprobic index results identified as β-mesosabrobic, β-mesosabrobic conditions are attributed to moderately polluted but still high dissolved oxygen concentrations in aquatic environments [24]. These findings were corroborated with the Ith class water quality according to measured environmental variables.

Cladoceran C. sphaericus determined as the indicator species of the studied sites in the present study (TWINSPAN) was reported from many lakes and ponds with different trophic levels from oligotroto eutrophic [49,50]. Frequent encountering of C. sphaericus in different sampling points was based on its eurytopic character [51] and also wide physiological tolerance to the wide range of abiotic conditions such as pH, temperature, trophic conditions (Table 2.3 and 8) [52, 53, 50]. Limnological results in the present study suggested that studied sites are not eutrophic yet, but, when it is considered that C. sphaericus evaluate as a eutrophication indicator in temperate region lakes, these areas may be under a threat.

Table 7: Species richness of zooplankton fauna of reservoirs in different regions of Turkey.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of reservoirs</th>
<th>Number of studies</th>
<th>Rotifera</th>
<th>Cladocera</th>
<th>Copepoda</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmara</td>
<td>5</td>
<td>3</td>
<td>34</td>
<td>12</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>6</td>
<td>7</td>
<td>64</td>
<td>25</td>
<td>14</td>
<td>103</td>
</tr>
<tr>
<td>Aegean</td>
<td>5</td>
<td>4</td>
<td>59</td>
<td>23</td>
<td>12</td>
<td>94</td>
</tr>
<tr>
<td>Black Sea Region</td>
<td>2</td>
<td>1</td>
<td>23</td>
<td>11</td>
<td>8</td>
<td>42</td>
</tr>
<tr>
<td>Central Anatolia</td>
<td>18</td>
<td>19</td>
<td>106</td>
<td>28</td>
<td>16</td>
<td>150</td>
</tr>
<tr>
<td>Eastern Anatolia</td>
<td>9</td>
<td>13</td>
<td>69</td>
<td>15</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>Southeastern Anatolia</td>
<td>4</td>
<td>6</td>
<td>66</td>
<td>23</td>
<td>17</td>
<td>106</td>
</tr>
</tbody>
</table>

*as total number of species
### TABLE 8
Zooplankton species richness and some environmental variables of previously studied reservoirs in Turkey. (Ref: reference number)

<table>
<thead>
<tr>
<th>Study area</th>
<th>Species richness*</th>
<th>Temperature °C</th>
<th>pH</th>
<th>DO mg/L</th>
<th>EC μS/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankara/ Kesikkö</td>
<td>MR 9 4 2</td>
<td>20 6 4</td>
<td>20 8.80</td>
<td>8.20</td>
<td>-</td>
</tr>
<tr>
<td>Hatay/ Karamanlı</td>
<td>MR 13 2 2</td>
<td>20.7 7.60</td>
<td>5.25</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hatay/ Yarseli</td>
<td>MR 20 11 7</td>
<td>7.1-28.0</td>
<td>7.04-8.90</td>
<td>3.00-11.50</td>
<td>250-690</td>
</tr>
<tr>
<td>Adana/ Catalan</td>
<td>MD 10 6 4</td>
<td>25.1 8.94</td>
<td>7.84</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>Adana/ Vagizlar</td>
<td>MD 9 4 2</td>
<td>20 8.00</td>
<td>8.20</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adana/ Catalan</td>
<td>MD 8 2 10</td>
<td>14.30-30.78</td>
<td>7.82-8.20</td>
<td>5.18-7.54</td>
<td>-</td>
</tr>
<tr>
<td>Hatay/ Kampüs</td>
<td>MD 24 4 6</td>
<td>21.4-29.4</td>
<td>8.03-9.41</td>
<td>5.10-8.00</td>
<td>-</td>
</tr>
<tr>
<td>Osmaniye/ Aslantaş</td>
<td>MD 35 - 11</td>
<td>11.04-30.92</td>
<td>7.79-8.97</td>
<td>7.37-9.48</td>
<td>-</td>
</tr>
<tr>
<td>Adana/ Catalan</td>
<td>MD 25 - -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Eskişehir/ Kunduzlar</td>
<td>CAR 6 4 2</td>
<td>10.0-14.0</td>
<td>-</td>
<td>7.70-8.60</td>
<td>325-327</td>
</tr>
<tr>
<td>Eskişehir/ Çatören</td>
<td>CAR 7 5 2</td>
<td>9.0-19.0</td>
<td>-</td>
<td>8.08-10.0</td>
<td>231-316</td>
</tr>
<tr>
<td>Ankara/ Kesikköprü</td>
<td>CAR 16 3 -</td>
<td>9.1-21.5</td>
<td>7.40-8.50</td>
<td>7.10-10.30</td>
<td>-</td>
</tr>
<tr>
<td>Ankara/ Bıyıtepe</td>
<td>CAR 5 3 1</td>
<td>4.3-25.0</td>
<td>7.80-8.30</td>
<td>6.10-7.70</td>
<td>735-778</td>
</tr>
<tr>
<td>Ankara/ Kesikköprü</td>
<td>CAR 11 - -</td>
<td>4.0-24.0</td>
<td>7.20-8.60</td>
<td>8.20-12.0</td>
<td>-</td>
</tr>
<tr>
<td>Kayseri/ Samaskı</td>
<td>CAR 33 - -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ankara/ Kurtboğazı</td>
<td>CAR 15 7 4</td>
<td>4.0-24.0</td>
<td>7.59-9.19</td>
<td>4.50-10.10</td>
<td>187.3-212</td>
</tr>
<tr>
<td>Ankara/ Çokludere</td>
<td>CAR 15 6 3</td>
<td>4.0-26.0</td>
<td>7.69-8.67</td>
<td>6.30-10.20</td>
<td>137.3-166</td>
</tr>
<tr>
<td>Kırşehir/ Hırlandı</td>
<td>CAR 18 9 4</td>
<td>6.0-27.0</td>
<td>7.00-8.70</td>
<td>8.30-11.00</td>
<td>-</td>
</tr>
<tr>
<td>Ankara/ EğrekKayaya</td>
<td>CAR 7 4 2</td>
<td>3.6-24.2</td>
<td>7.10-9.00</td>
<td>1.80-12.30</td>
<td>129-204</td>
</tr>
<tr>
<td>Ankara/ Kesikköprü</td>
<td>CAR 12 9 8</td>
<td>4.1-24.1</td>
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<td>8.00-11.50</td>
<td>-</td>
</tr>
<tr>
<td>Kırşehir/ Hırlandı</td>
<td>CAR 7 4 3</td>
<td>2.9-27.0</td>
<td>7.00-8.63</td>
<td>5.14-15.40</td>
<td>20-2180</td>
</tr>
<tr>
<td>Yozgat/ Gelingüllü</td>
<td>CAR 54 9 2</td>
<td>11.2-23.5</td>
<td>8.33-9.51</td>
<td>6.13-11.12</td>
<td>**409.7</td>
</tr>
<tr>
<td>Ankara/ Sarsyar</td>
<td>CAR 24 9 4</td>
<td>4.3-31.0</td>
<td>7.12-14.00</td>
<td>4.70-15.50</td>
<td>475-890</td>
</tr>
<tr>
<td>Sivas/ Çokludere</td>
<td>CAR 11 7 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Kayseri/ Samaskı</td>
<td>CAR 33 10 2</td>
<td>4.0-25.0</td>
<td>7.00-9.30</td>
<td>5.60-13.00</td>
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<tr>
<td>Ankara/ Asaritepe</td>
<td>CAR 43 3 2</td>
<td>10.45-23.50</td>
<td>8.50-9.16</td>
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<td>306-450</td>
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<td>-</td>
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<tr>
<td>Eskişehir/ Porsuk</td>
<td>CAR 10 4 1</td>
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<td>7.60-9.40</td>
<td>526-603</td>
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<tr>
<td>Eskişehir/ Porsuk</td>
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<td>24.30</td>
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<td>1177</td>
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<tr>
<td>Eskişehir/</td>
<td>Asaçıkçukrubinli</td>
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<td>-</td>
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<tr>
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<td>20.80</td>
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<td>9.37</td>
<td>139</td>
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<td>21.70</td>
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</tr>
<tr>
<td>Ankara/ Çubuk</td>
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<td>20.30</td>
<td>9.24</td>
<td>7.16</td>
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</tr>
<tr>
<td>Elazığ/ Keban (Güllüler)</td>
<td>EAR 27 - -</td>
<td>7.00-27.00</td>
<td>7.50-9.60</td>
<td>3.90-11.90</td>
<td>-</td>
</tr>
<tr>
<td>Elazığ/ Cip</td>
<td>EAR 15 - -</td>
<td>2.90-27.00</td>
<td>7.60-8.50</td>
<td>7.80-12.10</td>
<td>-</td>
</tr>
<tr>
<td>Elazığ/ Keban (Čemisgezek)</td>
<td>EAR 17 - - -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Elazığ/ Keban (Pertek)</td>
<td>EAR 20 - - -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Elazığ/ Kepektas</td>
<td>EAR 11 - -</td>
<td>7.00-25.00</td>
<td>7.90-8.50</td>
<td>5.70-11.20</td>
<td>-</td>
</tr>
<tr>
<td>Elazığ/ Keban (Güllüler)</td>
<td>EAR 23 - -</td>
<td>6.30-24.20</td>
<td>7.20-8.20</td>
<td>4.60-9.80</td>
<td>-</td>
</tr>
<tr>
<td>Malatya/ Karakaya</td>
<td>EAR 19 - -</td>
<td>7.00-20.00</td>
<td>7.10-7.90</td>
<td>7.10-10.30</td>
<td>-</td>
</tr>
<tr>
<td>Van/ Zemek</td>
<td>EAR 13 2 2</td>
<td>8.30-25.00</td>
<td>7.20-8.20</td>
<td>-</td>
<td>370-398</td>
</tr>
<tr>
<td>Malatya/ Sürmii</td>
<td>EAR 31 10 6</td>
<td>8.00-24.00</td>
<td>6.90-9.10</td>
<td>7.00-11.20</td>
<td>-</td>
</tr>
<tr>
<td>Elazığ/ Kalesıck</td>
<td>EAR 25 11 4</td>
<td>6.10-27.20</td>
<td>7.20-8.00</td>
<td>6.20-10.30</td>
<td>-</td>
</tr>
<tr>
<td>Tunceli/ Uzanayar</td>
<td>EAR 15 6 2</td>
<td>8.90-24.80</td>
<td>6.60-8.90</td>
<td>7.40-11.30</td>
<td>-</td>
</tr>
<tr>
<td>Elazığ/ Beyhan</td>
<td>EAR 24 5 3</td>
<td>7.10-20.10</td>
<td>6.80-8.10</td>
<td>4.60-10.10</td>
<td>-</td>
</tr>
<tr>
<td>Diyarbakır/ Devegeçidi</td>
<td>SAR 33 - -</td>
<td>3.00-28.00</td>
<td>7.60-8.60</td>
<td>4.20-12.50</td>
<td>-</td>
</tr>
<tr>
<td>Diyarbakır/ Güksu</td>
<td>SAR 28 16 3</td>
<td>5.00-27.00</td>
<td>7.56-8.65</td>
<td>6.00-13.00</td>
<td>-</td>
</tr>
<tr>
<td>Diyarbakır/ Devegeçidi</td>
<td>SAR - 12 5</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>
locations. Also, other reason for the rare distribution quantity, and geographical features of the sampling quality characteristics, available food quality and (36 taxa). This could be attributed to other water previous studies (163 taxa) and in Kocaeli Province of occurrence among the sites

However, a large number of rare species (frequency temperature, DO, pH, EC) were significantly found that Central Anatolia was the richest with 150 taxa of some species among the stations may be related to water temperature, which is the limiting factor of zo-

plankton abundance and distribution [110] and can

Some essential environmental variables (water temperature, DO, pH, EC) were significantly found relevant to zooplankton presence and they generally showed similarity among the sites (Table 2 and 8). However, a large number of rare species (frequency of occurrence among the sites ≤5) was found in the previous studies (163 taxa) and in Kocaeli Province (36 taxa). This could be attributed to other water quality characteristics, available food quality and quantity, and geographical features of the sampling locations. Also, other reason for the rare distribution of some species among the stations may be related to water temperature, which is the limiting factor of zooplankton abundance and distribution [110] and can be varied due to different sampling times and the number of sampling. Among-region comparison of zooplankton taxa of reservoirs in Turkey revealed that Central Anatolia was the richest with 150 taxa (Table 7). Common species of all studied localities are B. angularis, E. dilatata, K. cochlearis, P. dolichoptera, T. (D.) similis, B. longirostris, and D. cucullata. These species are the indicators of productive habitats [44,28], and P. dolichoptera and K. cochlearis are perennial species [28]. On the other hand, eutrophic aquatic environments of the same re-

cophtera


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FRUITS, SEEDS AND POLLEN MORPHOLOGY OF ALYSSUM L. (BRASSICACEAE) AND THEIR TAXONOMIC VALUE

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ABSTRACT

In the current study, macro- and micromorphological characters of fruits, seeds, and pollen in Alyssum L. (14 to 19 species) were examined by stereo, light (LM) and scanning electron microscope (SEM) to evaluate the taxonomic significance of these characters. Detailed description of features of Turkish Alyssum species are provided with illustrations. Generally, the pollen grains are radially symmetrical and isopolar. The fruit surface is glabrous or stellate trichome. The seeds are ovate, spheroidal or oblong in shape among the studied species. The colors of the seed vary between light brown, dull yellow-brownish, bright red brownish, dark brownish, dull light reddish, bright brownish, bright red brownish, dull brownish and dull yellowish. The surface of the seed is reticulate (normal reticulate, reticulate-foveate, reticulate-lineolate, reticulate-rugose), ruminate, rugose, lineolate, blister and colliculate in SEM. The pollen grains of the genus are subprolate or prolate. The pollen grains are tricolpate. Exine ornamentations are mainly reticulate. Ornamentations, pollen shape and size, aperture type, colpus length and width, fruit and seed shape, size and color, seed anti and periclinal walls have been observed as significant morphological characters.

KEYWORDS:
Alyssum L., Fruit, Pollen, Seed, Light microscope, Turkey.

INTRODUCTION

The Brassicaceae or Cruciferae family is a monophyletic group of about 325 genera and over 3740 species distributed worldwide [1]. Turkey has at least 606 Brassicaceae species, of which 226 are native [2, 3, 4]. The genus Alyssum L. is one of the largest genera, which includes about 195 species in the world with main distribution in Turkey and Eastern Europe [5]. There are about 100 species of the genus Alyssum in Turkey [6].

Industrialization, urbanization and due to the rapid growth of the world population has caused heavy metal pollution so that caused global problem over time [7, 8, 9]. The use of plants for the cleaning and remediation of contaminated areas is a safe way of ensuring natural balance [9, 10]. Many wild Brassicaceae species are known to hyperaccumulate heavy metals and have genes for tolerance to the toxic effects of a broad range of metals [11]. Alyssum species can grow in very high metal concentration soils. High Ni levels cause toxic effects on seed germination [12]. The effects of nickel on seed germination, root and hypocotyl elongation of the nickel hyperaccumulator some Alyssum species demonstrated in the study carried out by Pavlova et al. [12] distributed on the Albania. The determination of heavy metal distribution in plants is one of the most exert attribute related to environmental remediation [13]. Soil metal concentration ranges were reported from the studies carried out by Sağlam [14] and Bani et al. [15]. Branković et al. [16] reported that exhibit different metal concentration in Alyssum species, depending on kind of metals and plant species. Hyperaccumulator Alyssum species could be successfully used for the further biogeochemical prospecting and environmental monitoring [17]. Furthermore, some Alyssum species can be used as a precursor in erosion studies because they are not selective in terms of drought or soil requirements [18].


Pollen morphology of some Alyssum species was investigated by İnceoğlu and Karamustafa [26]. Khan [27] studied the pollen morphology 7 species of the Alyssum. Orcan and Binzet [28] investigated
TABLE 1
Collection data of *Alyssum* L. species examined for this study.

<table>
<thead>
<tr>
<th>Collector Number</th>
<th>Taxon</th>
<th>Gps</th>
<th>Altitude (m)</th>
<th>Collection Date</th>
<th>Palynogeographic Region</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>4807</td>
<td><em>A. murale</em> Waldst. &amp; Kt. subsp. murale var. alpinum Boiss. ex Nyár.</td>
<td>K 39 28 01.7</td>
<td>D 039 29 43.7</td>
<td>2100</td>
<td>2014</td>
<td>Irano-Turanian Endemic</td>
</tr>
<tr>
<td>5938</td>
<td><em>A. virgatum</em> Nyár.</td>
<td>K 39 23 46.9</td>
<td>D 039 29 11.4</td>
<td>2133</td>
<td>2014</td>
<td>Endemic</td>
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<tr>
<td>5588</td>
<td><em>A. peltaroides</em> Boiss. subsp. virgatiforme (Nyár.) T.R.Dudley</td>
<td>K 39 31 41.4</td>
<td>D 039 53 26.2</td>
<td>1900</td>
<td>2014</td>
<td>Endemic</td>
</tr>
<tr>
<td>4381</td>
<td><em>A. condensatum</em> Boiss. &amp; Hausskn. subsp. flexile(Nyár) T.R.Dudley</td>
<td>39° 26'07.7&quot;</td>
<td>36° 50'26.2&quot;</td>
<td>1750</td>
<td>2014</td>
<td>- -</td>
</tr>
<tr>
<td>5907</td>
<td><em>A. murale</em> Waldst. &amp; Kt. subsp. muralevar. haradjani (Rech.) T.R.Dudley</td>
<td>K 39 21 30.6</td>
<td>D 039 20 52.2</td>
<td>1400</td>
<td>2014</td>
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<tr>
<td>4869</td>
<td><em>A. pateri</em> Nyár.</td>
<td>K 39 2648.4</td>
<td>D 040 08 39.3</td>
<td>2080</td>
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<tr>
<td>7964</td>
<td><em>A. corsicum</em> Duby</td>
<td>37° 03'06.4&quot;</td>
<td>28° 55'20.1&quot;</td>
<td>1100</td>
<td>2017</td>
<td>E.mediter-ranean</td>
</tr>
<tr>
<td>7356</td>
<td><em>A. caricium</em> T.R.Dudley &amp; Hub. Mor.</td>
<td>36° 59'32.4&quot;</td>
<td>28° 39'15.3&quot;</td>
<td>7</td>
<td>2017</td>
<td>Endemic</td>
</tr>
<tr>
<td>7794</td>
<td><em>A. leptidotum</em> Boiss.</td>
<td>37° 19'1.6&quot;</td>
<td>28° 22'27.4&quot;</td>
<td>1618</td>
<td>2017</td>
<td>Endemic</td>
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<td>37° 05'30.9&quot;</td>
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<td>2017</td>
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<td>28° 53'43.0&quot;</td>
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<td>2017</td>
<td>Endemic</td>
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<tr>
<td>4123</td>
<td><em>A. callicloum</em> Boiss. &amp; Balansa</td>
<td>K 38 51 54.5</td>
<td>D 039 39 02.0</td>
<td>1250</td>
<td>2014</td>
<td>Endemic</td>
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<tr>
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<td><em>A. discolor</em> T.R.Dudley &amp; Hub. Mor.</td>
<td>36° 52'25.8&quot;</td>
<td>28° 16'29.6&quot;</td>
<td>81</td>
<td>2017</td>
<td>E.mediter-ranean</td>
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<tr>
<td>4274</td>
<td><em>A. ochroleucum</em> Boiss. &amp; A. Huet</td>
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<td>D 039 28 36.5</td>
<td>2120</td>
<td>2014</td>
<td>Endemic</td>
</tr>
<tr>
<td>7353</td>
<td><em>A. sibiricum</em> Wild.</td>
<td>37° 12'53.1&quot;</td>
<td>28° 23'4.3&quot;</td>
<td>736</td>
<td>2017</td>
<td>- -</td>
</tr>
<tr>
<td>6803</td>
<td><em>A. strigoss</em> Banks &amp; Sol.</td>
<td>37° 52'31.1&quot;</td>
<td>41° 44'1.3&quot;</td>
<td>726</td>
<td>2017</td>
<td>- -</td>
</tr>
<tr>
<td>7673</td>
<td><em>A. baumgartnerianum</em> Bornm. Ex Baumg.</td>
<td>37° 05'14.1&quot;</td>
<td>28° 49'35.0&quot;</td>
<td>1985</td>
<td>2017</td>
<td>- -</td>
</tr>
<tr>
<td>4477</td>
<td><em>A. simplex</em> Rudolph</td>
<td>K 39 00 19.1</td>
<td>D 039 11 24.1</td>
<td>1350</td>
<td>2014</td>
<td>- -</td>
</tr>
</tbody>
</table>

on *Alyssum floribundum* morphological, anatomical and palynological. Pakravan et al. [29] investigated bio systematically on the four varieties of *Alyssum minus* in Iran. Pavlova et al. [30] gathered information on pollen morphology and localization of Ni in stamens and pollen grains of eight Ni hyperaccumulator *Alyssum* taxa. Pnar et al. [31] examined pollen and seed morphology of 35 specimens representing 25 Turkish species of the taxonomically complex genus *Hesperis* L. by light and scanning electron microscopes. The structure of the seed coat is less influenced by the external environmental conditions, therefore it is a more stable character for evolutionary and taxonomic studies [32, 33, 34, 35]. Bona [36] investigated the seed exomorphic characteristics of 14 taxa of *Lepidium* L. in Brassicaceae. Hani et al. [37] studied production and morphological characteristics of seeds in Brassicaceae from Algeria. Karcz et al. [38] investigated micromorphological typing of seed surfaces using scanning electron microscopes.
RESULTS

The fruit, seed and pollen morphological characters for 14 to 19 species of the genus Alyssum are summarized in Tables 1–4 and image by SEM, stereomicroscope and light microscopy are showed in Figures 1–13.

Fruit Characters. Fruit shapes of the studied taxa are found to be orbicular, ovoid, elliptical or obcordate. Most common shape is elliptical seen in 6 taxa. The fruit size ranges from 1.67 to 7.41 mm in length and from 0.95 mm to 7.61 mm in width. The smallest fruits (A. callichrosum) have 1.67 mm length and 0.95 mm width. The largest fruits (A. virgatum) have 7.61 mm length and 7.61 mm width (Table 2). Most studied species have trichome on surface of silicules. A. virgatum, A. peltariaoides, A. corsicum, A. caricum, A. masmeneaum and A. discolor have glabrous on surface of silicules.

Seed Characters. The characters of seed of the examined Alyssum species are summarized in Table 3 and 4 by using stereomicroscope and SEM data and show in Figs 2 and 3.

The shape of seed varies from elliptical to spheroidal. Seeds are spheroidal in A. virgatum, A. lepidotum, A. strictum and A. strigosum, oblongin A. peltariaoides subsp. virgatiforme, and A. ochroleucum. Seed size also varies among the examined species; the largest ovate seeds in A. peltariaoides subsp. virgatiforme have a diameter of 2.04 mm length and 1.73 mm width the smallest seeds 0.6 mm length and 0.36 mm width in A. sibiricum, while the rest of the species have slightly larger seeds. The colors of the seeds give a supplemental indication for distinguished between species of Alyssum. Seed colour of examined taxa are found to be yellow or brown. However most of the seeds colour varies from light yellowish to dark brownish. Hilum position of examined species is mostly terminal. A. murale subsp. murale var. alpinum, A. peltariaoides subsp. virgatiforme, A. corsicum, A. caricum, A. masmeneaum, A. ochroleucum are subterminal. In addition hilum size can be one of the diagnostic characters in Alyssum species. The hilum size among the examined species showed wide range of variations. Except A. condensatum subsp. flexibile and A. sibiricum all examined taxa have wing of seed. Seed wings are generally prominent and relatively wide. The size of wings ranges from 0.05 to 0.97 mm in length.

MATERIALS AND METHODS

The specimens were collected from natural populations in Turkey. A list of taxa and full voucher data is provided in Table 1. Macro and microphotographs which showed the general view of fruit, seed, and pollen surface were taken by JeolTescan model electron microscope in Bartın University Central Research Laboratory.

For SEM, specimens were mounted directly onto stubs, using single-sided adhesive tape, and coated with gold. Measurements and optical observations of fruit and seed colours were carried out under aOlympus SZ2- LGB stereomicroscope. Photographs were taken with digital imaging system. Pollen slides were prepared using the method of Wodehouse [48], and photographed by the Leica DM 750 digital imaging system. The mean and standard deviations of the measurements were calculated. The terms used for describing the seed surface patterns and pollen morphology adopted by [49, 50, 51, 52].


Garb et al. [46] reported fruit, seed morphology and seed coat sculpturing were recorded for 10 species belongs to 9 genera and five tribes of Brassicaceae in eastern region of Saudi Arabia. Satil et al. [47] detailed in 9 genera and five tribes of Brassicaceae in eastern region of Saudi Arabia. Satil et al. [47] detailed investigated of fruit, seed and pollen macro and micromorphological structures of Turkish Chorispora species.

Understanding and explain the evolutionary relationships among species of Brassicaceae is difficult with the naked eye or lens. It is the reason why micromorphological structures have been observed on surface of the seeds, trichome types, fruit shape and pollen morphology. The present study aimed to examine Alyssum species from Turkey based on macro and micro morphology of fruit, seed coat, and pollen features by using scanning electron microscopy (SEM), stereo and light microscopy and to provide new insight into its potential taxonomic value.

Fruit Characters. Fruit shapes of the studied taxa are found to be orbicular, ovoid, elliptical or obcordate. Most common shape is elliptical seen in 6 taxa. The fruit size ranges from 1.67 to 7.41 mm in length and from 0.95 mm to 7.61 mm in width. The smallest fruits (A. callichrosum) have 1.67 mm length and 0.95 mm width. The largest fruits (A. virgatum) have 7.61 mm length and 7.61 mm width (Table 2). Most studied species have trichome on surface of silicules. A. virgatum, A. peltariaoides, A. corsicum, A. caricum, A. masmeneaum and A. discolor have glabrous on surface of silicules.

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### TABLE 2
Fruit morphology of *Alyssum* (values in mm)

<table>
<thead>
<tr>
<th>Fruit length</th>
<th>Fruit width</th>
<th>Shape</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Std Min Max</td>
<td>M Std Min Max</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. murale subsp. murale var. alpinum</em></td>
<td>3.51 0.17 3.19 3.74 2.84 0.35 2.35 3.32</td>
<td>Obcordate</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. virgatum</em></td>
<td>5.76 0.36 5.04 6.7 7.61 0.6 6.3 8.67</td>
<td>Ovoid</td>
<td>Glabrous</td>
</tr>
<tr>
<td><em>A. peltariorum subsp. virgaformum</em></td>
<td>7.41 0.88 4.48 8.74 4.86 1.01 0.49</td>
<td>Glabrous</td>
<td></td>
</tr>
<tr>
<td><em>A. bulbatarum</em></td>
<td>5.55 0.38 5.12 5.83 4.82 0.28</td>
<td>Ovate</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. condensatum subsp. flexibile</em></td>
<td>3.27 0.08 3.17 3.37 1.49 0.16</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. murale</em></td>
<td>2.94 0.17 2.64 3.42 2.68 0.2</td>
<td>Orbicular</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. corsicum</em></td>
<td>1.98 0.34 1.15 2.47 1.41 0.27</td>
<td>Ovoid</td>
<td>Glabrous</td>
</tr>
<tr>
<td><em>A. caricarum</em></td>
<td>5.8 0.32 5.05 6.41 5.46 0.44</td>
<td>Ovadate</td>
<td>Glabrous</td>
</tr>
<tr>
<td><em>A. lepidotum</em></td>
<td>4.56 0.57 3.61 5.48 4.81 0.81</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. massemense</em></td>
<td>5.48 0.44 4.41 6.26 3.02 0.25</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. calliclormum</em></td>
<td>1.67 0.49 1.15 2.93 0.95</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. discolor</em></td>
<td>4.23 0.51 2.85 4.92 2.2</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. ochroleucum</em></td>
<td>2.62 0.33 2.3 3.1 2</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. sibiricum</em></td>
<td>3.17 0.11 2.98 3.53 2.68</td>
<td>Ovadate</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. stipitum</em></td>
<td>3.92 0.2 3.35 4.33 2.35 0.2</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. stigmosum</em></td>
<td>3.7 0.33 3.31 4.04 4.31</td>
<td>Orbicular</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. patens subsp. prostratum</em></td>
<td>3.2 0.18 2.72 3.47 1.89</td>
<td>Elliptical</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. baumgartenianum</em></td>
<td>4.64 0.42 4.19 5.03 3.48</td>
<td>Orbicular</td>
<td>Hairy</td>
</tr>
<tr>
<td><em>A. simplex</em></td>
<td>4.17 0.28 3.7 4.6 3.94</td>
<td>Orbicular</td>
<td>Hairy</td>
</tr>
</tbody>
</table>

*M: Mean, Std: Standard Deviation, Min: Minimum, Max: Maximum*

### TABLE 3
Seed morphology of *Alyssum* (values in mm)

<table>
<thead>
<tr>
<th>Fruit length</th>
<th>Fruit width</th>
<th>Shape</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Std Min Max</td>
<td>M Std Min Max</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. murale subsp. murale var. alpinum</em></td>
<td>0.28 0.42 0.25 0.17</td>
<td>0.47 0.21 0.42</td>
<td>0.48 0.15</td>
</tr>
</tbody>
</table>

*M: Mean, Std: Standard Deviation, Min: Minimum, Max: Maximum*
FIGURE 1
Stereomicroscope micrographs of fruit in *Alyssum* species.

FIGURE 2
Scanning electron micrographs (SEM) of fruit in *Alyssum* species.
FIGURE 3

Scanning electron micrographs (SEM) of fruit in *Alyssum* species.
FIGURE 4
Scanning electron micrographs (SEM) of fruit in *Alyssum* species.
The surface ornamentation, periclinal and anticlinal walls of seeds are studied by SEM. Examination species demonstrated 6 different types of seed coat pattern. The most common types is reticulate (Figure 6-10).


2) Ruminate in *A. bulbotrichum* and *A. strictum*.

3) Rugose in *A. discolor* and *A. baumgartnerianum*.

4) Lineolate in *A. murale*.

5) Blister in *A. lepidotum*.

6) Colliculate in *A. masmenaeum*.
FIGURE 6
Stereomicroscopic micrographs of seed in *Alyssum* species.
FIGURE 7
Scanning electron micrographs (SEM) of seed in Alyssum species.
1-3A. murale subsp. murale var. alpinum, 4-6A. virgatum, 7-9A. peltarioides subsp. virgatiforme, 10-12A. bulbosichum, 13-15A. condensatum subsp. flexible.
FIGURE 8
Scanning electron micrographs (SEM) of seed in *Alyssum* species.
FIGURE 9
Scanning electron micrographs (SEM) of seed in *Alyssum* species.
**FIGURE 10**
Scanning electron micrographs (SEM) of seed in *Alyssum* species.

**TABLE 4**
The seed morphological characters as seen by LM and SEM between taxa of *Alyssum*

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Fig No</th>
<th>Seed Shape</th>
<th>Ratio L/W</th>
<th>Seed Colour</th>
<th>Seed Margin</th>
<th>Hilum Position</th>
<th>Seed Surface</th>
<th>Anticlinal</th>
<th>Periclinal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. murale</em> subsp. <em>murale</em> var. <em>alpinum</em></td>
<td>1</td>
<td>Ovate</td>
<td>1.38</td>
<td>Light Brown</td>
<td>Winged Subterminal</td>
<td>Reticulate Lineolate</td>
<td>Raised Flat</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. virgatum</em></td>
<td>2</td>
<td>Spherical</td>
<td>1.12</td>
<td>Dull Yellow Brownish</td>
<td>Winged Terminal</td>
<td>Reticulate-Foveate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pektinaroides</em> subsp. <em>virgataforme</em></td>
<td>3</td>
<td>Oblong</td>
<td>1.17</td>
<td>Bright Red Brownish</td>
<td>Winged Subterminal</td>
<td>Reticulate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. bulbosichium</em></td>
<td>4</td>
<td>Ovate</td>
<td>1.39</td>
<td>Dark Brownish</td>
<td>Winged Terminal</td>
<td>Reticulate-Foveate</td>
<td>Raised Indefinite</td>
<td>Concave</td>
<td></td>
</tr>
<tr>
<td><em>A. condensatum</em> subsp. <em>flexible</em></td>
<td>5</td>
<td>Ovate</td>
<td>1.55</td>
<td>Dull Light Reddish</td>
<td>Wingless Terminal</td>
<td>Reticulate-Foveate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. murale</em></td>
<td>6</td>
<td>Ovate</td>
<td>1.53</td>
<td>Bright Brownish</td>
<td>Winged Terminal</td>
<td>Lineolate</td>
<td>Sunken Convex</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. corsicum</em></td>
<td>7</td>
<td>Ovate</td>
<td>1.31</td>
<td>Bright Red Brownish</td>
<td>Winged Subterminal</td>
<td>Reticulate-Foveate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. caricum</em></td>
<td>8</td>
<td>Ovate</td>
<td>1.64</td>
<td>Bright Brownish</td>
<td>Winged Subterminal</td>
<td>Reticulate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. lepidotum</em></td>
<td>9</td>
<td>Spherical</td>
<td>1.08</td>
<td>Dull Brownish</td>
<td>Winged Terminal</td>
<td>Blister</td>
<td>Sunken Convex</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. massenianum</em></td>
<td>10</td>
<td>Ovate</td>
<td>1.05</td>
<td>Dull Red Brownish</td>
<td>Winged Subterminal</td>
<td>Colliculate</td>
<td>Sunken Convex</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. discolor</em></td>
<td>11</td>
<td>Ovate</td>
<td>2.02</td>
<td>Dull Yellowish</td>
<td>Winged Terminal</td>
<td>Rugose</td>
<td>Indefinite</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. ochroleucum</em></td>
<td>12</td>
<td>Oblong</td>
<td>1.69</td>
<td>Dull Yellow Brownish</td>
<td>Winged Subterminal</td>
<td>Reticulate-Rugose</td>
<td>Sunken Indefinite</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. sibiricum</em></td>
<td>13</td>
<td>Ovate</td>
<td>1.57</td>
<td>Dull Yellowish</td>
<td>Wingless Terminal</td>
<td>Reticulate-Foveate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. strictum</em></td>
<td>14</td>
<td>Spherical</td>
<td>1.22</td>
<td>Dull Yellow Brownish</td>
<td>Winged Terminal</td>
<td>Reticulate-Foveate</td>
<td>Indefinite</td>
<td></td>
<td></td>
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<tr>
<td><em>A. strigosum</em></td>
<td>15</td>
<td>Spherical</td>
<td>1.18</td>
<td>Bright Yellow Brownish</td>
<td>Winged Terminal</td>
<td>Reticulate-Foveate</td>
<td>Raised Concave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pateri</em> subsp. <em>prostratum</em></td>
<td>16</td>
<td>Ovate</td>
<td>1.55</td>
<td>Bright Brownish</td>
<td>Winged Terminal</td>
<td>Reticulate</td>
<td>Raised Flat</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. baumgartnerianum</em></td>
<td>17</td>
<td>Ovate</td>
<td>1.86</td>
<td>Dull Brownish</td>
<td>Winged Terminal</td>
<td>Rugose</td>
<td>Indefinite</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. simplex</em></td>
<td>18</td>
<td>Ovate</td>
<td>1</td>
<td>Dark Brownish</td>
<td>Winged Terminal</td>
<td>Reticulate-Rugose</td>
<td>Raised Indefinite</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pollen morphology of *Alyssum* (values in μm)

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>Polar axes</th>
<th>Equatorial axes</th>
<th>Colpus length</th>
<th>Exine thickness</th>
<th>Intine thickness</th>
<th>Apocolpium</th>
<th>Mesocolpium</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>20.3</td>
<td>14.4</td>
<td>14.6</td>
<td>0.97</td>
<td>0.43</td>
<td>3.71</td>
<td>4.31</td>
</tr>
<tr>
<td>Std</td>
<td>3.75</td>
<td>3.02</td>
<td>3.97</td>
<td>0.21</td>
<td>0.14</td>
<td>0.72</td>
<td>1.04</td>
</tr>
<tr>
<td>Min</td>
<td>14.5</td>
<td>10.1</td>
<td>10.7</td>
<td>0.72</td>
<td>0.35</td>
<td>1.3</td>
<td>2.85</td>
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<tr>
<td>Max</td>
<td>24.1</td>
<td>15.4</td>
<td>15.6</td>
<td>1.57</td>
<td>0.58</td>
<td>2.67</td>
<td>6.01</td>
</tr>
</tbody>
</table>

Polar axes: M = Mean, Std: Standard Deviation, Min: Minimum, Max: Maximum

The pollen characters of the examined *Alyssum* species are summarized in Table 4 by using light microscope and SEM data, and shown in Figs 5 and 6. The pollen grains of *Alyssum* are isopolar and radial symmetric. Pollen shape, calculated as a ratio between polar and equatorial diameter (P/E). Four taxa have subprolate pollen grain, observed in *A. peltaroides* subsp.

**TABLE 6**

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Fig No</th>
<th>P/E ratio</th>
<th>Pollen shape</th>
<th>Pollen Size</th>
<th>Lumen (μm)</th>
<th>Muri (μm)</th>
<th>Ornamentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. murale</em> var. <em>alpinum</em></td>
<td>1</td>
<td>1.57</td>
<td>Prolate</td>
<td>Small</td>
<td>307</td>
<td>295</td>
<td>Microreticulate</td>
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<tr>
<td><em>A. virgatum</em></td>
<td>2</td>
<td>1.34</td>
<td>Prolate</td>
<td>Small</td>
<td>197</td>
<td>563</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. peltaroides</em> subsp. <em>virginiforme</em></td>
<td>3</td>
<td>1.26</td>
<td>Subprolate</td>
<td>Small</td>
<td>1818</td>
<td>334</td>
<td>Reticulate</td>
</tr>
<tr>
<td><em>A. bulbichicum</em></td>
<td>4</td>
<td>1.26</td>
<td>Prolate</td>
<td>Medium</td>
<td>1013</td>
<td>460</td>
<td>Reticulate</td>
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<tr>
<td><em>A. condensatum</em> subsp. <em>flexible</em></td>
<td>5</td>
<td>1.36</td>
<td>Prolate</td>
<td>Small</td>
<td>531</td>
<td>321</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. pateri</em></td>
<td>6</td>
<td>1.39</td>
<td>Prolate</td>
<td>Small</td>
<td>291</td>
<td>180</td>
<td>Microreticulate</td>
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<tr>
<td><em>A. corisicus</em></td>
<td>7</td>
<td>1.17</td>
<td>Subprolate</td>
<td>Small</td>
<td>537</td>
<td>428</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. caricum</em></td>
<td>8</td>
<td>1.48</td>
<td>Prolate</td>
<td>Small</td>
<td>None</td>
<td>None</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. baumgartenianum</em></td>
<td>9</td>
<td>1.49</td>
<td>Prolate</td>
<td>Medium</td>
<td>None</td>
<td>None</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. callichroum</em></td>
<td>10</td>
<td>1.37</td>
<td>Prolate</td>
<td>Small</td>
<td>682</td>
<td>361</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. discolor</em></td>
<td>11</td>
<td>1.39</td>
<td>Prolate</td>
<td>Small</td>
<td>760</td>
<td>450</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. ochroleucum</em></td>
<td>12</td>
<td>1.43</td>
<td>Prolate</td>
<td>Medium</td>
<td>597</td>
<td>371</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. sibiricum</em></td>
<td>13</td>
<td>1.24</td>
<td>Subprolate</td>
<td>Small</td>
<td>559</td>
<td>324</td>
<td>Microreticulate</td>
</tr>
<tr>
<td><em>A. strigosum</em></td>
<td>14</td>
<td>1.19</td>
<td>Subprolate</td>
<td>Medium</td>
<td>790</td>
<td>510</td>
<td>Microreticulate</td>
</tr>
</tbody>
</table>

**Pollen Characters.** The characters of pollen grains of the examined *Alyssum* species are summarized in Table 6 by using light microscope and SEM data, and shown in Figs 5 and 6. The pollen grains of *Alyssum* are isopolar and radial symmetric. Pollen shape, calculated as a ratio between polar and equatorial diameter (P/E). Four taxa have subprolate pollen grain, observed in *A. peltaroides* subsp.
virgatifor-me, A. corsicum, A. sibiricum, A. strigosum, and prolate in rest of the examined taxa. Erdtman [52] categorized the different pollen size classes based on expressed as length of the longest axis. According to this classification examined species are small or medium size. Pollen grains show a wide variety in their sizes. The pollen grains are monad, polar length and equatorial diameter ranging from 17.38 (in A. corsicum) to 38.17 µm (in A. baumgartnerianum). The highest mean value of polar length and equatorial diameter is P= 38.17 µm and E=25.59 µm in A. baumgartnerianum, and the smallest mean value of polar length and equatorial diameter are 17.83 µm in A. corsicum and 12.91 µm in A. murale subsp. murale var. alpinum.

FIGURE 11
LM micrographs of pollen in Alyssum species.
FIGURE 12
LM micrographs of pollen in Alyssum species.
Pollen grains are tricolpate. The colpus is long, flat, slowly narrowing towards the poles. Colpus length and colpus width diameter ranging from 13.88 (in *A. corsicum*) to 33.86 µm (in *A. baumgartnerianum*). The highest mean value of colpus length is clg = 33.86 µm in *A. baumgartnerianum*, and the lowest mean value of clg = 13.88 µm in *A. corsicum*. The highest mean value of colpus
width is $cl=l=1.64 \mu m$ in *A. ochroleucum*, and the lowest mean value of $cl=0.77 \mu m$ in *A. murales* subsp. *murale* var. *alpinum*. The exine ornamentation of the examined *Alyssum* pollen was microreticulate. Reticulate ornamentation consisting of muri and lumina bigger than 1 $\mu m$. Reticulate ornamentation is tricolporate but the pollen grains are usually radially symmetrical and isopolar. The fruit surface is usually glabrous or pubescent. The seeds are ovate, spheroidal or oblong in shape among the studied species. The hilum position is terminal or subterminal. They show variations in color; light brown, dull yellow, brownish, bright red brownish, dark brownish, dull light reddish, bright brownish, bright red brownish, dull brownish, and dull yellowish. Several types of ornamentations have been observed on surface structures: reticulate (normal reticulate, reticulate-foveate, reticulate-lineolate, reticulate-rugose), tunicate, rugose, lineolate, blister, and colliculate. The pollen grains of the genus are subprolate or prolate. The pollen grains are mainly reticulate. Exine ornamentation is tricolporate. Exine ornamentation as a diagnostic character for species level.

**REFERENCES**


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THE EFFECTS OF BIOCHAR AND RAISING MYCORRHIZA USAGE ON SEVERAL MINERAL INGREDIENT OF PAK CHOI

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ABSTRACT

This study aims to determine the effect of biochar and increasing mycorrhiza applications on some mineral material content of pak choi plant. In the beginning of the experiment, 300 g/m² parcel biochar was used for each parcel one month before seedling planting. Then, increasing mycorrhiza doses per plant (0, 15, 20, 30 and 40 mL) were used to the parcels undergoing biochar application and the harvest occurred 54 days after seed sowing. The findings revealed important increases in some macro and micro nutrient element contents of Pak Choi plant with biochar and increasing mycorrhiza applications. The contents were found as P (0.41 %, 0.46 %, 0.47 %, 0.49 % and 0.56 %), K (4.33 %, 4.19 %, 4.06 %, 4.17 % and 4.59 %), Ca (2.03 %, 2.24 %, 2.17 %, 2.25 % and 2.47 %), Mg (0.15 %, 0.16 %, 0.17 %, 0.17 % and 0.20 %), Fe (569 mg/kg, 973 mg/kg, 1016 mg/kg, 1016 mg/kg and 1094 mg/kg), Cu (5.02 mg/kg, 6.26 mg/kg, 6.58 mg/kg, 7.04 mg/kg and 13.16 mg/kg), Mn (62.73 mg/kg, 74.03 mg/kg, 78.00 mg/kg, 85.96 mg/kg and 114.10 mg/kg) and Zn (37.23 mg/kg, 41.36 mg/kg, 42.03 mg/kg, 43.53 mg/kg and 52.83 mg/kg), respectively. Some macro (P, K, Ca and Mg) and micro (Fe, Cu, Zn and Mn) element contents were determined statistically significant at the level of 5 %. The best nutrient element contents of pak choi plant were obtained at V. dose: 40 mL/plant mycorrhiza applications.

KEYWORDS:

INTRODUCTION

In the world, brassicas are grown for their edible and terminal buds and present a preferable variety of leafy vegetables in cooking as well [1]. The world has eventually known this green vegetable by the help of the travelers and migrants [2, 3, 4]. Pak choi: syn. *Brassica Chinensis* L. (1759), *Brassica Campestris* L. subsp. *Chinensis* (L.) Makino, (1912) [5], *Brassica rapa* L. subsp. *Chinensis* (L.) Hanelt, (1986) [6] since it is evolved in China, the cultivation records start in the 5th century AD and it has been widely grown in China and Taiwan. It is rather a new vegetable for Japan as it is named as ‘Chinese vegetable’ [7]. Pak choi is rather found as an exotic and a worthy in the world [8, 9, 10]. There is a commonly known symbiotic relationship between the Mycorrhiza fungi and the roots of plants in natural circumstances. Mycorrhizal colonization of plants is significantly favorable for growth, nutrient uptake and yield as well. However, the activity of mycorrhizal fungi is not predictable easily or useful in agricultural ecosystems for either low input or intensively managed ones [11]. Nevertheless, the reference [12] cites the combination of mycorrhizal inoculation with applications of other bio fertilizers or bio pesticides as a promising future strategy in the cultivation of vegetables. There are many developments in the methods for the selection of suitable commercial products or production of mycorrhizal inoculation as challenging in the future including the practice in horticulture.

The impact of salt (0 and 100 mg NaCl kg⁻¹) and raising zinc usages (0, 25, 50 mg Zn kg⁻¹) on up taking in maize (*Zea mays* L.) plant with mycorrhizal and non-mycorrhizal conditions and mycorrhiza inoculated applications reported a significant increase in fresh weight, dry weight along with the content of zinc and phosphorus contents compared to the non-mycorrhizal practices [13]. The acquisition and detection of the effects of mycorrhiza inoculation on growth, yield, and quality of tomato plants in greenhouse conditions along with the efficiency of different doses of symbiont VAM as mycorrhiza source could be cited as well [14]. The infection in the root increases with symbiont VAM inoculation and in favor for plant growth and for total and marketable yield. Increases were found for fruit diameter, red color (a), vitamin C and pH of fruit juice; however titratable acidity, and electrical conductivity of fruit juice, and firmness were rather determined as decreasing [15].
It has been found that after biochar soil mixing in agricultural production has important effects on improving plant growth [16, 17]. There are studies on the effect of increasing doses of biochar applications on the nutrient content of the soil where available phosphorus, exchangeable potassium, and calcium were found as increased during the increasing biochar applications. Biochar has also a high capacity in energy production, it also develops soil fertility and organic material content along with the removal of heavy metals both from water and soil [18, 19, 20, 21, 22].

The impact of vermicompost, mycorrhiza, and both on pepper growth and mineral nutrition was also revealed in greenhouse conditions where mycorrhiza (0, 1 and 2 g pot⁻¹) and vermicomposting doses (0, 2.5, 5 and 10 g pot⁻¹) were used. The results inform that vermicomposting and mycorrhiza applications had positive effects on fresh, dry weights, and plant nutrition on pepper [23].

Another study on rising doses of mycorrhiza usages had shown that a rise occurs in selected macro (N, P, K, Ca and Mg) and trace (Fe, Cu, Zn and Mn) contents in pak choi plant [24].

This study, however, aims to determine the effect of biochar and raised doses of mycorrhiza applications on some mineral material content of pak choi.

**MATERIALS AND METHODS**

The study used a high tunnel cold greenhouse which was covered by polyethylene (PE) with UV additive and which belongs to Namik Kemal University, Vocational College of Technical Sciences, Plant and Animal Production Department. The whole process of the experiments was conducted in autumn of 2016, Tekirdag city (40°98’ N, 27°48’ E) Turkey.

The research design was determined as a typical three replications with randomized blocks. The white mini variety of pak choi was selected and used for the experimental design (Figure 1). Seeds were sown in multi-celled trays with peat in October. Some specifications of the peat are as follows: 160-260 mg L⁻¹ N, 180-280 mg L⁻¹ P₂O₅, 200-150 mg L⁻¹ K₂O, 80-150 mg L⁻¹ Mg, pH: 6, 70 % organic matter, and 35 % C. At the time the seedlings became 2 to 3 true leaves (on the 21st day for pak choi after seed sowing) they were planted in the high tunnel cold greenhouse with 10 cm x 10 cm intervals and adjusting the design as 10 plants for each parcel (Figure 1).

As the organic material, the hazelnut shell from Ordu Province, Turkey had been converted into biochar. With respect to biocidal production, the production temperature is +380 °C with a period of 270 minutes.

Beginning of the experiment, 300 gr m⁻² biochar applied to each parcel precisely in one month before seedling planting. Then mycorrhiza doses were applied in five different doses per plant (I: 0 mL, II: 15 mL, III: 20 mL, IV: 30 mL and V: 40 mL) a month before harvesting and plants were harvested just 54 days after seed sowing. The harvested plants were brought to the laboratory immediately, washed with distilled water two times and they were dried in 65 °C drying-oven till their weights get stabilized. They were ground and prepared for the analysis. Total N contents of plants was carried out with Kjeldahl method, and for their P, K, Ca, Mg, S, Fe, Cu, Zn and Mn analysis, ICP-OES device was used [25]. Thereafter, pH, lime amount, organic matter amount, total nitrogen, available phosphorus, exchangeable potassium, calcium and magnesium in the soil samples of research area were assessed [26] along with the texture in soil sample [27]. Some trace elements which are available in the soil such as Fe, Cu, Zn and Mn were also tested with DTPA method [28].

Then the findings of the experiments were evaluated by the use of tests in SPSS 21 statistical software. On the research results, ANOVA variance analysis and Duncan multiple comparison tests were conducted.

During the growing period of the plants, climate data were recorded inside the tunnel (Figure 2). No diseases and pests, and no pesticides were applied in the growing period.

**RESULTS AND DISCUSSION**

Some chemical and physical specifications of the soil sample of the experiment is given in Table 1. Some properties of biochar, which is acquired from hazelnut shell, are demonstrated and highlighted in Table 2.

Table 1 explains the pH value of soil alkaline, low saltiness, insufficient organic content, little lime, medium available phosphorus, exchangeable K, Ca and Mg contents, and available trace elements (Mn, Cu, Fe and Zn) and their contents are all found sufficient. The texture class of experiment area soil sample is clay.

The effect of biochar and raising mycorrhiza usages on several macro nutrient element ingredient of pak choi are displayed in Table 3.

In the Table 3, P ingredient of pak choi was as 0.41-0.56 % for I. dose and VI. dose, serially. K ingredient of pak choi was 4.06-4.59 % for I. dose and VI. dose, serially. Ca ingredient of pak choi was 2.03-2.47 % for I. dose and VI. dose, serially. Mg ingredient of pak choi was determined as 0.15-0.20 % for I. dose and VI. dose, serially. K, Ca and Mg ingredients of pak choi increased as 0.15-0.20 % for I. dose and VI. dose, serially. P, K, Ca and Mg ingredients of pak choi increased with biochar and raising mycorrhiza usages as seen in the Table 3. These increases were found...
FIGURE 1
Trial area of research and post-harvest pak choi plant (original)

FIGURE 2
Some average climate data in unheated greenhouse during the experiment months.

TABLE 1
Some specifications of the soil

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.0</td>
</tr>
<tr>
<td>Salinity*</td>
<td>0.07</td>
</tr>
<tr>
<td>CaCO3*</td>
<td>2.7</td>
</tr>
<tr>
<td>Organic matter*</td>
<td>1.3</td>
</tr>
<tr>
<td>Exchangeable Ca*</td>
<td>0.5</td>
</tr>
<tr>
<td>Available P**</td>
<td>36.4</td>
</tr>
<tr>
<td>Exchangeable K**</td>
<td>253.8</td>
</tr>
<tr>
<td>Exchangeable Mg**</td>
<td>473.1</td>
</tr>
<tr>
<td>Available Mn**</td>
<td>5.6</td>
</tr>
<tr>
<td>Available Cu**</td>
<td>0.8</td>
</tr>
<tr>
<td>Available Fe**</td>
<td>7.4</td>
</tr>
<tr>
<td>Available Zn**</td>
<td>0.9</td>
</tr>
<tr>
<td>Texture class</td>
<td>Clay</td>
</tr>
</tbody>
</table>

*: (%), **: mg kg⁻¹

TABLE 2
Some specifications of biochar

<table>
<thead>
<tr>
<th>Material</th>
<th>C, %</th>
<th>N, %</th>
<th>pH</th>
<th>K, mgkg⁻¹</th>
<th>P, mgkg⁻¹</th>
<th>Mg, mgkg⁻¹</th>
<th>Fe(mgkg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochar</td>
<td>81</td>
<td>1.17</td>
<td>9.24</td>
<td>0.33</td>
<td>-</td>
<td>1690</td>
<td>10.40</td>
</tr>
</tbody>
</table>
Pak choi was got 5.02-13.16 mg kg\(^{-1}\) for I. dose and VI. dose, serially. Cu ingredient of pak choi was got 569-1094 mg kg\(^{-1}\) are listed in Table 4. According to the Table 4, Fe practice on some trace nutrient elements of pak choi 37.23-52.83 mg kg\(^{-1}\) for I. dose and VI. dose, serially. Zn ingredient of pak choi was got 4.06-0.38 for I. dose and VI. dose, serially. Mn ingredient of pak choi was got 62.73-114.10 mg kg\(^{-1}\) for I. dose and VI. dose, serially. Cu ingredient of pak choi rises 569-1094 mg kg\(^{-1}\) for I. dose and VI. dose, serially. Zn ingredient of pak choi rises 4.06-0.38 mg kg\(^{-1}\) for I. dose and VI. dose, serially. Mn ingredient of pak choi rises 62.73-114.10 mg kg\(^{-1}\) for I. dose and VI. dose, serially.

In the references [39, 40], mycorrhizas have led to positive results in terms of the protection for the environment. Since excessive mycorrhizas or form other mycorrhizas. In addition, mycorrhizas can be seen in Table 4. These raises were assessed as statistically significant at 5 %. The results of the experiments are compatible with the results of former researchers [23, 35, 38].

Similarly, mycorrhizal applications also increased concentrations of Fe, Cu and Zn in tomato plant [39]. In another reference [35] mycorrhizas were given as contributing to the uptake of micro elements, such as copper and zinc. Those findings are parallel with the results of this study.

In the reference [34] it is reported that there are favorable effects of mycorrhiza on plant growth by an increase in the intake of micro nutrients, particularly Zn, for leek plant. The similar result was given by another study [40] for tomato and for green pepper plants in the reference [41]. Biochar which is used for the soil therefore affects the micro nutrient uptake positively and it has also impact on the agronomic development of the plant [42].

Nevertheless, in the case of reformed nutrient supply up to 20 % of the assimilated carbon from the plant is translocated to the root for the formation, existence, and function of the mycorrhizas [43]. Biochar has therefore a positive effect on such cases.

In the references [35, 44, 45], the Brassicaceae (including canola, mustards, cabbages, etc.) do not have arbuscular mycorrhizas or form other categories of mycorrhizas or lack mycorrhizas. In the cultivation of pak choi, as a member of Brassicaceae, increased mycorrhiza and biochar application have led to positive results in terms of the presence for plant nutrients.

### CONCLUSIONS

This experiment results ensure the statistical significance at the level of 5 % on both of some macro along with trace nutrient elements. According to our findings in this study, it would be better to use mycorrhiza, biochar with mycorrhiza, and other microbial and organic fertilizers or for the agricultural soils so as to attain higher yield and quality in plants, sustainable soil fertility ensuring protection for the environment. Since excessive

### TABLE 3

Findings on some macro nutrient elements, %, *, **

<table>
<thead>
<tr>
<th>Mycorrhiza doses***</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.41±0.22c</td>
<td>4.33±0.18b</td>
<td>2.03±0.58c</td>
<td>0.15±0.28c</td>
</tr>
<tr>
<td>II</td>
<td>0.46±0.11bc</td>
<td>4.19±0.38bc</td>
<td>2.24±0.10ab</td>
<td>0.16±0.33b</td>
</tr>
<tr>
<td>III</td>
<td>0.47±0.42bc</td>
<td>4.06±0.38e</td>
<td>2.17±0.91b</td>
<td>0.17±0.30ab</td>
</tr>
<tr>
<td>IV</td>
<td>0.49±0.40bc</td>
<td>4.17±0.23bc</td>
<td>2.25±0.10ab</td>
<td>0.17±0.15ab</td>
</tr>
<tr>
<td>V</td>
<td>0.56±0.75a</td>
<td>4.59±0.52a</td>
<td>2.47±0.29a</td>
<td>0.20±0.68a</td>
</tr>
</tbody>
</table>

*: Mean of three replications, **: each element was evaluated individually and values in the same column with different letters, significant at 5 %, ***: (Per plant I: 0 mL, II: 15 mL, III: 20 mL, IV: 30 mL and V: 40 mL).

### TABLE 4

Findings on some trace elements of pak choi, mg kg\(^{-1}\), *, **

<table>
<thead>
<tr>
<th>Mycorrhiza doses***</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>569±15.3e</td>
<td>5.02±0.06e</td>
<td>37.23±0.10c</td>
<td>62.73±0.4c</td>
</tr>
<tr>
<td>II</td>
<td>973±29.7b</td>
<td>6.26±0.19bc</td>
<td>41.36±0.10bc</td>
<td>74.03±0.2bc</td>
</tr>
<tr>
<td>III</td>
<td>1016±49.9ab</td>
<td>6.58±0.03bc</td>
<td>42.03±0.02bc</td>
<td>78.00±0.4bc</td>
</tr>
<tr>
<td>IV</td>
<td>1016±41.6ab</td>
<td>7.04±0.07b</td>
<td>43.53±0.10b</td>
<td>85.96±0.1b</td>
</tr>
<tr>
<td>V</td>
<td>1094±23.3a</td>
<td>13.16±0.10a</td>
<td>52.83±0.30a</td>
<td>114.10±0.5a</td>
</tr>
</tbody>
</table>

*: Mean of three replications, **: each element was evaluated individually and values in the same column with different letters, significant at 5 %, ***: (Per plant I: 0 mL, II: 15 mL, III: 20 mL, IV: 30 mL and V: 40 mL).
chemical fertilizer application on agricultural land will have damaging effects on soil fertility and quality, mycorrhiza and other organic materials should be applied to agricultural lands to achieve the sustainability in soil fertility and high-quality plant production.

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PHYSIOLOGICAL CHARACTERISTICS OF NARBON VETCH \((V\text{\textit{ICIA NORBONENSIS}} \text{\textit{L.}})\) PLANTS

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ABSTRACT

Physiological characteristics should be defined for better assessment of plant growth and development. Such characteristics play a significant role in finding out optimum intervention time. Vetch is commonly used in animal feeding. Therefore, it is quite significant to work on vetch yield and quality. Vetch constitutes about 10% of feed crop production of Turkey. Therefore, vetch cultivars and lines were used and physiological characteristics of Narbon vetch \((V\text{\textit{ICIA NORBONENSIS}} \text{\textit{L.}})\) plants were determined. Leaf area index, leaf area ratio, relative leaf growth ratio, relative growth ratio, net assimilation ratio and plant growth ratio were determined.

Present findings revealed that leaf area index was a significant parameter for growth and development of all genotypes. Leaf area ratio and net assimilation ratio were also identified as the other effective physiological factors. Leaf growth ratio did not have significant effects. Correlations of dry matter yield with physiological characteristics revealed that leaf area index was significant in all parameters and it was followed by leaf growth ratio. Present findings revealed that Narbon vetch genotypes had significant differences in their physiological characteristics.

KEYWORDS:
Vetch, growth, physiology, livestock production

INTRODUCTION

Feed is the greatest input in animal production activities. There are several alternatives for animal feed. Besides several feed sources successfully produced ecologically by farmers, there are some others not able to be produced successfully. Serious decreases are experienced in production quantities together with decreasing production sites. Problems are then evident in meeting feed demand of animals. Therefore, some feed sources were started to be exported to meet current deficits in animal feed.

Pasture and meadowlands should be reclaimed to have sufficient and quality feed supply for animals. However, it is assumed that Turkey was not successful in this issue. Therefore, feed crops culture should widespread. Yield and quality should always be kept in mind while increasing feed crops cultivation lands. Turkey is quite lucky in this issue and should use this chance [1]. Vetch can be grown successfully both under dry and irrigated conditions. It can be used as a great source of roughage. Vetch is quite rich in protein and supplied to animals as fresh and dry roughage and silage [2]. Soya [3], Jimenez et al. [4] and Singh and Gill [5] indicated that seeds had significant effects on herbage and dry matter yields. Besides the use of high-yield and quality genotypes, physiological characteristics and adaptation of plants to current environmental conditions should also be taken into consideration. In this way, more successful productions will be possible [6]. Plants react against stress factors like temperature, drought, excessive precipitation as to minimize potential damages on plants [7, 8]. Measures to be taken against these stressors reflect on physiological characteristics. Relative growth should be defined to determine the increase in dry matter per unit area [9, 10].

Physiological growth can be defined by relative growth ratio, relative growth rate, net assimilation ratio, leaf area ratio and leaf area index [11, 12]. All these attributes can be used as an indicator whether or not plants exhibit a healthy growth and development [13]. Lighting, shading and similar factors have significant effects on physiological factor [14]. It was reported in previous studies that leaf ratios decreased, dry matter and crude protein yields increased with increasing plant heights; crude protein ratios relatively decreased with decreasing leaf ratios [15-19].

Plant physiological characteristics should be determined for better comprehension of plant growth and development. Every kind of changes in environmental conditions between the emergence and flowering periods greatly influences plant growth and development [20]. Charles-Edwards et al. [9] carried out plant growth studies and mostly pointed out the significance of dry weight and leaf areas. Beadle [21] indicated dry matter quantity as a significant indicator of relative growth and thus it was used to determine plant response to different
treatments. This study was conducted to determine physiological characteristics of vetch genotypes collected from the nature. In this way, a significant progress will be achieved in breeding studies. Since physiological parameters will be known, reliable further steps can be taken in further breeding studies.

MATERIALS AND METHODS

Experiments were conducted over the experimental fields of Bingöl University Agricultural Faculty. Six Narbon vetch lines (IFVN 564, IFVN 565, IFVN 575, IFVN 567, IFVN 116 and IFVN 562) and four Narbon vetch cultivars (Tarman, Halilbey, Karakaya and Görkem) were used as the plant material. Experimental soils were slightly loamy with a pH of 7.5, organic matter content of 1.44%, phosphorus content of 4.9 kg/da, nitrogen content of 2.1 kg/da and potassium content of 15 kg/da. Experiments were conducted in the years 2014 and 2015. In randomized blocks design with 3 replications. Row spacing was 30 cm and on-row plant spacing was 10 cm. Each plot had 6 rows. Irrigation was not applied to treatments. A manual weeding was performed right after emergence. Measurements were performed on randomly selected 5 plants from each plot. From the emergence, regular measurements were made and recorded in every 10 days. Weights were measured with a precise balance (±0.0001 g) (Sartorius). Means were compared with Duncan’s multiple range test.

Plant samples were subjected to leaf area index (LAI), leaf area ratio (LAR), average relative leaf growth ratio (RLGR), relative growth ratio (RGR), net assimilation ratio (NAR) and plant growth ratio (PGR) analyses [9, 20, 22, 23, 24].

Leaf area index is calculated as the ratio of total above-ground leaf area of a plant to total ground surface area; \[ \text{LAI} = \frac{\text{TL}_{\text{A}}}{G} \] where; LAI is leaf area index, TL_A; total leaf area of a plant at time “t” and G is total ground surface area.

Leaf area ratio is calculated as the ratio of unit leaf area to total dry matter production; \[ \text{LAR} = \frac{L_A}{A} \] where; LAR is leaf area ratio, L_A is total leaf area at time “t” and A is plant final weight at time “t”.

Leaf growth ratio is calculated as the ratio of unit leaf area to total leaf area; \[ \text{LGR} = \frac{SL_A}{TL_A} \] where; LGR is leaf growth ratio, SL_A is single leaf area at time “t” and TL_A is total leaf area at time “t”.

Plant growth ratio is calculated as the ratio of unit leaf weight to total leaf weight. However, in practice, leaf area index is multiplied by net assimilation ratio to get plant growth ratio; \[ \text{PGR} = \text{LAI}.\text{NAR} \] where; PGR is plant growth ratio, LAI is leaf area index and NAR is net assimilation ratio.

Relative growth ratio is calculated as the ratio of unit plant weight to total plant dry weight. However, in practice, leaf area ratio is multiplied by net assimilation ratio to get relative growth ratio; \[ RGR = \frac{\text{LA}_1.\text{NAR}}{Y_{\text{AO}}} \] where; RGR is relative growth ratio, YAO is leaf area index and NAR is net assimilation ratio.

Net assimilation ratio is calculated as the ratio of unit leaf weight to increase in dry weight; \[ \text{NAR} = \frac{1}{Y_{\text{AO}}} \frac{\text{dW}}{\text{dt}} \] where; NAR is net assimilation ratio, dW is derivative of weight and dt is derivative of time.

RESULTS AND DISCUSSION

Some physiological characteristics of vetch genotypes are provided in Table 1. The average LAI was identified as 0.59 for vetch lines and as 0.69 for vetch cultivars and the differences in LAI values were found to be significant. General average was calculated as 0.63. The greatest LAI (0.78) was obtained from Tarman cultivar and it was followed by Karakaya (0.70) cultivar. The lowest LAI (0.55) was obtained from IFVN 575 genotype. Since leaf area index expresses the ratio of total leaf area to total ground surface area, greater areas indicate greater leafing. Greater number of leaves brings about a photosynthetic advantage to the plant, but also brings about decreased generative development. Thus, the balance between them should be well-preserved.

There were significant differences in leaf area ratios of the vetch lines and cultivars. Leaf area ratio was identified as 2.55 for vetch lines and as 2.74 for vetch cultivars. General average was calculated as 2.63. The greatest leaf area ratio (2.85) was obtained from Görkem cultivar and it was followed by Halilbey (2.84) cultivar. The lowest leaf area ratio (2.51) was obtained from IFVN 116 line. Since leaf area ratio expresses the ratio of unit leaf area to total dry matter production, it can reveal significant information about plant growth and development. Increasing leaf area ratios indicate increasing dry matter accumulation. Görkem and Halilbey cultivars had slightly greater values, but the difference was found to be significant.

Leaf growth ratios did not have significant variations and general average was calculated as 0.11. Such a value indicated more stable nature of leaf growth ratios than the other physiological attributes. The greatest leaf growth ratio (0.12) was obtained from IFVN 564 and IFVN 565 genotypes and the lowest leaf growth ratio (0.09) was obtained from IFVN 575 and IFVN 562 genotypes. Similar leaf growth ratios of the cultivars indicated that
stability was generated in cultivars. However, variations in leaf growth ratios of the vetch lines were significant. Since leaf growth ratio is calculated as the ratio of unit leaf area to total leaf area of a plant, it can reveal significant information about leaf growth and development. Closer values of the genotypes indicated similar responses of the genotypes to ecological conditions.

There were significant differences in relative growth ratios of the genotypes. The average value was calculated as 0.24 for the vetch cultivars and as 0.21 for the vetch lines. General average was calculated as 0.22. The greatest relative growth ratio (0.26) was obtained from Görkem cultivar and it was followed by Tarman cultivar (0.24). The lowest relative growth ratio (0.18) was obtained from IFVN 567 genotype. Relative growth ratio is calculated through multiplying leaf area ratio by net assimilation ratio. Thus, directly influenced by leaf area ratio and net assimilation ratio. Increase or decrease in any of these parameters will automatically increase or decrease.

Net assimilation ratios were generally stable. The average net assimilation ratio was identified as 0.09 for the vetch cultivars and as 0.08 for vetch lines. The difference was not found to be significant. The general average was calculated as 0.08. The greatest net assimilation ratio (0.09) was obtained from IFVN 564 and IFVN 562 genotypes and Tarman and Görkem cultivars. The lowest value (0.07) was obtained from IFVN 567 genotype. Net assimilation ratio is calculated as the ratio of unit leaf weight to increase in total dry weight. Thus, it is a quite significant indicator of plant growth and development. The decrease in net assimilation ratio indicates a decrease in plant growth and development and vice-versa.

There were not large variations in plant growth ratios, but these variations were found to be significant. Plant growth ratio was identified as 0.05 for vetch lines and as 0.06 for vetch cultivars. General average was calculated as 0.05. The greatest plant growth ratio (0.07) was obtained from Tarman cultivar and the lowest value (0.04) was obtained from IFVN 575 and IFVN 567 genotypes. Plant growth ratio is calculated through multiplying leaf area index by net assimilation ratio. Therefore, any change in leaf area index and net assimilation ratio will influence plant growth ratios.

The dry matter yields of vetch lines were slightly greater than the dry matter yields of the vetch cultivars. The average value was identified as 507 kg/da for vetch cultivars and as 499 kg/da for vetch lines. The difference was not found to be significant. General average was calculated as 503 kg/da. The greatest dry matter yield (512 kg/da) was obtained from IFVN 564 genotype and the lowest value (495 kg/da) was obtained from Halilbey cultivar. Dry matter yield reveals significant information about plant growth and development. It is the most significant indicator of stress factors. Dry matter yields decrease, thus plant growth and development recess with increasing stress factors.

Correlations between physiological characteristics and dry matter yield are provided in Table 2. There were significant correlations between leaf area index and dry matter yield of all genotypes. Therefore, leaf area index can be stated as the most significant factor for dry matter production of vetch plants. Any changes in leaf area index will directly influence dry matter yield of the plants. Breeders and producers should definitely consider these parameters for successful operations. Net assimilation ratio is considered as another significant parameter. This parameter was identified as a parameter with significant effects on dry matter yield of 7 genotypes. Besides these two parameters, leaf growth ratio and plant growth ratio also had varying impacts on dry matter yield of the plants.

### TABLE 1
Physiological Characteristics of the Vetch Genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Leaf Area Index</th>
<th>Leaf Area Ratio</th>
<th>Leaf Growth Ratio</th>
<th>Relative Growth Ratio</th>
<th>Net Assimilation Ratio</th>
<th>Plant Growth Ratio</th>
<th>Dry Matter Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFVN 564</td>
<td>0.58 c</td>
<td>2.54 cd</td>
<td>0.12 a</td>
<td>0.23 ab</td>
<td>0.09</td>
<td>0.05 ab</td>
<td>512</td>
</tr>
<tr>
<td>IFVN 565</td>
<td>0.61 c</td>
<td>2.59 c</td>
<td>0.12 a</td>
<td>0.21 b</td>
<td>0.08</td>
<td>0.05 ab</td>
<td>506</td>
</tr>
<tr>
<td>IFVN 575</td>
<td>0.55 d</td>
<td>2.58 c</td>
<td>0.09 b</td>
<td>0.21 b</td>
<td>0.08</td>
<td>0.04 b</td>
<td>496</td>
</tr>
<tr>
<td>IFVN 567</td>
<td>0.58 cd</td>
<td>2.52 d</td>
<td>0.10 ab</td>
<td>0.18 c</td>
<td>0.07</td>
<td>0.04 b</td>
<td>507</td>
</tr>
<tr>
<td>IFVN 116</td>
<td>0.59 c</td>
<td>2.51 d</td>
<td>0.11 a</td>
<td>0.20 bc</td>
<td>0.08</td>
<td>0.05 a</td>
<td>510</td>
</tr>
<tr>
<td>IFVN 562</td>
<td>0.61 c</td>
<td>2.59 c</td>
<td>0.09</td>
<td>0.23 b</td>
<td>0.09</td>
<td>0.06 a</td>
<td>508</td>
</tr>
</tbody>
</table>

| Line Average  | 0.59 B         | 2.55 B         | 0.11 A            | 0.21 B               | 0.08                   | 0.05 A             | 507              |
| Tarman        | 0.78 a         | 2.69 b         | 0.10 ab           | 0.24 ab              | 0.09                   | 0.07 a             | 498              |
| Halilbey      | 0.65 bc        | 2.84 a         | 0.11 a            | 0.23 b               | 0.08                   | 0.05 ab            | 495              |
| Karakaya      | 0.70 b         | 2.59 c         | 0.11 a            | 0.21 b               | 0.08                   | 0.06 a             | 500              |
| Görkem        | 0.64 bc        | 2.85 a         | 0.11 a            | 0.26 a               | 0.09                   | 0.06 a             | 501              |

| Cultivar Average | 0.69 A      | 2.74 A         | 0.11 A            | 0.24 A               | 0.09                   | 0.06 A             | 499              |
| General Average  | 0.63         | 2.63           | 0.11              | 0.22                 | 0.08                   | 0.05               | 503              |

*Values were rounded.
TABLE 2

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Correlations (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFVN 564</td>
<td>LAI<em>DMY=0.512**, NAR</em>DMY=0.634**, LGR<em>DMY=0.499</em></td>
</tr>
<tr>
<td>IFVN 565</td>
<td>LAI<em>DMY=0.394</em>, NAR*DMY=0.547**</td>
</tr>
<tr>
<td>IFVN 575</td>
<td>LAI<em>DMY=0.448</em>, RGR<em>DMY=0.408</em>, LGR<em>DMY=0.628**, PGR</em>DMY=0.492**</td>
</tr>
<tr>
<td>IFVN 567</td>
<td>LAI*DMY=0.617**</td>
</tr>
<tr>
<td>IFVN 516</td>
<td>LAI<em>DMY=0.557**, NAR</em>DMY=0.674**</td>
</tr>
<tr>
<td>IFVN 562</td>
<td>LAI<em>DMY=0.594**, LGR</em>DMY=0.439*, PGR*DMY=0.548**</td>
</tr>
<tr>
<td>Tarman</td>
<td>LAI<em>DMY=0.491**, LGR</em>DMY=0.601**, NAR<em>DMY=0.572**, PGR</em>DMY=0.488**</td>
</tr>
<tr>
<td>Halibey</td>
<td>LAI<em>DMY=0.537**, RGR</em>DMY=0.534**, NAR*DMY=0.489**</td>
</tr>
<tr>
<td>Karakaya</td>
<td>LAI<em>DMY=0.654**, RGR</em>DMY=0.577**, NAR<em>DMY=0.536**, PGR</em>DMY=0.567**</td>
</tr>
<tr>
<td>Görkem</td>
<td>LAI<em>DMY=0.581**, NAR</em>DMY=0.629**, PGR*DMY=0.567**</td>
</tr>
</tbody>
</table>

* Significant at 0.05, ** significant at 0.01, NAR: Net Assimilation Ratio, LAR: Leaf Area Ratio, PGR: Plant Growth Ratio, LAI: Leaf Area Index, DMY: Dry Matter Yield

In general, genotypes had physiological differences. Plants usually exhibit a rapid growth in initial stages. Then, growth and development is shaped around environmental factors. Plants tend to increase number of leaves especially in initial stages to have sufficient photosynthesis rates and to get sufficient nutrients. Leaf area in this case becomes prominent and significant. Leaf area index and leaf area ratio initially exhibit rapid development, then decrease later on. Significant decreases are observed together with pod-set period after flowering.

Shalaby and Muhammed [25] reported rapid increases in leaf area index and leaf area ratio of soybean plants throughout the initial growth stages and reported decreases in both parameters later on. Karadavut and Tozluca [6] also reported rapid increases in leaf area index and leaf area ratio of rye plants during the initial stages of growth. Present findings comply with those earlier reports. Singh et al. [26] reported different results for leaf growth ratio. Researchers reported quite slow leaf growth ratios of broad bean plants in the initial growth stage and accelerated ratios toward to flowering period. Such differences were probably resulted from differences in climate and sowing time.

Growth ratios generally decreased in time. However, such decreases were not sharp, thus did not negatively influenced plants. Rapid changes induced by biotic and abiotic conditions may sharpen changes and then negatively influence plant growth and development. Effects of biotic and abiotic factors have already known. Physiological attributes may easily change with environmental factors. Vegetation duration also significantly influence plant growth and development. Dong et al. [8] and Menske [28] indicated that vegetation duration had significant effects on development and differentiation of physiological attributes.

Karadavut et al. [29] reported that leaf area increased in time in all genotypes, but leaf area index, leaf growth ratio and relative growth ratio decreased in time. Researchers also indicated that vegetation duration had also significant effect on such changes. Karadavut et al. [30] indicated that relative growth ratio, net assimilation ratio and leaf area ratio exhibited faster increase during the initial growth stages and carbon accumulation ratios of leaf areas were quite high throughout the initial growth stages, but decreased later on. Present findings comply with those earlier reports. Despite the several attributes to determine plant growth and development, relative growth ratio is the most significant one [30]. However, this ratio alone is not sufficient. Leaf area index and net assimilation ratio could also be used together with relative growth ratio to assess plant growth and development.

Leaves are the assimilation organ of the plants, therefore, leaf growth and development is a significant issue for net assimilation ratios [31]. The greater the leaf development especially in initial growth stages, the greater the photosynthesis capability and resultant dry matter accumulation will be. Such a case will positively influence genotype performance [32]. Kozlawski et al. [33] pointed out the significance of dry matter accumulation instead of growth and development of seedlings. Present findings also revealed the significance of dry matter accumulation. Haase and Rose [34] also reported similar findings.

Present findings revealed significant differences in physiological characteristics of Narbonne vetch genotypes. Plants tend to produce leaves as much as possible during the initial growth stages to get greater sun exposure. Their capability in sun exposure will then significantly influence their efficiencies in other physiological processes. The present study was performed in two groups, thus it is for the benefit of the breeders to take the differences between the genotypes and between the cultivars into consideration. With regard to cultivation, timing of agronomic practices should also be taken into consideration since they may influence the effects and development of physiological factors.
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EFFECT OF CLINOPTILOLITE-RICH ZEOLITE ON THE PROPERTIES OF WATER BASED DRILLING FLUID

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ABSTRACT

Wellbore instability is an important problem in drilling operation, especially in high temperature and pressure shale formations. This problem has been addressed using zeolite or other particles as drilling fluid additives. In the present study, the effect of clinoptilolite-rich zeolite (CZ) as an additive (in the range of 1 to 7 % w/v) on the rheological and filtration properties of the water based Na-bentonite drilling fluid was investigated. The chemical composition, crystallinity and the morphological properties of the plain drilling fluid and the CZ added drilling fluid were analyzed using X-ray powder diffraction (XRD), Fourier transform infrared (FTIR) and scanning electron microscope (SEM). The rheological properties such as apparent viscosity (AV), plastic viscosity (PV), yield point (YP) and gel strength of the CZ added drilling fluid were determined with a FANN 35 viscometer. The filtration properties were also examined using an API Filter Press. All physical measurements were carried out according to the American Petroleum Institute (API) standards. The results showed that the CZ was a good candidate as a drilling fluid additive due to the reduction in the loss of filtrate (30 %) and the increasing PV (33 %), AV (45 %), YP (62.5 %) and gel strengths (10-second 127 % and 10-minute 48.5 %).

KEYWORDS:
Clinoptilolite-rich zeolite, Water based drilling fluid, Rheological property, Filtration property

INTRODUCTION

Drilling fluid (also called "drilling mud") has many functions such as wellbore cleaning, cooling and lubricating of the bit and drill string, formation of an impermeable cake, checking high formation pressures and corrosion protection in drilling operations. Drilling fluids are generally categorized in three main classes, named water-based muds, oil-based muds, and air drilling fluid [1]. The water-based drilling fluid is frequently used and consists of mainly water, bentonite, viscosifier, fluid loss reducer and lubricants [2]. It is known that a high quality drilling fluid makes drilling operations easier and cost effective. For this purpose, many studies have been reported to improve the related properties of drilling fluids by using some additives, usually polymers, ore and nano-materials, at different compositions [3, 4, 5].

In the literature some industrial minerals have been applied as a mud additive to improve rheological properties of the drilling fluid. Recently, cationic-anionic organo-sepiolite is used as an additive and the results indicate that due to the surface characteristics, properties and the pore structure of the sepiolite, the organic surfactants are adsorbed onto the surface, improving the thermal stability and the rheological properties of the drilling fluid [6, 7]. Organomontmorillonite and organo-palygorskite (OPal) are some other industrial minerals which are usually preferred as rheological additives in oil-based drilling fluids [8].

Zeolite has been recently used to solve the wellbore instability problem relying also on its surface area, pore structure and surface properties. It improves the quality of mud cake and rheological properties, decreases the frictional resistance, minimizes the risk of pipe sticking and also prevents the reservoir from being damaged. Zeolites have different forms and chemical formulae which originate from various reactions of aluminum and silica containing minerals. They are the important industrial minerals which are used in a variety of fields such as pollution control, energy, agriculture, animal husbandry, mining and metallurgy [9].

Zeolites may be represented by the crystallographic unit cell formula:

\[ \text{Ma/n [(AlO}_2)_a (SiO}_2)_b \cdot x\text{H}_2\text{O} \]

Where M represents one or more cations such as Na, K, Mg, Ca, Sr, Li or Ba for natural zeolites and NH4, CH3NH3, (CH3)3NH, (CH3)4N, Ga, Ge and P for manmade zeolites; n represents the cation valence; the ratio of b:a is in a range from 1 to 5; and x represents the moles of water entrained into the zeolite framework [10].

In the present study, effects of CZ as an additive on rheological properties, mud cake thickness and water loss of a water based drilling fluid were investigated in detail. The filtration and the rheolog-
ical properties such as apparent viscosity (AV), plastic viscosity (PV), yield point (YP), gel strengths, of the plain water based drilling fluid and the CZ added drilling fluid were carried out according to the American Petroleum Institute (API) 13 B1 [11] recommendations for testing WBDF.

MATERIALS AND METHODS

Water-based drilling fluid is the most popular drilling mud used in the petroleum industry [12]. Water is the continuous phase, providing the initial rheological properties of the drilling fluid. In this study, firstly a water based drilling fluid (WBDF) system was prepared using fresh water, Na-bentonite (6 w/v %) (particle size lower than 75 \( \mu \)D) type) according to API standards 13A (API- 13A, 2004). The pH of the fluid was adjusted by sodium hydroxide (NaOH) (pH range of drilling mud 9.5 - 10). After preparation of the mud, the system was mixed for 30 minutes in total. This drilling fluid was used as an arbitration specimen. Secondly, the same drilling fluid was prepared and different amounts of CZ (1, 2, 3, 4, 5, 6 and 7 w/v %) was added into it. The mixture was kept mixing for 3 minutes and then aged for 16 hours (average temperature 25 °C). Conventional WBDF and CZ added WBDF formulations are given in Table 1.

The physical and chemical differences between the arbitration specimen and the CZ added drilling fluid were determined and compared with each other. The elemental compositions of the Na-bentonite and CZ were determined using XRF and the chemical compositions, crystallinity, functional groups and the morphological properties, as well as the change of the plain drilling fluid and the CZ added drilling fluid were investigated by X-ray diffraction (Rigaku MiniFlex 600 with Cu K\( \alpha \)) and LeO EVO40 scanning electron microscope. In addition, the BET (Brunauer-Emmett-Teller) surface area of the CZ was obtained from the adsorption isotherms by A Tri Star 3000 (Micromeritics, USA).

The filtration and the rheological properties such as apparent viscosity (AV), plastic viscosity (PV), yield point (YP), and gel strengths the drilling fluids were determined in line with the American Petroleum Institute (API) 13 – B1 recommendations. A FANN 35 viscometer was used to measure rheological properties of the drilling fluids. The filtration properties (filtrate loss and mud cake thickness) of drilling fluids was measured using an API filter press device.

RESULTS AND DISCUSSION

Characterization. The elemental compositions of the Na-bentonite and clinoptilolite-rich zeolite were determined by X-ray fluorescence (XRF) which are shown in Tables 2. As expected, the results showed that SiO\(_2\) and Al\(_2\)O\(_3\) are the main components in the structures and at the same time Na-bentonite of suitable for the API Specification 13A standard [12, 13].

The XRD patterns of the water based drilling fluid and 1 % and 7 % CZ additive drilling fluids are given in Figure 1.

The XRD pattern of the dried drilling fluid indicated that the structure is mainly composed of montmorillonite (with the characteristic peaks at 14.29 A° and 4.49 A°) and kaolinite and quartz were also observed [14, 15]. On the other hand, it was seen that with the increasing CZ concentration in the drilling fluid, the intensity of peaks increased. The two sharp peaks with highest intensity (Figure 1.c) could be attributed to clinoptilolite (JCPDS files card No. 025-134). The morphology of the CZ (Figure 2.a), the drilling fluids (Figure 2.b), and 1 % and 7 % CZ drilling fluids (Figure 2.c and Figure 2.d, respectively) are evaluated using SEM.

Figure 2.a showed the irregular shape prismatic cristobalite in the boundary of a pore which were filled with authigenic and euhedral crystals of clinoptilolite [16]. While the concentration of CZ increasing in the drilling fluid, the clinoptilolite particles were observed in the mud, supporting with the XRD results. The BET surface area of the CZ was found to be 31.249 m\(^2\)/g.

### TABLE 1

<table>
<thead>
<tr>
<th>Materials</th>
<th>WBDF</th>
<th>Clinoptilolite-rich Zeolite added WBDF (w/v %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>CZ (g)</td>
<td>-</td>
<td>4.06</td>
</tr>
<tr>
<td>Fresh water (g)</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Bentonite (w/v %)</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

### TABLE 2

The elemental composition of the Na-bentonite and CZ.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Na(_2)O</th>
<th>MgO</th>
<th>Al(_2)O(_3)</th>
<th>SiO(_2)</th>
<th>P(_2)O(_5)</th>
<th>K(_2)O</th>
<th>CaO</th>
<th>TiO(_2)</th>
<th>MnO</th>
<th>FeO(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-Bentonite</td>
<td>2.48</td>
<td>2.44</td>
<td>20.9</td>
<td>65.85</td>
<td>-</td>
<td>0.75</td>
<td>1.44</td>
<td>0.29</td>
<td>-</td>
<td>5.85</td>
</tr>
<tr>
<td>CZ</td>
<td>0.7</td>
<td>1.6</td>
<td>13.5</td>
<td>71.9</td>
<td>&lt;0.1</td>
<td>3.9</td>
<td>5.5</td>
<td>0.3</td>
<td>&lt;0.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>
FIGURE 1
XRD pattern of dried drilling fluids: (a) plain fluid; (b) fluid containing 1% CZ and (c) fluid containing 7% CZ.

FIGURE 2
SEM images: (a) pure CZ, (b) plain fluid, (c) fluid containing 1% CZ; and (d) fluid containing 7% CZ.

TABLE 3
Rheological results of the drilling fluids

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WBDF</th>
<th>Clinoptilolite-Rich Zeolite added WBDF (w/v %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% 1</td>
</tr>
<tr>
<td>600 (rpm)</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td>300 (rpm)</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>Plastic viscosity (cp)</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Yield Point (lb/100 ft²)</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Apparent viscosity (cp)</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Gel at 10-second</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Gel at 10-minute</td>
<td>35</td>
<td>37</td>
</tr>
</tbody>
</table>
Rheological Results. Flow characteristics (viscosity and gel strengths) of the drilling fluid were determined with a FANN 35 viscometer by dial readings at 300 and 600 rpm in terms of shear rate and shear stress.

The viscosities are defined as follows:
- \( \text{PV} = \phi_{600} - \phi_{300} \)
- \( \text{PV} = \) plastic viscosity, cp
- \( \text{AV} = \frac{1}{2} \phi_{600} \)
- \( \text{AV} = \) apparent viscosity, cp
- \( \text{YP} = \phi_{300} - \text{PV} \)
- \( \text{YP} = \) yield point, lb/100 ft²

The experimental results are given in Table 3. It is shown that the rheological properties in terms of plastic viscosity (PV) and yield point (YP), which are important for good cuttings suspension characteristics when drilling is stopped, was improved with addition of the CZ. The maximum value was recorded for 7 % w/v. CZ added into the drilling fluid (see Figure 3).

After that there was no any change was observed. In addition, the gel strengths versus the CZ concentration graph is given in Figure 4.

It is known that the gel strengths are correlated to the pressure which is required to initiate flow after the mud [1-6]. As shown in Figure 3, the apparent viscosity increased with increasing CZ concentration in the structure. This is a very good result for thixotropy property of the drilling fluid.

Filtration Results. Filtration is a very important property for hole stability. Low filtration is highly desired for water based drilling fluids which contributes to wellbore stability as they will not penetrate the formation and/or can deposit quality filter. Figure 5 shows the filtration loss characteristics of
Minimum filtration loss was obtained at the addition of the 7 % w/v CZ and after that there was no any change recorded. The same observation was obtained in the rheological section. Therefore in the present study, the 7 % w/v CZ in the drilling fluid was considered as a maximum concentration for the water based drilling fluid. It was also found that the CZ addition decreases the filtrate loss of drilling mud by about 30%. The loss of filtration resulting from naturally fractured, crevices and channels [17] is important in terms of the cost and the required time for the drilling to reach the target depth [18].

Thick filtrate cake is not desirable for a stable and low cost wellbore operation. The cake thickness versus the CZ concentration in the drilling fluid is plotted in Figure 6, indicating that the cake thickness increased with increasing CZ dosage.

This showed that the addition of CZ into the drilling fluid might generate a impervious barrier which decreased the filtration loss. The results of the filtrate loss and cake thickness are given in Table 4.

Tight borehole along with increased cake thickness and the damage to the rig pump resulting from pressure increase cause the undesirable situations such as high cost and long rig time [19]. A thick mud cake is not favorable for drilling operation, however a lower filtration loss can decrease the cost of drilling operation.

CONCLUSIONS

In the present study, the effects of CZ on rheological and filtration properties of the water based drilling fluid were investigated. The experimental results showed that CZ is a promising additive for improvement of the rheological properties of the drilling fluids, especially plastic viscosity, apparent viscosity, yield point and gel strengths.

The effects of the CZ addition on the rheological properties of the drilling mud as below:
- increasing plastic viscosity (PV) by 33%,
- increasing apparent viscosity (AV) by 45%,
- increasing yield point (YP) by 62.5 %,
- increasing 10-s and 10-m gel strengths by 127 % and 10-minute 45.6 % respectively.

The results of API filtration test suggested that filtration loss decreased with increasing CZ concentration while mud cake thickness increased.

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INVESTIGATION OF ELECTROMAGNETIC PROPERTIES OF GLASS FIBER REINFORCED EPOXY COMPOSITES CONTAINING PAN NANOFIBERS WITH MWCNT/GRAPHENE ADDITIVE

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ABSTRACT

In this work; the differences between of electromagnetic properties of glass fiber reinforced (GFR) -epoxy laminate composites with various nanoparticle doped polyacrylonitrile (PAN) fibers were investigated. The laminated composites that made in specific molds were obtained by putting woven glass fibers between every PAN nano fiber mats. PAN nano fibers have three different nano modified configurations which pure, added multi-walled carbon nanotubes (MWCNTs) and added graphene. The prepared solution for electrospinning process contains 10wt% PAN. The solvent dimethylformamide was preferred to use as an excellent solvent for PAN materials. The morphologies of the PAN nano fiber mats were viewed by scanning electron microscope (SEM). The radar absorption efficiencies of the composites in the individual and different combinations were measured in the X band frequency range (3-10 GHz). Experimental results show that the produced graphene-added fiber laminated composite material has excellent microwave absorption effect at many points in the 3-10 GHz band.

KEYWORDS:
Electrospun, Polyacrylonitrile (PAN) Nano-Fibers, MWCNTs, Graphene, Radar Absorption.

INTRODUCTION

Nowadays, polymer-matrix composites reinforced with carbon fibers are increasingly used for many applications, i.e. aviation sector, space, automotive, defence industry, ship industries and railway industries to solve main troubles such as cost, weight, strength and resistance. Composite materials are used in the body coatings of these sectors and nanotechnology has been incorporated into composites to achieve good results in terms of mechanical properties, electrical conductivity or insulation, radiation protection, UV light resistance, wear and fatigue life. Expected properties of composite materials; mechanical properties such as low impact resistance, strength-toughness, electrical conductivity and thermal strength of the workings are better than the average. However, it is also important that they are light and flexible. To improve these properties, reinforcing materials are used fiber such as glass, carbon, aramid and basalt in the production of composite and the polymer matrix can be combined with organic or inorganic compounds to design multi-phase matrices such as nano composites. Amongst them, multi-walled carbon nanotubes (MWCNTs) and graphene are especially promising for targeting multi-scale reinforcement as they can be combined with micrometer-scale fibers [1-4]. These fibers are generally used in polymers and the epoxy resin is preferred as the connecting element. In laminated composite structures, physical disunities and mismatches can cause problems like delamination. Delamination means separation of laminated plies that stuck together in a composite structure. Several methods have been developed to solve this problem [5-8]. The nanoparticle reinforcement of laminated composite materials are straightly connected to reinforce the interlaminar region, and many studies endeavors have viewed that the out-of-plane properties of such composites are increased significantly [9-11]. Nowadays, electrospinning method is one of the best generally used methods for producing nanofiber materials [12-13]. Electrospinning method provides production of nanofibrous mats with a single fiber diameter ranging from nanometer to micrometer that allowing the combining of different type of nanostructures [14-15]. Polyacrylonitrile, Polyamide, Polyvinyl Chloride, polyurethane, Chitosan and so on. materials can be electrospun to obtain nanofibers [16-18]. Nanoparticle reinforced nano fiber composite materials are used in many fields due to their extraordinary mechanical and electrical properties compared to pure fiber composite materials. Particularly, the carbon nanotube or graphene modified nano composites electrospun fibers have a significant potential of energy absorption due to the perfectly mechanical properties of MWCNTs or gra-
phene such as high specific strength, flexibility, resilience, and aspect ratio [19]. CNTs and carbon nanostructures, for example; Carbon Nanoparticles (CNPs), Graphene Nano Platelets (GNPs) and others, which combine unusual electrical, thermal, mechanical and chemical properties make them very valuable in many applications [20-23]. Nowadays, Daelemans et al. [24] research arrayed nanofibrous structures as interlayers to identify hardening mechanisms during Mode II crack increase propagation. In their research revealed that varied micro-mechanic toughening mechanisms revealed to the fracture toughness interconnecting on the nanofiber orientation. Saghafi et al. [25] declared to increase of the modes I and II energy release rates (GI and GII) as 21% and 56% with the joining of electrospon ny- 
lon 6,6 and polycaprolactone (PCL) nanofiber mats as interlayers in the mid-plane of unidirectional glass/epoxy laminates. Lutz et al. reported that they investigated the synergies between carbon nanotubes and conventional carbon fibers to design multi-scale and multifunctional composites. In their studies were compared with ex-PAN carbon fibers and in enlarged form CNTs fibers which direct added to the epoxy-carbon fiber composites. They reported Inter- 
facial shear strength (IFSS) improvement of 150% was obtained for sized carbon fibers. Furthermore, they added MWCNTs grown on carbon fibers demonstrate an IFSS improvement of 87% and 104% depending on the neat carbon fiber and the stress transfer efficiency is improved by 8 times [26]. Research has demonstrated that; MWCNTs the load carriers bear ballistic transport and additionally have electrical conductivity (1.85x10^3 S.cm^-1) [27-33]. Current densities that nearing 10^9 A° cm^-2 have also been recorded [34-39]. Similarly; the graphene, which with a single layer of sp2 hybridized carbon atoms and perfect electron mobility in π conjugation system, supply a possibility for the electron transmis- 
sion in graphene composites [40-41].

Radar is the abbreviated word of "radio detection and ranging" and uses electromagnetic (EM) waves to detect faraway materials [42]. Since EM waves used by radars move at the speed of light to detect airplanes, the speed of modern aircraft is slower than the speed of the radar. Moreover, over the years, radar technology has been greatly developed with the use of high-power broadband transmitters, so the technologies that "hidden" technologies use for radar detection have become even more important [43-46]. Reducing the detectability of targets, such as planes or rockets, is an important parameter that will affect not only task success, but also survival in enemy territory [47]. Detectability of targets is a measure of the radar cross section (RCS). Decreasing the RCS decreases the detectability to the same extent. In general, three categories have been proposed techniques for RCS reduction: shaping of the target, radar absorbing material (RAM) and radar absorbing structures (RAS) [48]. In the beginning, many researchers focused on the reduction of RCS by shaping and RAM. However, in today's world, studies on researching radar absorbing structures (RAS) on fiber reinforced polymeric composite materials become popular research field. The electromagnetic properties of the fiber reinforced polymeric composites can be increased since some electromagnetic powders such as carbon black, ferrite, carbonyl iron, etc. can be added to the matrix of the polymeric composites, and accordingly the RAS having the desired performance can be manufactured [49-53].

Wang and Zhu reported that they fabricated; high performance radar absorber structures with poly- 
imide as matrix, SLF-SiCf as reinforcement and carbon filler (CNTs and GNP) as absorbent. As a re- 
sult of their work; the complex permeability of GNP- SLF-SiCf / polyimide composites was reported to be higher than that of CNTs-SLF-SiCf polyimide composites. In addition; although the attenuation constant of GNP-SLF-SiCf / polyimide composites is higher than that of CNTs-SLF-SiCf / polyimide composites, weak impedance matching properties have also reported that they reduce absorbent properties. Finally; According to the complex permeability tested, the double layer composites reported that they had designed and simulated, the thickness of the slip- pery layer and the impedance layer being 1.5 mm to 1.5 mm, and the minimum reflection loss value reached to ~29.8 dB at 8.7 GHz [54].

In this study, nanoparticle doped fiber layer composite materials were developed to improve the radar absorbing structure (RAS). The glass and nano- 
ofiber reinforced laminated composite plates were produced by using special mold. The PAN nano- 
fibers which were added nanoparticles such as MWCNTs and graphene used in the produced com- 
posites were obtained by electrospinning method. Produced composites have three different PAN fiber configurations; pure PAN fibers, MWCNTs doped PAN fibers and graphene doped PAN fibers. Their morphological structures were examined with SEM. Radar absorption efficiencies of materials for radar processing was measured at a frequency range of 3-18 GHz.

**MATERIALS AND METHODS**

Firstly part, PAN pellets were dissolved in N,N-Dimethylformamide (DMF) with a concentra- 
tion of 10% wt. PAN pellets were supplied from Sigma Aldrich. The solution was kept in an ultrasonic bath for about 8 hours to provide homogeneity. Secondly part, PAN/DMF (1:9 wt/wt) solutions were prepared by mixing on a magnetic stirrer for 3 h at room temperature to produce nanofibers. Next, 1% graphene nanoparticles were added to the first solu- 
tion by weight of the PAN, and 1% by weight of MWCNTs nanoparticles were added to the second
solution. And then, these solutions were put in an ultrasonic bath for about 8 hours to provide homogeneity. Homogenized solutions were sequentially drawn into a 10 mL syringe and placed in the pump.

The electrospinning unit be composed of a high voltage power supply (Glassman HV 0 to 50 kV), grounded collector plate (aluminium sheet) and a syringe pump (Univertor 801 Syringe Pump). The voltage applied during the electrospinning process is 25 kV, the feed rate is 4.00 mL/h and the temperature is 23 °C. The distance between the cylindrical collector and the needle was set to 20 cm. SEM images of the obtained pure PAN nanofibers, graphene added PAN nanofibers and MWCNT added PAN nanofibers are given in Fig. 1.

FIGURE 1
SEM images of a) pure PAN nanofiber mats, b) MWCNTs added PAN nanofiber mats, c) Graphene added PAN nanofibers mats

The epoxy resin codenamed L160/H160 which was provided from Dost Kimya Company was used. The PAN/glass fiber reinforced epoxy composites are layered as shown in Fig. 3 and then cast into special molds as shown in Fig. 2. The three different laminated composite specimens were produced. The first series of composites were made with pure PAN fibers. The second and third series of composites were obtained by adding MWCNTs and graphene respectively to PAN fibers. For the nanofiber modified ones; one nanofiber mat was interleaved in two glass fiber layers and then placed into epoxy and three nanofiber mats were interleaved in two glass fiber layers and then placed into epoxy as well, whose simplified cross-sections are presented in Fig. 3. Pure PAN nanofiber mats are called “PPNF Mats.”. MWCNTs added PAN nanofiber mats are called “CNTPNF” and graphene added PAN nanofiber mats are called ”GPNF” in those figures. Also, the composite materials produced are shown in Fig. 4.

FIGURE 2
Special mold used in composite production

FIGURE 3
The cross-section of the layers produced in the composite; (a) pure polymer, (b) MWCNTs modified polymer, (c) graphene modified polymer
Electromagnetic Response of Composite Plates- Grounded by Metallic Sheet. Microwave absorption of a composite material is related with both impedance matching between incident wave side and material and lossy characteristic of composite medium. The absorption coefficient of a material can be retrieved by using reflection and transmission of incident wave from the composite medium. For a highly absorber medium, these transmission and reflection values needs to be around zero. The remaining power of incident wave is absorbed by the composite medium. Hence, the absorbed power in the material can be obtained depending on the reflected and transmitted power;

\[ A(\omega) = 1 - R(\omega) - T(\omega) \]

The reflected and transmitted powers have been measured by using two horn antennas in the system. Since, the antennas measure scattering parameters, reflection coefficient and transmission coefficients are calculated from the measured scattering parameters of \( S_{11} \) and \( S_{21} \) by the equations of \( R(\omega) = S_{11}^2 / 2 \) and \( T(\omega) = S_{21}^2 / 2 \). As can be seen from these equations both reflection and transmission coefficients are frequency dependent. Since, scattering parameters are also dependent to frequency of incident wave. Therefore, the frequency dependent absorption magnitude of the power in the structure, can be written in terms of scattering parameters;

\[ A(\omega) = 1 - S_{11}^2 (\omega) - S_{21}^2 (\omega) \]

The square definition of the scattering parameters stem from the relation between measured electric field collected by antenna and power transformation. Whereas scattering parameters are in terms of field, power is the square of the measured field.

The minimization of the measurement process and reduction of errors have been provided by placing metallic sheet to the backside of the sample composite. In this case, the reflection coefficient measurement is sufficient to evaluate the absorptivity. Since, the transmitted wave magnitude and scattered electric field value will be around zero for microwave frequency range. This is due to exact inverse electric polarization of metallic sheet and overall incident wave will be reflected by this sheet. Therefore, the absorbed power is only related with the reflected wave coefficient and the equation can be simplified;

\[ A(\omega) = 1 - S_{11}^2 (\omega) \]

The measurements have been realized by using two horn antennas. One of these antennas is the source antenna which generates the incident wave and the second antenna situated near it measures the reflected electric field value. Hence, transmitter and receiver antennas are placed one side and metallic sheet is placed to the other side of the sample. Each antenna is connected to a vector network analyzer operating in the frequency range of 3-18 GHz. The measurement medium is given in Fig.5.
metallic sheet at peak levels is clear. The frequency shift results from the difference between electromagnetic behaviour of graphene and air. It is well known that the wave velocity of EM signal is related with both relative permittivity and permeability of a medium. This scattering parameter is related with reflection coefficient and hence there is no transmitted wave, absorption depends on this reflection coefficient. The reflection coefficient of the graphene+metallic sheet sample is significantly lower than that of metallic sheet at the peak points. It can be observed that there are eleven peak points for each sample between 3-10 GHz. The reflection magnitudes and calculated approximate absorptions has been given at the related frequencies in Table 1. The maximum value of absorption is seen at fifth peak. In this frequency point, the scattering values of reflection is 0.60 and 0.46 for graphene+metallic sheet and metallic sheet, respectively. Therefore, the absorption level is evaluated by the equation of $A(\omega)=1-S11^2(\omega)$. As it is mentioned, the higher absorptivity depends on higher impedance matching between the boundaries of mediums. The absorption mechanism operates in the step that, EM wave enters to the sample material, travel in the medium and exactly reflected from the backside metallic sheet. The higher loss tangent of the material results in stronger absorption of the penetrated wave. Some of the waves which does not reflected from the first penetration or scattered when travelling between front and backside also return to the antenna as a reflected wave. Beside this, a low part of the wave produce a surface wave and absorbed by backside metallic sheet. In addition, around 15% absorption is also observed at the first and sixth peaks. Hence, a graphene based layer can be used as an absorber materials. A negligible absorption has been seen at some peak points such as seventh peak. This very low level of absorption at the related frequency points give opportunity to the researches to design highly efficient novel radomes based on graphene.

The reflection magnitude comparison is also investigated between MWCNTs+metallic sheet and metallic sheet in the same frequency range (Fig. 7). Exactly same measurement system has been used for the samples responses. It can be seen from the reflection responses of the samples, whereas the MWCNTs+metallic sheet reflection coefficient is significantly lower than that of metallic sheet at some frequencies (3.5GHz, 5.8GHz and 6.3GHz), the reflection responses of both samples are nearly same with each other. The absorption ratio of the MWCNTs is considerable at the mentioned frequency points. Hence, the improvement of the absorption can provide to researches to design dielectric microwave absorbers as a stealth technology. In addition, the minimum change of the reflection coefficient give chance to design novel radome applications. Since, the two characteristics of a radome are strength and low loss. The electromagnetic response

![FIGURE 7](image)

**FIGURE 7**

Measured reflection coefficient of MWCNTs grounded by metallic sheet and metallic sheet

<table>
<thead>
<tr>
<th>Peak</th>
<th>Frequency (GHz)</th>
<th>Reflection</th>
<th>Frequency (GHz)</th>
<th>Reflection</th>
<th>Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak-1</td>
<td>3.40</td>
<td>0.780</td>
<td>3.30</td>
<td>0.74</td>
<td>15.52</td>
</tr>
<tr>
<td>Peak-2</td>
<td>3.92</td>
<td>0.480</td>
<td>3.90</td>
<td>0.57</td>
<td>0.46</td>
</tr>
<tr>
<td>Peak-3</td>
<td>4.43</td>
<td>0.250</td>
<td>4.41</td>
<td>0.46</td>
<td>0.58</td>
</tr>
<tr>
<td>Peak-4</td>
<td>5.01</td>
<td>0.49</td>
<td>5.75</td>
<td>0.46</td>
<td>23.19</td>
</tr>
<tr>
<td>Peak-5</td>
<td>5.82</td>
<td>0.60</td>
<td>6.10</td>
<td>0.46</td>
<td>15.97</td>
</tr>
<tr>
<td>Peak-6</td>
<td>6.30</td>
<td>0.58</td>
<td>6.70</td>
<td>0.48</td>
<td>3.38</td>
</tr>
<tr>
<td>Peak-7</td>
<td>7.10</td>
<td>0.46</td>
<td>7.30</td>
<td>0.45</td>
<td>9.30</td>
</tr>
<tr>
<td>Peak-8</td>
<td>7.70</td>
<td>0.52</td>
<td>7.85</td>
<td>0.45</td>
<td>0.47</td>
</tr>
<tr>
<td>Peak-9</td>
<td>8.30</td>
<td>0.48</td>
<td>8.60</td>
<td>0.47</td>
<td>0.44</td>
</tr>
<tr>
<td>Peak-10</td>
<td>9.30</td>
<td>0.46</td>
<td>9.35</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Peak-11</td>
<td>9.80</td>
<td>0.48</td>
<td>9.85</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>
of the MWCNTs backed by a metallic sheet must provide highly matching intrinsic impedance with free space and strong lossy characteristics at the frequency point of significant absorption. In future applications, the frequency independency of the structure must be provided both application types of absorber and radome.

The reflection scattering values of PAN+metal and metal samples have also been measured at the same frequency region as can be given in Fig.8. The measurements have been carried out in an anechoic chamber same with the studies mentioned above. It can be seen that there is a frequency shift at entire frequency range due to PAN sample placed front side of metal. This shift results from the phase velocity shift of EM wave in the sample with a different relative permittivity and permeability with respect to free space. Beside this, the reflection coefficient of PAN+metal is significantly lower than that of metallic sheet especially at some frequency point such as 5.1GHz, 7.8GHz and 7GHz. Since the reduction of the reflection demonstrates the frequency dependent absorption characteristic of the PAN sample backed by metallic sheet. The realization of the significant absorption depends on both impedance matching between two medium and strong lossy characteristic at the related frequencies. Therefore, the PAN+metal system can be used in microwave absorber systems. At some frequency points, the existence of PAN sample has not changed the reflection characteristic of the metallic sheet. This response indicates that the electric and magnetic loss of the sample is at a negligible level. Hence, this sample can be used for radome applications by taking into consideration of mechanical properties.

![FIGURE 8](image)

**FIGURE 8**
Measured reflection coefficient of pure PAN fibers grounded by metallic sheet and metallic sheet

**CONCLUSIONS**

In this study, the electromagnetic absorption and reflection properties of pure PAN/epoxy composite mats, MWCNTs doped PAN/epoxy composite mats and graphene doped PAN/epoxy composite mats were investigated. It was determined that pure PAN/epoxy composite mats have absorption characteristic, but not more than graphene doped PAN/epoxy composite mats. It is determined that graphene doped structure has certain absorption characteristic up to 23.19% at a constant frequency. In addition, other high absorption frequency points have also been observed between 3-10 GHz. The broadband absorption can be realized by using cascaded graphene doped layers. It is observed that compared the reflections coefficient of MWCNTs doped PAN/epoxy composite mats that combined with the metallic sheet to metallic sheet were almost the same in some points in the frequency range. Hence, it can be emphasized that MWCNTs based nano composites can be used to design novel radomes and materials for stealth technology.

**REFERENCES**


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CHANGING APPLE PRODUCERS’ BEHAVIOUR IN PEST CONTROL THROUGH PHEROMONE TRAPS: THE CASE OF BALKIRI VILLAGE IN EGIRDIR DISTRICT OF ISPARTA, TURKEY

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ABSTRACT

The aim of the study was to address effects of pheromone traps using on changing apple producers’ behaviour in pest control and determine their attitudes to biotechnical control. The study was carried out in the village of Balkiri in Egirdir district in Isparta, Turkey in 2016, based on data collected through face-to-face interviews with 100 apple producers corresponding to 65% of the total apple producers in the village that has been one of the pilot areas with a state support scheme for pheromone traps for codling moth control in apple production since 2010. Fifty farmers received state support in 2014-2015 in the village and all of these were involved in the research study, in addition to 18 farmers who used pheromone traps on their own initiative and 32 farmers who used only chemical pest control methods. The most important factor was its effects on apple yield, followed by easy implementation and affordable price. The majority of those who used pheromone traps but did not benefit from the state support schemes stated that they did not use it because was not worth the bother of applying for the support and dealing with the bureaucracy. However, all producers were satisfied with the results of the pheromone traps, as their input cost was low and they increased the quality of the product. The study is that there was a significant decrease in the use of chemicals by farmers even though only three years had passed. Not only the farmers who were involved in the interviews but most farmers in fruit production tend to over apply pesticides. The results show that most of the apple producers sprayed between five and eight times, and some of them even more than eight times. However, the use of pheromone traps contributed to a significant decrease in the number of sprayings. While only one farmer sprayed one time before using pheromone traps, the number of farmers in this first group increased to 37 farmers, corresponding to more than half of the total number of farmers. While there were 15 farmers in the third group who sprayed more than eight times before using pheromones, no farmer left this group after pheromone trap use. Considering that overuse and misuse of agricultural inputs and specifically of pesticides is very common high in the region, this is not a final achievement but a good start in changing farmers’ behaviour.

KEYWORDS: Apple, biotechnological control, pest control, codling moth, pheromone traps

INTRODUCTION

Apples have the largest production area of any fruit in the world. In 2016, the total apple production of the world was 89 329 179 tonnes in an area of 5 293 340 hectares. World apple production has doubled in twenty-five years. The biggest share of this change comes from China, where apple production has increased by ten times. China has rapidly increased its apple production area and production, and now provides half of the world’s production, an increase from 10% in the 1990s. During the same period, France, Germany and USA have significantly lost their share in world production, although their production amount has fallen only slightly. Countries which have increased their production and kept their share in world production are Argentina, Brazil, Turkey and the Russian Federation, while Poland has increased both its production and its share in the world. Turkey and Poland’s apple
production are close to each other but Poland has advanced slightly since 2014, and is now in ahead of Turkey. Recently, Turkey has been the fourth biggest apple producer, with a share of 3% of world production (Table 1).

The changes in apple production are explained by changes in production area, technology, yield and consumer demand. In the case of China, where apple harvesting areas have increased by 46%, production by 926%, and yield by 604% between 1990 and 2016, it is mostly through advanced technology and high yield. Demand-oriented changes in apple production have emerged, as changes in income, pleasures, living conditions and healthy nutrition affect consumer preferences. The main factors which determine consumer demand for apples are price, income level, geographic area and conditions, food safety issues and supply-related factors such as technology, new varieties and product diversification.

The most favourite apple varieties in the world can be listed as follows: Golden Delicious, Red Delicious, Gala, Fuji, Granny Smith, Idared, Jonagold, Braeburn, Pink Lady and Jonathan [2]. Geographical area and the level of economic development of countries affect consumers’ apple preferences.

Previous studies referred to success in the world market in two areas: finance and an effective marketing system [3]. Taking into account these two areas, Turkey cannot be considered as a competitive country for apples. In terms of the internal market, although Turkey is an important apple producing country, most of its production is still conducted by means of conventional production methodologies. The small scale of agricultural enterprises and apple orchards make the transition to intensive production methods difficult, but it is observed that new plantations are being established in accordance with intensive cultivation methods, and that new varieties are being brought into cultivation [2].

Total apple production in Turkey is about three million tonnes (2 925 828 tonnes in 2016, 3 032 164 tonnes in 2017 and an average of 2 759 256 in the last decade). Despite the fact that apples can be cultivated everywhere in Turkey, the provinces where apple production is developed in a commercial sense are, in order of volume of production, Isparta, Karaman, Niğde, Denizli and Antalya. Isparta stands out not only in production but also for storage, processing and R&D capacity [2]. The province has attracted attention in the apple industry as it merges small conventional apple farms with newly established big modern orchards, university and public research facilities and private sector initiatives. Isparta has 23 151 hectares of apple orchard area and produces 20% of the total apple production of the country [4]. However, the Isparta apple has not yet gained a reputation on the market or in consumers’ recognition.

Intensive agricultural production in the region has brought with it the intensive use of pesticides. According to a study conducted in 2010 in the region, the pesticide group most used in Isparta with an important place in apple production is fungicides, followed by insecticides and acaricides, and common fungicides in the region were diniconazole, copper sulphate, pencyclocazole, propinb, trifloxystrobin and metallic copper; commonly used insecticides are deltamethrin, thiacyclorid, endosulfan, cypermethrin, methidathion, diazinone, methyl-parathion, chlorpyrifos, phosalone and carbosulfan, and widely used acaricides are fenprozimate and propargite [5]. However, some of these substances were forbidden latterly (Appendix 1). Currently, producers in the study area use mostly deltamethrin, thiacyclorid, indoxacarb+abamectin, bifenthrin and thiacyclorid.

### TABLE 1

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>4 331 922</td>
<td>14 017 142</td>
<td>20 437 065</td>
<td>24 016 881</td>
<td>33 265 186</td>
<td>42 614 319</td>
<td>44 448 575</td>
</tr>
<tr>
<td>USA</td>
<td>4 380 000</td>
<td>4 798 282</td>
<td>4 799 416</td>
<td>4 384 832</td>
<td>4 210 290</td>
<td>4 537 693</td>
<td>4 649 323</td>
</tr>
<tr>
<td>Poland</td>
<td>812 340</td>
<td>1 288 289</td>
<td>1 450 376</td>
<td>2 074 951</td>
<td>1 877 906</td>
<td>3 168 818</td>
<td>3 604 271</td>
</tr>
<tr>
<td>Turkey</td>
<td>1 900 000</td>
<td>2 100 000</td>
<td>2 400 000</td>
<td>2 570 000</td>
<td>2 600 000</td>
<td>2 569 759</td>
<td>2 925 828</td>
</tr>
<tr>
<td>Italy</td>
<td>2 050 070</td>
<td>1 940 007</td>
<td>2 232 100</td>
<td>2 192 000</td>
<td>2 204 972</td>
<td>2 473 608</td>
<td>2 455 616</td>
</tr>
<tr>
<td>Russia</td>
<td>1 200 000</td>
<td>1 832 000</td>
<td>1 786 000</td>
<td>n.a.</td>
<td>992 000</td>
<td>1 612 700</td>
<td>1 843 544</td>
</tr>
<tr>
<td>France</td>
<td>2 326 000</td>
<td>2 063 809</td>
<td>2 130 274</td>
<td>1 829 166</td>
<td>1 751 269</td>
<td>1 968 628</td>
<td>1 819 762</td>
</tr>
<tr>
<td>Brazil</td>
<td>543 515</td>
<td>686 373</td>
<td>1 153 269</td>
<td>850 535</td>
<td>1 279 124</td>
<td>1 264 651</td>
<td>1 049 251</td>
</tr>
<tr>
<td>Germany</td>
<td>2 222 019</td>
<td>573 300</td>
<td>3 136 800</td>
<td>891 402</td>
<td>834 960</td>
<td>973 462</td>
<td>1 032 913</td>
</tr>
<tr>
<td>Argentina</td>
<td>975 600</td>
<td>1 416 000</td>
<td>833 322</td>
<td>1 206 210</td>
<td>720 000</td>
<td>950 000</td>
<td>967 847</td>
</tr>
<tr>
<td>Top 10</td>
<td>19 222 351</td>
<td>28 612 829</td>
<td>38 372 031</td>
<td>37 959 232</td>
<td>47 736 583</td>
<td>59 918 987</td>
<td>62 779 832</td>
</tr>
<tr>
<td>The rest</td>
<td>21 823 634</td>
<td>20 237 870</td>
<td>20 754 573</td>
<td>23 947 564</td>
<td>23 455 562</td>
<td>26 303 263</td>
<td>26 549 347</td>
</tr>
<tr>
<td>World</td>
<td>41 045 985</td>
<td>48 850 699</td>
<td>59 126 604</td>
<td>61 906 796</td>
<td>71 192 145</td>
<td>86 222 250</td>
<td>89 329 179</td>
</tr>
</tbody>
</table>

Source: [1]
Apple codling moth - Cydia pomonella L., is one of the most damaging pests of the apple orchards, can develop also the third generation, the adults flight extent became wider, the attack of the second generation of larva is more aggressive and under some particular orchard conditions resistance to various groups of insecticides are noticed [6]. Previous studies in the region and other important apple producing regions of Turkey show that producers overuse chemical treatment in order to control codling moths and other pests and diseases [7, 8, 9]. This causes severe food safety problems and environmental pollution. This fact and threat together make it necessary to find alternative methods of pest and disease control.

The best alternative control methods which can be used in combination with chemical control are biotechnical control methods. Using these methods in line with the principle of integrated control helps to increase the success of plant protection activities and contributes to the preservation of the natural balance and to the solution of the problem of pesticides in plant products. In Turkey, the Ministry of Agriculture and Forestry has supported biological and alternative control methods since 2010. The state supports biotechnical control targets to expand integrated control in the long term, to expand the biotechnical control sector and to specialise in this area. The use of pheromone for controlling pest insects requires three items: a pheromone chemical, a trap, and a support to hang the trap in the field. Technically sex pheromones can be used in three principal ways which are detection and monitoring, mass trapping and mating disruption. Pheromone traps are very sensitive that they attract insects present at very low densities [10].

As of 2017, this support scheme covers certain vegetables (tomatoes, peppers, eggplants, cucumbers and zucchini) in greenhouses, tomatoes in open fields, grapes, olives, apricots, pomegranates and apples. Support for apple production includes pheromone diffusors. Pheromone traps are successful methods for controlling the codling moth, which causes economic loss in apple production. There are researches previously conducted on pheromone traps using in apple production which addressing damage severities, environment protection and economic aspects. For instance, in a study conducted in the study area, fluctuations of codling moth population were monitored with pheromone traps and found that damage severity was 6-38 % for early apple cultivars, 13-74% for mid-season season apple cultivars and 36-77 % for late season apple cultivars [11]. Another study was carried out in Romania using mating disruption method where ISOMATE C pheromone dispensers (E,E-8,10-dodecadiene-1-ol 52.4%, 1-dodecanol 30.6%, 1tetradecanol 7.1%) combined with a reduced number of treatments with insecticides were used to control apple codling moth. The study results reported that mating disruption is an efficient alternative method to control apple codling moth which contribute to environment protection and cleaner high quality fruits production [6]. Kilic et al. [12] compared trapped codling moths through sex trap which was placed in the middle of the each treatment and evaluated the number of damaged and undamaged fruits. Results reported that 2 adult males were trapped in pheromone treatment, 58 adult males were trapped no pesticides treatment and 15 adult males were trapped in last part in which registered agrochemicals were applied; in the next year of the study 0, 26 and 3 adult males were trapped respectively. They also found that 17.2% damaged fruit in pheromone treatment, 3.7% in registered agrochemicals treatment and 40.9% in no pesticide treatment in the first year while there were 28.7, 3.8 and 62.1% damaged fruit was counted respectively in the second year of the study. Having pheromone effects as 57.5% and 52.8% successively were stated promising by the authors [12].

However, in this study we addressed only the effects of pheromone traps on changing apple producers’ behaviour in pest control because the aim of the study was to determine the attitudes to biotechnical control of the apple producers in the village of Balkiri in Egirdir district in Isparta, Turkey, and to analyse the preliminary results of biotechnical implementation started after the implementation of state support, in order to develop suggestions for policy decisions.

RESEARCH

Research Area. Balkiri Village. Balkiri village is in the Egirdir district of Isparta in the West Mediterranean Region of Turkey. It is located 12 km from the administrative centre, and has 240 households and 593 inhabitants. The main economic activity of the village is apple growing. There are 155 apple producers in Balkiri and 240 hectares of irrigated arable land. Balkiri was selected for this study because the village is one of the pilot areas for pheromone trap use. In 2014 there were 50 registered farmers who benefitted from the state support of the pheromone traps in Balkiri. The total apple production area under the control of these 50 farmers was about 60 hectares and the average size of an apple orchard was 1.2 hectares. All of the farmers who benefitted from the state pheromone trap support scheme were involved in the research study.
MATERIALS AND METHODS

The data of the study were collected using questionnaire forms in face-to-face interviews with 100 apple producers out of 155 total apple producers in Balkiri village in 2016. In the village, similar to the region, all farmers use chemical pest management while none of them does apply biological control and they have used sex pheromone mating disruption combine with chemical control which is called biotechnical control since the implementation of state supports. Farmers apply insecticides for both larva and adults while some of them currently support pest management with sex pheromone mating disruption which helps to decrease number of insecticides uses.

Based on this information the producers interviewed were classified into three groups: 1) producers using pheromone traps with state support (50 farmers); 2) producers using pheromone traps but not benefitting from state support (18 farmers); 3) producers not using pheromone traps at all (32 farmers). Thus, the study covered 68 farmers who were applying biotechnical methods through using pheromone traps and 32 farmers who were using only chemical control for codling moths.

The producers were asked about their reasons for choosing control methods and their levels of knowledge regarding pest control in general and more specifically pheromone traps for codling moth control. In addition to two-choice and multiple-choice questions, the five-point Likert scale was used to identify farmers’ perspectives, knowledge and behaviour regarding pheromone use. The effects of state support on pheromone use and its effects on farmers’ pesticide use were analysed in the study through descriptive statistics, ratios and analysis of significance was done by ANOVA. It is important to see the first results of the pheromone traps with only a three-year history of use in apple orchards as a preliminary analysis for future policy decisions.

RESULTS

The study primarily aimed to identify farmers’ knowledge of pest control as it determines their behaviour. The first part of the questionnaire was prepared for all participants. All apple producers were asked which kind of pest control they used, why they preferred that kind of pest control, how they would describe their level of knowledge regarding pest control, and whether they knew about
pheromone traps. The farmers who did not use pheromone traps (32 farmers) only applied chemical control, and the others (68 farmers) applied biotechnical control, which consisted of pheromone traps and chemical control. The most important factor which affected farmers’ decisions on pest control was its effects on apple yield, followed by easy implementation and affordable price. Nevertheless, no farmer stated one factor alone other than “its positive effect on apple yield” (Table 2).

**TABLE 2**
Resume of Findings

<table>
<thead>
<tr>
<th>Questions and Answers</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors affecting farmers’ pest and disease control preferences</td>
<td></td>
</tr>
<tr>
<td>Positive effects on apple yield</td>
<td>66.18</td>
</tr>
<tr>
<td>Easy implementation and positive effects on apple yield</td>
<td>19.12</td>
</tr>
<tr>
<td>Affordable cost and positive effects on apple yield</td>
<td>2.94</td>
</tr>
<tr>
<td>Reasonable cost and the other factors</td>
<td>8.82</td>
</tr>
<tr>
<td>The other factors</td>
<td>2.94</td>
</tr>
<tr>
<td>Knowledge level of farmers on biotechnical control methods</td>
<td></td>
</tr>
<tr>
<td>I know little (low)</td>
<td>44.12</td>
</tr>
<tr>
<td>I know what it is (middle)</td>
<td>54.41</td>
</tr>
<tr>
<td>I know it very well (high)</td>
<td>1.47</td>
</tr>
<tr>
<td>Where did you hear about pheromone traps?</td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>1.47</td>
</tr>
<tr>
<td>Ministry</td>
<td>1.47</td>
</tr>
<tr>
<td>Other farmers</td>
<td>19.12</td>
</tr>
<tr>
<td>Agricultural input sellers</td>
<td>63.24</td>
</tr>
<tr>
<td>Both other producers and input sellers</td>
<td>11.76</td>
</tr>
<tr>
<td>Other sources</td>
<td>2.94</td>
</tr>
<tr>
<td>Have you ever had any training regarding the pheromone traps?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>67.65</td>
</tr>
<tr>
<td>No</td>
<td>32.35</td>
</tr>
<tr>
<td>The best timing to start pheromone traps using according to farmers</td>
<td></td>
</tr>
<tr>
<td>25 April</td>
<td>23.53</td>
</tr>
<tr>
<td>1 May</td>
<td>70.59</td>
</tr>
<tr>
<td>10 May</td>
<td>5.88</td>
</tr>
<tr>
<td>Do you benefit from state support for pheromone traps</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>73.53</td>
</tr>
<tr>
<td>No</td>
<td>26.47</td>
</tr>
<tr>
<td>For how long have you been in the pheromone support scheme?</td>
<td></td>
</tr>
<tr>
<td>1 year</td>
<td>8.82</td>
</tr>
<tr>
<td>2 years</td>
<td>44.12</td>
</tr>
<tr>
<td>3 years</td>
<td>20.59</td>
</tr>
<tr>
<td>Not in the scheme</td>
<td>26.47</td>
</tr>
<tr>
<td>Why don’t you benefit from the state support?</td>
<td></td>
</tr>
<tr>
<td>It is not worth the bother</td>
<td>80.88</td>
</tr>
<tr>
<td>I missed the application deadline</td>
<td>13.24</td>
</tr>
<tr>
<td>I will apply for it depending on the first year results of pheromones</td>
<td>5.88</td>
</tr>
<tr>
<td>Why do farmers use pheromone traps in general?</td>
<td></td>
</tr>
<tr>
<td>Economic benefit</td>
<td>19.12</td>
</tr>
<tr>
<td>Success in controlling codling moths</td>
<td>80.88</td>
</tr>
<tr>
<td>The number of chemical sprayings for codling moth before/after pheromone traps</td>
<td>Before</td>
</tr>
<tr>
<td>&lt;5 times</td>
<td>1.47</td>
</tr>
<tr>
<td>5-8 times</td>
<td>76.47</td>
</tr>
<tr>
<td>&gt;8 times</td>
<td>22.06</td>
</tr>
</tbody>
</table>
Results of ANOVA test also confirm that the decrease in the number of chemical sprayings after pheromone trap use is at the 99 percent confidence level ($F_{h}>F_{c}, 14.981>7.029$) (Table 3). Farmers stated the reason of using pheromones trap as being success in codling moth control (81%) and economic benefit (19%), and they said that they would like to continue in the future as their input cost had decreased and the yield of the product had increased (Table 2). In addition to these statements, the main finding of the study was the significant change in the pesticide use behaviour of farmers, which came about through spraying less after using pheromone traps. The long-term results of this behavioural change will be very positive for food safety and the environment, and also for farmers’ own safety at work.

### TABLE 3

**ANOVA for changes in the number of chemical sprayings after pheromone trap use**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2.427</td>
<td>1</td>
<td>2.427</td>
<td>14.981</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>10.691</td>
<td>66</td>
<td>.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13.118</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

Although the use of pheromone traps in biotechnological control in agriculture is not new, application accelerated in the 1980s. The most successful results have been obtained for grape berry moth control in vineyards and codling moths in apple production. It has also been used for codling moths in Turkey, where they are a really big challenge for apple production, but use is still low compared to the use of chemical pest control. A state support scheme started by the Ministry of Agriculture and Forestry in 2010 attracts apple producers to start using biotechnological control, and results are promising. In this study also, we see that it not only contributes to their economic benefit but also to food safety and the environment, although this is not one of the prior concerns of the farmers.

Most of the farmers are aware of state support and its importance, and are satisfied with the results, so that they indicate that they want to continue to use pheromone traps. Pheromone traps improve the quality of apples and also have a positive effect on storing. As there are no codling moths and pesticide residue is decreased, apples can be saved in a better condition and kept longer in storage. However, given the fact that most of the information and training sources are indicated as “agricultural input supplier”, there may be a conflict of interest. For instance, 63% of the farmers had heard about the pheromone traps from agricultural pesticide dealers. Farmers should not be left to depend on input suppliers for information. The public body responsible should be involved more efficiently in
the monitoring and control mechanism. Another point which will help overall improvement in the region is expanding biotechnological control through the use of pheromone traps.

In conclusion, it is clear that the number of the sprayings decreased significantly after only three years. Considering that overuse and misuse of agricultural inputs and specifically of pesticides is very high in the region, this is not a final achievement but a good start in changing farmers’ behaviour. In addition to their economical welfare, the long-term results of this behavioural change will be much more positive for food safety and the environment, and also the safety at work of the farmers themselves.

APPENDIX I

List of the banned active substances which were used to apply in the the research area

<table>
<thead>
<tr>
<th>Name</th>
<th>Forbidden after production and import</th>
<th>Using</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbosulfan</td>
<td>31.08.2009</td>
<td>31.08.2011</td>
</tr>
<tr>
<td>Diazinon</td>
<td>31.08.2009</td>
<td>31.08.2011</td>
</tr>
<tr>
<td>Iprodione</td>
<td>19.02.2018</td>
<td>08.06.2018</td>
</tr>
<tr>
<td>Methidathion</td>
<td>30.06.2010</td>
<td>31.08.2011</td>
</tr>
<tr>
<td>Phosalone</td>
<td>30.06.2010</td>
<td>31.08.2011</td>
</tr>
<tr>
<td>Propined</td>
<td>01.01.2009</td>
<td>01.01.2011</td>
</tr>
<tr>
<td>Trifuralin</td>
<td>31.08.2012</td>
<td>30.06.2013</td>
</tr>
<tr>
<td>Diniconazole M</td>
<td>31.08.2012</td>
<td>30.06.2013</td>
</tr>
<tr>
<td>Propargite</td>
<td>31.05.2018</td>
<td>01.01.2019</td>
</tr>
</tbody>
</table>

Source: [13]

REFERENCES

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LOCAL-LEVEL AGRICULTURAL GREENHOUSE GAS EMISSIONS IN POLAND

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ABSTRACT

Agriculture in Poland is the source of 7.7% of national GHG emissions. Effective mitigation in agriculture and adaptation to climate change requires identification of size and structure of emission from this sector not only at national and regional level, but also locally. Therefore, we are the first to estimate the size of the carbon footprint from agriculture and present its statistical analyses and spatial distribution for all Polish communes (LAU level 2). We propose a solution that can be successfully applied using almost exclusively data available in public statistics. The annual value of the carbon footprint from agriculture in Polish communes varies from 0.01 to 289.48 thousand Mg CO$_2$eq, with a mean value of 13.85 thousand Mg CO$_2$eq and a standard deviation of 14.96 thousand Mg CO$_2$eq. The intensive animal production concentrated in the central, northern and northeastern parts of Poland means that the communes located in these areas are characterized by high emissions from enteric fermentation and animal faeces. The communes with large areas of organic soils and former State Agricultural Farm are characterized by high GHG emissions from agricultural land use. The obtained results should facilitate the planning and prioritisation of measures to reduce GHG emissions from agriculture.

KEYWORDS: Agriculture, GHG emissions, local-level GHG inventories, carbon dioxide equivalent, Polish communes

INTRODUCTION

Agricultural production has been identified as a major contributor to atmospheric greenhouse gases (GHG) on a global scale with about 14% of global GHG emissions coming from this sector [1]. According to Richards et al. [2], agriculture contributes an average of 30% of countries’ total GHG emissions. This is higher than the Intergovernmental Panel on Climate Change (IPCC) global estimate of agriculture’s contribution to emissions because of the large number of countries where agricultural emissions are low but relatively important in national greenhouse gas budgets. According to these authors, in 42 countries, agriculture contributes more than half of GHG emissions. In 91 countries agriculture contributes ≥20% of greenhouse gas emissions. The two regions with the highest average contribution of agricultural emissions are West and East Africa. However, agricultural emissions are also of high importance in Southern Asia and South America – regions that contribute substantially to global agricultural emissions.

According to the data presented in the Poland’s National Inventory Report 2017 [3], the total share of GHG emissions from agriculture in Poland is 7.7%. It is slightly lower than in many other European countries (in the EU, an average of 10.1% of greenhouse gases come from agricultural sources), which results from the fact that the Polish economy is based on coal and the role of energy industries in total emissions. It should be stressed, however, that agriculture in Poland is the source of 29.8% of national methane emissions and 78.0% of nitrous oxide emissions.

Effective mitigation in agriculture and hence global climate change mitigation requires identification of GHG sources or hotspots in agricultural production systems [4, 5]. Several methods exist to estimate GHG emissions and carbon sequestration in the agricultural and forestry sector. In parallel to IPCC guidelines, many software tools have been developed recently to assess GHG emissions from agriculture and forestry practices, also at the smaller scale (landscape-scale) [5-8]. However, so far, agricultural GHG emissions calculators are too complex and often require data that are hard to access at the local government level [9-12]. Meanwhile, in order to effectively transform the Polish economy (like other countries), appropriate actions at the local level should be planned. To this end, local low carbon economy plans have been created. These are important strategic documents which are to determine the vision of commune development towards a low carbon economy and to increase the chances of success for local authorities in applying for EU funds in the 2014–2020 financial perspective. They are equivalent to the Sustainable Energy Action Plans (SEAP) – key documents developed by the signatories of the Covenant of Mayors for Climate and Energy, an association of more than 6
thousand local governments from Europe and beyond. The tasks, which are included in these plans, should focus on low carbon and resource-efficient activities aimed at improving energy efficiency and use of renewable energy sources in all sectors of the economy with the participation of producers, consumers of energy, residents, local authorities, and institutions.

Research on the role and effectiveness of low-carbon economy plans adopted by local governments in order to program and coordinate activities for low-carbon development in Poland has shown that the methods used in these documents to calculate the carbon footprint are ineffective and do not allow to determine the actual level of GHG emissions [13-20]. The almost exclusive focus on CO2 emissions without taking other gases into consideration and their omission in the agricultural inventory means that the carbon footprint calculated for the low-carbon economy plans is generally underestimated. This problem has also been observed in other European countries [21-24].

This study attempts to estimate the size of the carbon footprint from agricultural sources and to indicate the share of individual sources with respect to total GHG emissions from agriculture for the whole country – in all Polish communes (according to Local Administrative Units – LAU level 2). This follows a pilot study of 48 selected communes, the results of which were presented in the earlier work, published in Geografisk Tidsskrift-Danish Journal of Geography [25]. The assessment of GHG emissions at local level for the whole of Poland has not been carried out so far. The inclusion of all Polish communes in the study allowed for detailed statistical analysis and spatial distribution of emissions from agricultural sources across the country, which was not in the pilot study. It also allowed to verify the accuracy of the emission assessment methodology used by comparing the estimated GHG emissions for all communes with the Poland's National Inventory Report 2017 [3], presenting the results of national greenhouse gas inventory, which has been prepared according to the United Nations Framework Convention on Climate Change (UNFCCC) guidelines. The results obtained for the whole country can provide a database for local governments, which will allow, not only to identify local emission sources related to agriculture, but also to properly plan and prioritize measures of its reduction. So far, such studies have not been conducted in Poland on a large scale. In addition, the estimation of GHG emissions from agricultural sources for all Polish communes will be an excellent basis for attempt to regionalize the needs of measures to reduce the impact of agriculture on emissions and climate change, which contribute to a low carbon development in local communities.

There are still some challenges with estimating local-level GHG emissions. First, the bottom-up approach commonly employed can be time consuming and expensive. Second, the sources, scope, and overall robustness of data changes from assessment to assessment. Thus, the source data, time, money, and skills needed to collect and analyze the information necessary for completing a GHG inventory can inhibit the progress of developing and implementing a climate action plans. However, the prospect of state government driven carbon emission reduction targets brings with it the prospect of local target setting. Such targets will need to be based on good quality estimates of energy demand and greenhouse gas emissions along with viable assumptions about reduction potential at the local level [26]. So there is a growing requirement for good quality emissions data at the level of local council areas. Different methods and approaches to assessing GHG emissions at the local level in selected European countries, United States and Australia were presented [26-29]. The assessment and analysis of GHG emissions for Polish communes, presented in this paper, may be an important elements to include in this discussion and example for other countries for research on estimating local-level GHG emissions from agriculture.

MATERIALS AND METHODS

Poland is characterised by a high share of agricultural land in total surface area (approx. 170,000 km², 54% of the country's area). Concentration of agricultural land is observed on areas with fertile soils and in the less industrialised central and eastern parts of the country. The lowest shares of agricultural land are observed on areas featuring natural conditions disadvantageous for farming, the highly forested north-western Poland, and the areas characterised by high degree of industrialisation and urbanisation. Arable land takes most important share in the structure of agricultural land (more than 75%). The zonal soils occupy in Poland around 74% of total area, with domination of Cambisols and Podzols. The mid-zonal soils, which include alluvial soils, half-bog soils and black earth, Calcaric Leptosols, Gleyic Phaeozems and Gleyic Podzols, occupy roughly 25% of the territory of Poland. The out-of-zone soils are represented in Poland mainly by the Chernozems, having developed on loess. The arable lands in Poland are mainly covered by soils classified as medium or poor quality [30, 31]. The productive potential on an average hectare of Polish soil is equal to the potential of 0.6 ha of arable lands in European Union [32]. The rate of carbon accumulation is reduces by the large proportion of acid soils (nationally, the share of soils with very high or high acidification is over 60%), as these have a low retention capacity and low humus content. In recent years, Poland has largely abandoned the cultivation of perennial crops.
– grasses or their mixtures with legume plants (Fabaceae). They leave a large amount of biomass in the form of plant residues which improves the balance of nitrogen in the soil. The structure of crops on arable lands is dominated by cereals, whose share amounts to approx. 72%. The shares of pulse, industrial and root crops are decidedly smaller. In Poland systems of large-scale management with crop monocultures and simplified crop rotation (especially on the grounds of the former State Agricultural Farm) is still in use, often employing inappropriate cultivation technologies. This method of management triggers erosion processes that contribute to the reduction of soil organic matter and limited soil carbon sequestration. In Poland there has been a successive decline in the number of pigs, limiting the possibility of using manure as an alternative to nitrogen fertilizers [30, 33].

In order to calculate GHG emissions, a method was chosen, which can be successfully applied by local government units, thereby enabling them to, estimate the carbon footprint and to monitor the impact of actions taken to reduce GHG emissions. This method has been described and implemented by Wiśniewski and Kistowski [25], who presented the pilot study to assess the GHG emissions from agricultural sources in order to plan for needs of low carbon economy for 48 selected Polish communes. The suggested solution can be successfully applied using almost exclusively data available in public statistics. This means that data can be accessed by local government units in order to self-assess the carbon footprint and to monitor the impact of the measures taken to reduce GHG emissions from this sector. The proposed solution is in line with the methodology and standard indicators of the Intergovernmental Panel on Climate Change [34, 35], and in order to obtain more accurate emissions data, the method takes into account the elements of national methodology and emission factors developed by the National Centre for Emission Management for the purposes of preparing annual inventory reports. The presented method combines the calculation of GHG emissions from agriculture per unit area (for soil management) and per unit product (for crops and livestock).

According to Wiśniewski and Kistowski [25], in order to estimate carbon footprint from agriculture in the Polish communes, the three main sources of greenhouse gas emissions from this sector in Poland were taken into account. These include: enteric fermentation of livestock (the main source of methane emissions), animal faeces (a source of methane and nitrous oxide emissions) and management of agricultural soils (a source of emissions of nitrous oxide). The burning of plant residues was also included (a source of methane and nitrous oxide emissions), although its share of GHG emissions is significantly lower. In order to estimate emissions from agricultural sources, the results of the National Agricultural Censuses (NAC) were used. These data are available from the Local Data Bank of the Central Statistical Office (LDB CSO) for 2010 and were used with regard to livestock (with distinction for dairy cows, other cattle, horses, pigs and poultry). Compared to the pilot study [25], the indicators used in the estimation of CH4 and N2O emissions from enteric fermentation and livestock manure, the burning of plant residues and use of agricultural soils were reviewed and updated. Thus, the latest indicators were applied, as specified in the national inventory report [3] as well as default indicators recommended by the IPCC [35] (Tables 1 and 2). Updated default indicators, compliant with the guidelines of IPCC [35] have also been used in case of: nitrous oxide emissions in the estimation of emissions from mineral fertilizers as well as in the calculation of nitrogen mass introduced into the soil as a result of biological binding by Fabaceae plants and plant residues (at a level of 0.01 kg N2O-N/kg N); share of nitrogen in faeces left by grazing animals on soils (at the level of 0.077 kg N2O-N/year); N2O emissions from nitrogen leaching from the soil (at the level of 0.0075 kg N2O-N/kg of leached nitrogen).

In order to harmonize the results and conduct statistical and comparative analyses, GHG emissions from the various types of agricultural activity, expressed in carbon dioxide equivalent (CO2eq) and assuming the global warming potential (GWP) the IPCC Fifth Assessment Report [1], were estimated. Based on the obtained results, the spatial distribution of emissions from agricultural sources across the country was also presented using GIS (MapInfo Pro software) tools. This has helped to identify and assess the spatial variability of the main anthropogenic sources of emissions associated with agricultural activities, which, in the future, should facilitate the planning and prioritisation of abatement.

### TABLE 1

<table>
<thead>
<tr>
<th>Animals</th>
<th>CH4 from enteric fermentation (kg/animal/year)</th>
<th>CH4 from livestock manure (kg/animal/year)</th>
<th>Nitrogen excreted in animal faeces (kg/animal/year)</th>
<th>Liquid (%)</th>
<th>Solid (%)</th>
<th>Pasture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy cows</td>
<td>122.00</td>
<td>11.87</td>
<td>70.26</td>
<td>10.5</td>
<td>79.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Other cattle</td>
<td>49.65</td>
<td>2.15</td>
<td>49.95</td>
<td>5.1</td>
<td>82.9</td>
<td>12.0</td>
</tr>
<tr>
<td>Horses</td>
<td>18.00</td>
<td>1.56</td>
<td>41.28</td>
<td>-</td>
<td>78.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Pigs</td>
<td>1.50</td>
<td>0.03</td>
<td>0.54</td>
<td>11.0</td>
<td>89.0</td>
<td>-</td>
</tr>
<tr>
<td>Poultry</td>
<td>-</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
measures. In order to calculate the Pearson correlation coefficients, the relationship between the emissions amount of CO$_2$eq emissions from agriculture and the variables such as of consumption mineral fertiliser, animal population, arable crop harvest, and organic soil surface were assessed.

RESULTS AND DISCUSSION

The calculations show that the values of the carbon footprint from agriculture in Polish communes (excluding the communes of Łęknica, Jastarnia and Dziwnów, for which there are no data in the public statistics necessary to calculate it) range from 0.01 thousand Mg CO$_2$eq/year in the urban-rural commune of Międzyzdroje to 289.48 thousand Mg CO$_2$eq/year in the rural commune of Wierzchowo, in the West Pomeranian Voivodeship (according to Nomenclature of Territorial Units for Statistics – NUTS level 2), with the average absolute value of 13.85 thousand Mg CO$_2$eq /year and a standard deviation of 14.96 thousand Mg CO$_2$eq/year (Fig. 1, Table 3). Per capita, these values range from 2 kg CO$_2$eq/year in Międzyzdroje to 67.15 Mg CO$_2$eq/year in Wierzchowo, with an average of 2.17 Mg CO$_2$eq/year and a standard deviation of 3.18 Mg CO$_2$eq/year. 69.7% of total emissions from agricultural sources come from rural communes, 28.4% from urban-rural communes and only 1.9% from urban communes. However, the highest average annual absolute emission from agriculture is observed in urban-rural communes. This amounts to 16.32 thousand Mg CO$_2$eq from agricultural sources from all Polish communes (without emissions resulted from energy use in agriculture), on the basis of own calculations, is 34280.88 thousand Mg CO$_2$eq. This is higher by 4630.99 thousand Mg CO$_2$eq than the Poland’s National Inventory Report 2017 [3] estimate. This may be due to the fact that estimation of GHG emissions at local level allows for using more detailed data and taking into account local environmental and economic conditions.

Estimates of agricultural emissions are highly uncertain. In a pilot study of 48 selected Polish communes [25], the uncertainty of emission value in the agricultural sector at the level ± 38% was obtained. This is due to the use of the simplified IPCC Tier 1 methodology using default CH$_4$ emission factors in the estimation of emissions from enteric fermentation – in case of horses and pigs. The Tier 1 methodology and the default emission factors were also used for estimation of CH$_4$ emissions from manure management of horses and poultry. As the emission factors for the Tier 1 method are not based on country-specific data, they may not accurately represent a country’s livestock characteristics, and may therefore be highly uncertain. The more detailed IPCC Tier 2 methodology was applied in calculation of methane emissions of enteric fermentation from cattle, and of manure management from cattle and pigs. The uncertainty under the Tier 2 approach depends on the accuracy of the livestock characterisation (e.g. homogeneity of livestock categories), and also on the extent to which the methods for defining the coefficients in the various relationships that make up the net energy approach correspond to national circumstances.

The analysis of the spatial distribution of communes and the carbon footprint from agriculture calculated for each of them shows that the higher level of GHG emissions from this sector is usually characteristic for communes located in northern, central and eastern Poland (Fig. 1). This is related to a higher share of large farms (over 15 ha) in these areas, intensive animal production and a

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**TABLE 2**

Coefficients used to estimate CH$_4$ and N$_2$O emissions from plant residue combustion and use of agricultural lands

<table>
<thead>
<tr>
<th>Crop</th>
<th>The ratio of non-agricultural to agricultural harvest</th>
<th>The share of dry mass in the aboveground biomass</th>
<th>The share of burned fraction</th>
<th>Combustion efficiency</th>
<th>Carbon content in biomass</th>
<th>Nitrogen content in biomass</th>
<th>The share of total above-ground crop biomass that is removed from the field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>0.90</td>
<td>0.85</td>
<td>0.005</td>
<td>0.90</td>
<td>0.4853</td>
<td>0.0068</td>
<td>0.70</td>
</tr>
<tr>
<td>Rye</td>
<td>1.40</td>
<td>0.86</td>
<td>0.005</td>
<td>0.90</td>
<td>0.4800</td>
<td>0.0053</td>
<td>0.70</td>
</tr>
<tr>
<td>Barley</td>
<td>0.80</td>
<td>0.86</td>
<td>0.005</td>
<td>0.90</td>
<td>0.4567</td>
<td>0.0069</td>
<td>0.70</td>
</tr>
<tr>
<td>Oats</td>
<td>1.10</td>
<td>0.86</td>
<td>0.004</td>
<td>0.90</td>
<td>0.4700</td>
<td>0.0075</td>
<td>0.70</td>
</tr>
<tr>
<td>Triticale</td>
<td>1.10</td>
<td>0.86</td>
<td>0.005</td>
<td>0.90</td>
<td>0.4853</td>
<td>0.0063</td>
<td>0.70</td>
</tr>
<tr>
<td>Cereal</td>
<td>0.90</td>
<td>0.86</td>
<td>0.004</td>
<td>0.90</td>
<td>0.4730</td>
<td>0.0071</td>
<td>0.70</td>
</tr>
<tr>
<td>Potato</td>
<td>0.10</td>
<td>0.25</td>
<td>0.100</td>
<td>0.85</td>
<td>0.4226</td>
<td>0.0203</td>
<td>0.01</td>
</tr>
<tr>
<td>Rapseseed</td>
<td>1.20</td>
<td>0.87</td>
<td>0.030</td>
<td>0.90</td>
<td>0.4500</td>
<td>0.0068</td>
<td>0.10</td>
</tr>
<tr>
<td>Maize</td>
<td>1.30</td>
<td>0.52</td>
<td>0.002</td>
<td>0.90</td>
<td>0.4709</td>
<td>0.0094</td>
<td>0.10</td>
</tr>
<tr>
<td>Legumes</td>
<td>0.90</td>
<td>0.86</td>
<td>0.001</td>
<td>0.90</td>
<td>0.4500</td>
<td>0.0180</td>
<td>0.10</td>
</tr>
</tbody>
</table>
system of large-area management with plant monocultures and simplified crop rotation still functioning there – especially on the lands of former State Agricultural Farm – which additionally contributes to the intensification of erosion processes, reduction of soil organic matter content and insufficient carbon sequestration in the soil [33]. The spatial distribution of GHG emissions from agriculture in Polish communes presented in Fig. 1 is consistent, among others, with the results of studies on methane and nitrous oxide emissions on a regional scale in Poland, presented in the paper by Wysocka-Czubaszek et al. [36].

### TABLE 3

<table>
<thead>
<tr>
<th>Emission sources</th>
<th>Rural communes</th>
<th>Urban-rural communes</th>
<th>Municipalities</th>
<th>All communes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Average</td>
<td>σ</td>
</tr>
<tr>
<td></td>
<td>thousand Mg CO₂eq/yr</td>
<td>thousand Mg CO₂eq/yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteric fermentation</td>
<td>0.00</td>
<td>59.22</td>
<td>6.45</td>
<td>7.40</td>
</tr>
<tr>
<td>Animal faeces</td>
<td>0.00</td>
<td>173.79</td>
<td>2.79</td>
<td>5.26</td>
</tr>
<tr>
<td>Agricultural lands</td>
<td>0.00</td>
<td>116.01</td>
<td>5.93</td>
<td>6.62</td>
</tr>
<tr>
<td>The burning of plant residues</td>
<td>0.00</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.05</td>
<td>289.48</td>
<td>15.17</td>
<td>15.47</td>
</tr>
<tr>
<td></td>
<td>thousand Mg CO₂eq/yr</td>
<td>thousand Mg CO₂eq/yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteric fermentation</td>
<td>0.00</td>
<td>4.90</td>
<td>0.47</td>
<td>0.71</td>
</tr>
<tr>
<td>Animal faeces</td>
<td>0.00</td>
<td>5.11</td>
<td>0.38</td>
<td>0.61</td>
</tr>
<tr>
<td>Agricultural lands</td>
<td>0.01</td>
<td>13.14</td>
<td>1.29</td>
<td>1.56</td>
</tr>
<tr>
<td>The burning of plant residues</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.01</td>
<td>15.04</td>
<td>2.13</td>
<td>2.43</td>
</tr>
</tbody>
</table>

**FIGURE 1**

The annual total GHG emissions from agriculture in Polish communes
More than half of the total emissions from agriculture in Polish communes are related to livestock farming, of which 41.2% comes from enteric fermentation and 18.7% from animal faeces. The management of agricultural soils (40.1%) is another essential source of emission, particularly direct
emissions from organic soil cultivation and the use of mineral fertilizers, as well as indirect emissions from the leaching of nitrogen compounds from the soil. A small share in total emissions from agricultural sources is also attributed to burning of plant residues (0.02%). The highest emission values and the share of enteric fermentation in the total GHG emission from agricultural sources are characteristic for communes in the central and northeastern part of Poland, where intensive animal production, including pigs and cattle, is concentrated (Fig. 2 and 3). Communes in the central part of the country (mainly in the Kuyavian-Pomeranian and Greater Poland Voivodships) are also characterized by a high level of emissions from livestock manure and its high share in the total emissions from agriculture (Fig. 4 and 5). This is due to the high importance of pigs in the animal husbandry structure of these areas. Faecal matter decomposition, which is an important source of GHG emissions, takes place during their storage and after application to the soil, and the amount of methane generated in pig slurry during storage is higher than in cattle slurry [37-39]. The highest GHG emissions related to agricultural land use are characteristic mainly for communes located in the northern and northeastern part of the country, where the share of organic soils is significant (Fig. 6). These results confirm the assumptions of Turbiak et al. [40], who indicate that among soil types, the organic soils may be one of the main sources of nitrous oxide emissions. They emphasise that following the use of organic soils for agricultural purposes, which entails the lowering of groundwater levels, there is an intensive organic matter mineralisation in these soils. According to Okruszko and Piaśek [41], in the climatic conditions of Poland, about 10 Mg/ha of organic matter is subjected to annual mineralisation, which results in the release of up to 400 kg/ha of mineral nitrogen into the environment. In terms of the size of GHG emissions from land use, compared to the whole country, the communes in southeastern Poland (the vicinity of Hrubieszów in the Lublin Voivodeship) are particularly distinguished (Fig. 6), where the largest complex of agriculturally used Chernozems, in Poland, is located. In addition, in this area, there are lands of former State Agricultural Farm [42], on which the agricultural large-area management system is still maintained, which is conducive to GHG emission from cultivation. On the other hand, the share of agricultural land use emissions in the total GHG emissions from agriculture – outside the above-mentioned areas – is the highest in the western and southern parts of the country and in the vicinity of Warsaw (Fig. 7), where intensive animal production is of much lesser importance. The size of emissions from the burning of plant residues and its share in the total GHG emission from agricultural sources are the highest in the communes of western and northern Poland (Fig. 8 and 9), characterized by the highest percentage of farms cultivating cereals and the highest average area of cereal crops in farms [30].
FIGURE 5
The share of animal feaces in total GHG emissions from agriculture in Polish communes

FIGURE 6
The annual GHG emissions from agricultural land use in Polish communes
FIGURE 7
The share of agricultural land use in total GHG emissions from agriculture in Polish communes

FIGURE 8
The annual GHG emissions from the burning of plant residues in Polish communes
The dominant impact of livestock farming on GHG emissions is observed in both rural communes (42.5% from enteric fermentation and 18.4% from animal faeces at 39.1% from agricultural land use), as well as in urban-rural communes (39.3% from enteric fermentation and 19.4% from animal faeces at 41.2% from agricultural land). In urban communes (municipalities), the land use is the predominant source of emissions from agriculture (60.2%). The urban livestock farming accounts for 39.8% of total emissions from agricultural sources.

The statistical analyses carried out indicate high and very high correlations, in particular, between the amount of greenhouse gas emissions from agriculture and the cattle population, the use of mineral fertilizers and the surface of organic soils (Table 4). The obtained Pearson correlation coefficients between these variables are similar,
among others, to the results of modelling of nitrous oxide emissions from agricultural sources, using the linear regression conducted by Kolasa-Więcek [43] and the results of research on the influence of land use on N₂O emissions conducted by Wiśniewski and Kistowski [44]. However, there are differences in the strength of correlation compounds depending on the type of commune. In the case of rural and urban-rural communes, a statistically significant correlation was observed between the amount of emissions and the cattle population. On the other hand, in urban communes, there are statistically significant correlations between emissions and cattle population, wheat and barley harvest as well as organic soil area.

**CONCLUSIONS**

The total amount of emissions from agricultural sources in Poland (without emissions resulted from energy use in agriculture), estimated, on the basis of own calculations, amounts to 34280.88 thousand Mg CO₂eq. This is higher by 4630.99 thousand Mg CO₂eq than the last Poland’s National Inventory Report estimate, presenting the results of national GHG inventory, which has been prepared according to the UNFCCC guidelines. This may be due to the fact that estimation of GHG emissions at local level allows for using more detailed data and taking into account local environmental and economic conditions. Absolute GHG emissions from agricultural sources in Polish communes range from 0.01 thousand Mg CO₂eq/year to 289.48 thousand Mg CO₂eq/year, with an average absolute value of 13.85 thousand Mg CO₂eq/year and a standard deviation of 14.96 thousand Mg CO₂eq/year. Calculated per capita, these values range from 2 kg CO₂eq/year to 67.15 Mg CO₂eq/year, with an average of 2.17 Mg CO₂eq/year and a standard deviation of 3.18 Mg CO₂eq/year.

Estimates of agricultural emissions are highly uncertain. In particular, when the IPCC Tier 1 methodology is used. The IPCC Tier 2 methodology gives better estimates, which is sufficient to approximate the scale of emissions from a particular source and which will accurately respond to local changes in use or behavior. The first step in collecting emission data should be to investigate existing national, regional and local statistics, industry sources, research studies and FAO statistics. The uncertainty associated emission values will vary widely depending on the source. Therefore, further research in this field is necessary, especially at the local level.

Almost 70% of emissions from the agricultural sector in Poland come from rural communes and slightly over 28% from urban-rural communes. Cities are the sources of 1.9% of agricultural greenhouse gas emissions. More than half of the total emissions from agriculture in Polish communes are related to livestock farming (the dominant influence of this source of GHG emissions is observed in rural and urban-rural communes), of which 41.2% comes from enteric fermentation, and 18.7% from animal faeces. Its significant source (especially in urban communes) is also the use of agricultural soils (40.1%), mainly direct emissions from the cultivation of organic soils and the use of mineral fertilizers, as well as indirect emissions from leaching nitrogen compounds from the soil. The burning of plant residues accounts for a small share of total emissions from agricultural sources (0.02%).

The statistical analyses carried out indicate high and very high correlations, in particular, between the amount of greenhouse gas emissions from agriculture and the cattle population, the use of mineral fertilizers and the surface of organic soils. However, there are differences in the strength of correlation compounds depending on the type of commune. In the case of rural and urban-rural communes, a statistically significant correlation was observed between the amount of emissions and the cattle population. On the other hand, in urban communes, there are statistically significant correlations between emissions and cattle population, wheat and barley harvest as well as organic soil area.

The intensive animal production concentrated in the central, northern and northeastern parts of Poland means that the communes located in these areas are characterized by high GHG emissions from enteric fermentation and livestock manure. On the other hand, communes with large areas of organic soils and former State Agricultural Farm are characterized by high GHG (mainly N₂O) emissions from agricultural land use. In communes with large areas of cereal cultivation in farms (mainly in the western and northern parts of Poland), an increase in the significance of the burning of plant residues, in the structure of agricultural sources of emission, is noticeable.

The obtained results confirm the appropriateness of including GHG emissions from the agricultural sector and related sources in low carbon economy plans. Identifying GHG emissions from this sector can help the reduction of these emissions and mitigate their negative effects on the atmosphere, climate and other elements of the ecosystem. This is of particular importance in rural and urban-rural communes, but should also be considered in municipalities, where a significant share of the land is agricultural land or is home to high biological activity. The results obtained for the whole country can provide a database for local governments, which will allow, not only to identify local emission sources related to agriculture, but also to properly plan and prioritize measures of its reduction. In addition, the estimation of GHG emissions from agricultural sources for all Polish communes is an
excellent basis for attempt to regionalize the needs of measures to reduce the impact of agriculture on emissions and climate change, which contribute to a low carbon development in local communities. The assessment and analysis of GHG emissions for Polish communes, presented in this paper, may also be an important elements to include in discussion on methods and approaches to assessing GHG emissions from agriculture at the local level and example for other countries for such research.

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A HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS IN DRINKING WATER SYSTEMS OF CENTRAL-HUNZA, GILGIT-BALTISTAN, PAKISTAN

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4Institute of Geographic Sciences and Natural Resources Research, University of Chinese academy of Sciences Beijing, China

ABSTRACT

Heavy metals (HMs) in drinking water can affect human health through the exposure pathways of oral ingestion, dermal contact, and inhalation. This study assesses the exposure of rural mountain communities to trace metals via oral ingestion. 192 water samples in two seasons from 8 rural villages in Central-Hunza, Northern Pakistan, were collected and analysed for 8 trace metals by Atomic Absorption Spectrophotometry (Perkins-Elmer 2000). Based on the concentration of heavy metals in water, the health-risks assessment such as hazard quotients (HQ) and chronic daily intake (CDI) were calculated. Results showed that in summer the average concentrations of Ar, Cd, Cu, Fe, Hg and Pb were higher than the WHO Drinking Water Guidelines while Zn and Cr exhibited elevated concentrations in both seasons. The findings also revealed that Hazard Quotient HQ were near to the acceptable limits, indicating non-carcinogenic risk to the recipients via oral intake of water however HMs i.e., Cd, Cr, Hg and Pb which could pose serious health risks to the local inhabitant in future. The calculated values for CDI were found in the order of Zn > Cu > Ar > Pb > Cd > Hg in summer and Zn > Cr > Pb > Ar > Cd > Fe > Hg during winter. The data provided in this study suggested that quick action should be taken to protect and control the drinking water from the different nonpoint pollution sources, especially the application of agricultural fertilizers.

KEYWORDS:
Health Risk Assessment, Heavy Metals Pollution, Environmental Health

INTRODUCTION

Water is the basic component of life. Freshwater comprises 3% of the total water on earth, of which, just 0.01% is available for human utilization [1]. Ironically, even this little extent of freshwater is severely stressed due to lithogenic and anthropogenic activities, predominantly as a result of rapid population growth, urbanization, mining, vehicular emission and unsustainable exploitation in agriculture and industries [2-4]. Subsequently highly concentrated toxic heavy metals accumulate in the aquatic system by means of several pathways, i.e., atmospheric deposition, assortment of bedrock, runoffs from agricultural and urban areas, industrial effluents, and water drainage [4-6]. Moreover, high concentrations of noxious metals defile the surface water and ground water, bringing about disintegration of irrigation and drinking water quality that not only influences aquatic-biological systems but also adversely affects human health and wellbeing [7]. Therefore, the influences of these water pollutions should be systematically evaluated, followed by appropriate measures to recover polluted water bodies.

Metals are found naturally in the earth’s crust and their compositions vary among different localities, resulting in spatial variations of surrounding concentrations [8]. As heavy metals are unique environmental toxicants, unable to transform into non-toxic forms [9]. Pollution of surface water bodies, resulting from groundwater discharges and runoffs, has been an issue of worldwide environmental concern [10], because once liberated into the environment through air, water, food or countless varieties of man-made chemicals and products, heavy metals are taken into the body via inhalation, ingestion and dermal absorption [11]. Dermal absorption and ingestion are the principle means of introduction in case of surface water situations [12-15]. If heavy metals enter and accumulate in body tissues faster than the body’s detoxification pathways can dispose of, then a gradual build-up of these toxins occurs. High concentration exposure is not a necessity to produce a state of toxicity in the body, as heavy metal accumulation occurs in the body tissues gradually and, over time, can reach toxic concentration levels, much beyond the permissible limits [16].
Therefore, a risk assessment of daily human exposure is essential. This involves the characterization of physical settings, identification of potential-exposure populations and pathways as well as estimating the exposure concentrations and chemical intakes. Because, Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons [17-19]. Some substantial metals at lower concentration i.e., Copper (Cu), Iron (Fe) and Zinc (Zn) assume an imperative part in the metabolic exercises of living life forms, but long-term exposure of metals such as chromium (Cr), Manganese (Mn), Lead (Pb) and Cadmium (Cd) are deemed toxic for human health [20, 21], including liver and kidney problems and genotoxic carcinogens [22, 23]. Heavy metals possess serious effects on human health and might cause various symptoms depending on the type and amount of metal involved [24]. For instance, Cd is a kind of carcinogenic, mutagenic HM and its long-term exposure can cause kidney and skeletal damage, diarrhoea, hair loss, depression and arteriosclerosis [20, 25]. Cu overdose is a causative factor for gastrointestinal cancers, mental diseases such as Alzheimer’s and kidney damage [26]. Similarly, Pb is a highly toxic and carcinogenic metal, and may cause chronic health risks including headache, loss of appetite, birth defects, mental retardation, hypertension, lung cancer and renal dysfunction [27]. Hg is also poisonous and might be associated with development of joint diseases, deterioration of mental status and disorders of speech, hearing, vision and movement [28].

Water reservoirs not only supply drinking water for domestic requirements but also contributing for irrigation, and major source of beautification and shaping landscape structure for ecotourism and cultural ecosystem services [29]. Besides all benefits water supply to human wellbeing, it also transports toxic elements. For successful appraisal of water quality it is critical to recognize potential impacts of contaminants in drinking water on human health. The conventional technique for assessing health effects is to directly compare the deliberated values and permissible limits; however it is not adequately reliable to present detailed hazard levels and distinguish contaminants of the most concern. Health risk assessment is a vital tool for evaluating the potential health impacts caused by a range of contaminants in aquatic ecosystems [30, 31]. This strategy has been applied to assess the potential adverse health effects by the exposure to contaminated water [19, 32, 33]. Seasonal progression of various components can be a key factor in formulating seasonal variations of trace metal concentrations in various water reservoirs and its relationships with environmental factors.

The health risk assessment is an efficient method for evaluating the relationship between the environment and people’s health, which can be quantitatively assessed in terms of hazard degree [34]. It is evident that anthropogenic and agricultural activities are generally responsible for the deterioration of surface water quality [35]. Although, there are numerous studies that focuses the physical and microbiological analysis of drinking water quality. But unfortunately, there is limited information on the effect different anthropogenic and agricultural activities on the quality of drinking water reservoirs of Northern Pakistan. Especially in the study area, so far no research work has been conducted on heavy metals contamination in drinking water sources and their effects on human health. This study aims to determine the concentration of the selected heavy metals (Ar, Cd, Cr, Cu, Fe, Hg, Pb and Zn) in drinking water samples collected from water supply resources (wells), water reservoirs and water distribution networks (tap-water, open water channels) and to evaluate the health risks associated with exposure to these trace metals via oral ingestion. We chose these eight noxious heavy metals as real cohorts of soil contamination from the utilization of composts/fertilizers and pesticides. It is expected that by comparing the results of this study with the HM’s oral reference dose (mg/kg/d) of the WHO (2017), the data will serve as an important reference for the management of the drinking water reservoirs in the targeted areas of District Hunza.

MATERIALS AND METHODS

Study Area. The Central-Hunza (36° 16’ N and 74° 44’ E), comprising of 14 villages with more than 4800 households and 20,000 inhabitants; is a picturesque mountainous valley, located in northern Pakistan at 3,626 meters above the sea level. Snow-capped mountains like Ullar (7388m) and Shisper (7611m), holding glaciers of the same name are the main source of drinking and irrigation water for the local inhabitants. In the last few years the applications of agrochemicals and fertilizers, incessant mining, episodic runoff events from the agricultural areas and small mechanical industries in the vicinity of water supply systems, seriously impacting the quality of drinking as well as irrigation waters [36].

Sampling and Analysis. The sampling was carried out from June – December, 2016. A total of 192 drinking water samples in triplicates were collected from eight selected villages (Murtazabad, Hassanabad, Aliabad, Ganish, Dourkhan, Karimabad, Haiderabad and Altit) of Central-Hunza, 96 each during summer and winter to assess the spatio-temporal variations of heavy metals concentration in drinking water samples. These samples were collected in pre-sterilized plastic bottles of 250ml from four main sources of drinking water i.e.,
community tapes (CTs), open water channels (OWCs), water containers (WCs) and traditional wells (TWs) by using WHO Guidelines [37]. The samples were then transported to the Geochemistry Laboratory of the National Centre of Excellence in Geology (NCEG), University of Peshawar, Pakistan for further processing and analysis.

The concentrations of selected heavy metals i.e., Arsenic (Ar), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Mercury (Hg) and Zinc (Zn) were analysed by using an Atomic Absorption Spectrophotometer (Perkins-Elmer 2000) under optimal analytical conditions [34, 37].

First, the concentrations and characteristics of heavy metals in drinking water samples were presented and, then the human health risks of these heavy metals were assessed.

Data Treatment and Multivariate Statistical Analysis. Analytical data were processed using STATISTICA software. Basic descriptive statistics such as minimum, maximum, mean, and relative standard deviation (RSD) were computed, along with the multivariate statistics i.e., Correlation Matrix, Principle Component Analysis (PCA/FA) and Cluster Analysis (CA) was used to identify the possible heavy metal sources [12, 35].

HUMAN HEALTH RISK ASSESSMENT INDICES

The exposure pathways of the metals in water include direct ingestion and dermal contact, but the later can be negligible in comparison to oral intake. The U.S. EPA Risk Assessment Methodology was used to assess health risk of the metals in surface water via ingestion route to the recipients [14, 15]. The exposure dose via ingestion route was calculated by using equation (i) adopted from the US Environmental Protection Agency (USEPA) [15, 28].

\[
\text{Exp}_{\text{ing}} = \frac{C_{\text{water}} \times DI \times EF \times ED}{BW \times AT} \quad (i)
\]

Where, \(\text{Exp}_{\text{ing}}\) is exposure dose through ingestion of water (mg/kg/d), while the other parameters for estimating human health risk assessment through different pathways are listed in Table 1. Likewise, the \(HQ_{\text{ing}}\) meant for non-carcinogenic risk and can be calculated by using the following equation.

\[
HQ_{\text{ing}} = \frac{\text{Exp}_{\text{ing}}}{\text{RfD}} \quad (ii)
\]

Where, \(HQ_{\text{ing}}\) is hazard quotient via ingestion (unit less); and \(\text{RfD}\) is oral reference dose (mg/kg/d) [12, 13, 28]. The uncovered population is assumed to be safe, when \(HQ < 1\). Chronic daily intake (CDI) is an index recommended by USEPA to estimate the oral intake, was calculated using equation (iii) modified from [27, 32, 36, 37].

\[
\text{CDI} = \frac{C \times DI}{BW} \quad (iii)
\]

Where, \(C\), \(DI\), and \(BW\) represent the concentration of heavy metal in water (mg/L), average daily intake rate was considered to be 2.2 L/day and average body weight 70 kg [13, 28, 38, 39].

RESULTS AND DISCUSSION

Spatio-temporal variations in trace Metal concentrations. This study focuses on eight potential trace metals, including Ar, Cd, Cr, Cu, Fe, Hg, Pb and Zn. The results summarized in Table 2 showed that the average concentration of Zn in drinking water samples was 2.684 mg/L, followed by Ar (0.2700 mg/L), Cu (0.5444 mg/L), Fe (0.0580 mg/L), Pb (0.1150 mg/L), Cd (0.0527 mg/L), Cr (0.2885 mg/L) and Hg (0.0046 mg/L) in summer and Zn (0.7687 mg/L), Ar (0.0387 mg/L), Cu (0.0331 mg/L), Fe (0.0262 mg/L), Pb (0.3220 mg/L), Cd (0.0038 mg/L), Cr (0.4030 mg/L) and Hg (0.0028 mg/L) during winter.

The comparative analysis of the mean heavy metals concentration in water resources revealed that there were significant differences observed among the total concentrations in different points during summer and winter. Better water quality was found during winters than that of summers. This variation in aquatic environment could be because of the geographical surface [40] and more agrarian (agricultural) exercises around water resources (open water channels and traditional wells) during summer season [33] particularly derived both from atmospheric deposition and from weathering of bedrocks and soil within the catchment [41]. The phenomenon of heavy metals accumulation along the watershed was earlier reported by Ntakirutimana et al. and Xu et al. [42, 43].

<table>
<thead>
<tr>
<th>Exposure factors</th>
<th>Unit</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of HMs in water ((C_{\text{water}}))</td>
<td>mg/L</td>
<td>-</td>
</tr>
<tr>
<td>Water ingestion rate (IR)</td>
<td>L/day</td>
<td>2.2</td>
</tr>
<tr>
<td>Exposure frequency (EF)</td>
<td>days/year</td>
<td>360</td>
</tr>
<tr>
<td>Exposure duration (ED)</td>
<td>year</td>
<td>30</td>
</tr>
<tr>
<td>Average body weight (BW)</td>
<td>kg</td>
<td>70</td>
</tr>
<tr>
<td>Average time (AT)</td>
<td>days</td>
<td>10,950</td>
</tr>
</tbody>
</table>
TABLE 2
Summary of Health Risk Assessment for HMs in water samples through Ingestion Pathway
during Summer and Winter

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>C&lt;sub&gt;water&lt;/sub&gt;</th>
<th>Exp&lt;sub&gt;ing&lt;/sub&gt;</th>
<th>RfD</th>
<th>HQ&lt;sub&gt;ing&lt;/sub&gt;</th>
<th>CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (Zn)</td>
<td>2.684</td>
<td>0.0844</td>
<td>3.0</td>
<td>0.0281</td>
<td>0.084</td>
</tr>
<tr>
<td>Arsenic (Ar)</td>
<td>0.2700</td>
<td>0.0085</td>
<td>0.05</td>
<td>0.17</td>
<td>0.009</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.5444</td>
<td>0.0171</td>
<td>0.05</td>
<td>0.342</td>
<td>0.017</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.0580</td>
<td>0.0018</td>
<td>0.001</td>
<td>1.8229</td>
<td>0.009</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.1150</td>
<td>0.0036</td>
<td>0.05</td>
<td>0.072</td>
<td>0.004</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.0527</td>
<td>0.0017</td>
<td>0.005</td>
<td>0.34</td>
<td>0.002</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.2885</td>
<td>0.0090</td>
<td>0.05</td>
<td>0.18</td>
<td>0.009</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>0.0046</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>C&lt;sub&gt;water&lt;/sub&gt;</th>
<th>Exp&lt;sub&gt;ing&lt;/sub&gt;</th>
<th>RfD</th>
<th>HQ&lt;sub&gt;ing&lt;/sub&gt;</th>
<th>CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (Zn)</td>
<td>0.7687</td>
<td>0.0235</td>
<td>3.0</td>
<td>0.0078</td>
<td>0.024</td>
</tr>
<tr>
<td>Arsenic (Ar)</td>
<td>0.0387</td>
<td>0.0012</td>
<td>0.05</td>
<td>0.024</td>
<td>0.001</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.0331</td>
<td>0.0010</td>
<td>0.05</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.0262</td>
<td>0.0008</td>
<td>0.001</td>
<td>0.8234</td>
<td>0.0008</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.3220</td>
<td>0.0101</td>
<td>0.05</td>
<td>0.202</td>
<td>0.010</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.0038</td>
<td>0.0001</td>
<td>0.005</td>
<td>0.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.4030</td>
<td>0.0127</td>
<td>0.05</td>
<td>0.253</td>
<td>0.013</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>0.0028</td>
<td>0.0001</td>
<td>0.001</td>
<td>0.1</td>
<td>0.00008</td>
</tr>
</tbody>
</table>

Multivariate Analysis for Source Apportionment. Inter associations between elements preserve more information about the pathways and heavy metals sources [44]. That’s why, multivariate techniques i.e., cluster analysis (CA) and principal component analysis (PCA) was applied to the datasets to yield comprehensive results.

Cluster analysis identified two main groups of metals for summer; cluster 1 comprised of Pb and Cr, and cluster 2 consists of Fe, Hg and Cd [Fig. 1]. Two clusters were also identified for winter season; cluster 1 has Fe and Ar, while Cd, Hg, Cr and Pb in cluster 2 [Fig. 2]. Factor analysis resulted in four factors with total 72.880 variance and eigenvalue > 2 [Table 4 and Fig. 3]. PCA/FA 1, 2, 3 and 4 accounted for 26.194%, 20.410%, 13.608% and 12.668% of total variance.

Correlation matrix among all parameters were created to check whether the concentration of one parameter affect the concentration of other. Correlation analysis exhibited in Table 3 showed that in summer season Zinc (Zn) was positively correlated with Ar (r = 0.402) and Hg (r = 0.273), while negatively correlated with Cu (r = -0.229) and Cd (r = -0.257); Arsenic (Ar) was positive-significantly correlated with Cr (r = 0.239), while negatively correlated with Fe (r = -0.205). Copper (Cu) illustrated positive-significant correlation with Fe (r = 0.403) and Cd (r = 0.414), while Iron (Fe) was negatively correlated with Cr (r = -0.230).
Similarly, during winter season Arsenic (Ar) was positive-significantly correlated with Zn (r = 0.311); Lead (Pb) showed positive-significant correlation with Zn (r = 0.425), Ar (r = 0.446) and Cu (r = 0.263), while negative-significant correlation with Fe (r = -0.263); Cadmium (Cd) exhibited positive correlation with Pb and Iron (Fe) exhibited negative correlation with Zn (r = -0.307), only.

Similarly, Chromium (Cr) was observed to be positive-significantly correlated with Cu (r = 0.276) and Fe (r = 0.700), while negatively correlated with Zn (r = -0.455), Pb (r = -0.368) and Cd (r = -0.232). The cluster analysis (CA) further supports the recorded/observed inter-metal relationships in both seasons (Fig. 1 and 2).

**TABLE 3**
Correlation matrix of select heavy metals in water samples during Summer (below the diagonal) and Winter (above the diagonal) n=192.

<table>
<thead>
<tr>
<th></th>
<th>Zn</th>
<th>Ar</th>
<th>Cu</th>
<th>Fe</th>
<th>Pb</th>
<th>Cd</th>
<th>Cr</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>1</td>
<td>.402*</td>
<td>-.229*</td>
<td>-.040</td>
<td>.181</td>
<td>-.237*</td>
<td>-.105</td>
<td>.273*</td>
</tr>
<tr>
<td>Ar</td>
<td>.311**</td>
<td>1</td>
<td>-.071</td>
<td>-.205*</td>
<td>-.041</td>
<td>-.105</td>
<td>.239*</td>
<td>.042</td>
</tr>
<tr>
<td>Cu</td>
<td>-.133</td>
<td>.004</td>
<td>1</td>
<td>.403**</td>
<td>.104</td>
<td>.414**</td>
<td>-.082</td>
<td>-.071</td>
</tr>
<tr>
<td>Fe</td>
<td>-.307**</td>
<td>-.185</td>
<td>.015</td>
<td>1</td>
<td>.075</td>
<td>-.096</td>
<td>-.230*</td>
<td>-.164</td>
</tr>
<tr>
<td>Pb</td>
<td>.425**</td>
<td>.446**</td>
<td>.263**</td>
<td>-.263**</td>
<td>1</td>
<td>-.077</td>
<td>-.134</td>
<td>.140</td>
</tr>
<tr>
<td>Cd</td>
<td>.046</td>
<td>.125</td>
<td>.020</td>
<td>-.049</td>
<td>.221*</td>
<td>1</td>
<td>-.108</td>
<td>.036</td>
</tr>
<tr>
<td>Cr</td>
<td>-.455**</td>
<td>-.174</td>
<td>.276**</td>
<td>.700**</td>
<td>-.368**</td>
<td>-.232*</td>
<td>1</td>
<td>-.084</td>
</tr>
<tr>
<td>Hg</td>
<td>.075</td>
<td>.143</td>
<td>-.024</td>
<td>-.163</td>
<td>.068</td>
<td>-.068</td>
<td>-.140</td>
<td>1</td>
</tr>
</tbody>
</table>

Bold correlations are significant at the 0.05 level

**TABLE 4**
Factor loading for selected HMs in water samples from Central-Hunza

<table>
<thead>
<tr>
<th>Factors</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>.689</td>
<td>-.429</td>
<td>-.250</td>
<td>-</td>
</tr>
<tr>
<td>Pb</td>
<td>.570</td>
<td>.139</td>
<td>-.228</td>
<td>-.438</td>
</tr>
<tr>
<td>Hg</td>
<td>.542</td>
<td>-.162</td>
<td>-</td>
<td>-.520</td>
</tr>
<tr>
<td>Cr</td>
<td>.534</td>
<td>-.212</td>
<td>.294</td>
<td>.466</td>
</tr>
<tr>
<td>Cu</td>
<td>.538</td>
<td>.690</td>
<td>.101</td>
<td>.241</td>
</tr>
<tr>
<td>Ar</td>
<td>.506</td>
<td>-.535</td>
<td>.213</td>
<td>.341</td>
</tr>
<tr>
<td>Cd</td>
<td>.261</td>
<td>.563</td>
<td>.657</td>
<td>-.215</td>
</tr>
<tr>
<td>Fe</td>
<td>.321</td>
<td>.529</td>
<td>-.632</td>
<td>.323</td>
</tr>
</tbody>
</table>

Eigenvalue | .2096 | 1.633 | 1.089 | 1.013 |
% Total variance | 26.194 | 20.410 | 13.608 | 12.668 |
% Cumulative variance | 26.194 | 46.604 | 60.212 | 72.880 |

Marked loadings are significant at > 0.50

**FIGURE 3**
Factor Analysis of the water samples from Central-Hunza
In the present study, the results of the PCA/FA [Table 4, Fig. 3] showed that concentrations of Zn, Pb, Hg, Cr, Cu and Ar in PC1 were strongly correlated with each other. In PC2, Cu, Cd and Fe exhibited close associations. In PC3 the only Cd was positively significant which suggests that these metals are from the same source, which may be attributed to the use of agrochemicals, insecticides, and run-offs from extensive farmed areas [45], while the Ar in PC2, Fe in PC3 and Hg in PC4 showed a significant negative correlation indicating their input sources from different activities (natural/anthropogenic). Correlation analysis and cluster analysis also verified the positive/negative correlations and strong/weak clusters between these metals [Table 3, Fig. 1 and 2]. The PCA/FA results suggested that Zn, Pb, Hg, Cr, Cu, Fe and Ar were the main contributors for ingestion and dermal exposures to the human environment and serve as an indirect marker of oxides content in the soil, which are known to affect the retention and chemical behavior of HMs in soil and water [46].

Exposure Assessment. The averaged exposure levels (mg/kg/day) of selected HMs in Central-Hunza via ingestion route during summer and winter are summarized in Table 2. The average Exp_{ing} values in summer season for the selected heavy metals (Zn, Ar, Cu, Fe, Pb, Cd, Cr, and Hg) were 0.0844, 0.0085, 0.0171, 0.0018, 0.0036, 0.0017, 0.0090 and 0.0002, respectively. Similarly, during winter the average Exp_{ing} indices for the selected heavy metals (Zn, Ar, Cu, Fe, Pb, Cd, Cr, and Hg) were calculated to be 0.0235, 0.0012, 0.0010, 0.0008, 0.0101, 0.0001, 0.0127 and 0.0001, respectively. Therefore, Exp_{ing} indices intended for HMs in the study area during summer and winter were found in the order: Zn > Cu > Cr > Ar > Pb > Fe > Cd > Hg and Zn > Cd > Hg > Cr > Pb > Ar > Cu > Fe, respectively. While, Cd = Hg.

Likewise, the average CDI values in summer for the selected heavy metals (Zn, Ar, Cu, Fe, Pb, Cd, Cr and Hg) were 0.084, 0.009, 0.017, 0.009, 0.004, 0.002, 0.009 and 0.0001, respectively. Similarly, during winter the average CDI indices for the selected heavy metals (Zn, Ar, Cu, Fe, Pb, Cd, Cr and Hg) were 0.024, 0.001, 0.001, 0.0008, 0.010, 0.0001, 0.013 and 0.00008, respectively. Therefore, CDI indices for heavy metals in the study area during summer and winter were found in the order: Zn > Cu > Ar > Pb > Cd > Hg and Zn > Cr > Pb > Ar > Cd > Fe > Hg respectively, where Ar = Fe and Cr during summer and Cu = Ar during winter.

The high CDI values of Pb and Cr could be because of the consumption of drinking water from old and corrosive diffused pipelines or means of their access to the distribution network. He et al. [47, 48] suggested that domestic sewage and agricultural practices such as fertilization and use of fungicides and run-off from these extensive farmed areas intended to increase the concentration of these HMs and were suspected to affect water quality and ecosystem biodiversity.

Risk Assessment. The values of hazard quotient (HQ_{ing}) indices of the study area for trace metals during summer season were found in the order of Fe > Cu > Cd > Hg > Cr > Ar > Pb > Zn. Similarly, the order of HQ_{ing} indices during winter season were: Fe > Cr > Pb > Hg > Ar > Cd > Zn. While, Cu = Cd.

These results also suggest that in the present study the levels of HQ_{ing} for all the metals during summer and winter were smaller than 1, except Fe (1.8229 mg/L) in summer indicating a negligible carcinogenic/non-carcinogenic risk which means that these metals could pose minimum hazard to local residents [13]. While, the reasons for increased Fe could be perpetual discharging wastewater of workshops and small industrial units to the environment inside the valley as well as vehicle traffic could be other reason of water resources pollution within the valley.

CONCLUSION

The aim of the study was to measure the concentrations of eight selected HMs (Ar, Cd, Cr, Cu, Fe, Hg, Pb and Zn) in drinking water reservoirs of Central-Hunza and to assess possible hazards to public health. Based on the results obtained, it can be concluded that the population settled in the lap of mountains and along the banks of Hunza River consume highly contaminated water as most of the HMs analysed in this study have concentrations above the safety limits set by WHO/EPA for drinking water. Though the values of CDI and HQ_{ing} indicated a negligible health risk via oral ingestion pathway, however precautions need to be taken to monitor the content of Cd, Cr, Hg and Pb as in the present investigations these metals are the main contributors to carcinogenic/non-carcinogenic health risks over a period of time. Furthermore, long-term and comprehensive plans should be established and implemented to meet the human needs of both affordable irrigation water and high quality drinking water.

As there is no any proper pipeline distribution system in the village and throughout the distribution there are probabilities of contamination. So, this study highly recommends the emphasis on utilization of agro-chemicals and waste management practices along with the construction of appropriate engineered sanitary landfill sites in order to minimize surface water contamination and control future risks in terms of environmental pollution and human health concerns. Similar studies are also recommended in other districts of Gilgit-Baltistan so that steadfast data could be on hand for the re-
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THE EFFECTS OF RHODOBACTER CAPSULATUS AND VERMICOMPOST APPLICATIONS ON AGRO-MORPHOLOGICAL TRAITS OF THE MALABAR SPINACH (BASELLA ALBA L.)

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ABSTRACT

This study was carried out to investigate the effects of bacteria and vermicompost applications on some agro-morphological traits of the Malabar spinach (Basella alba L.). The standard variety of Basella alba L. was used as plant material, Rhodobacter capsulatus DSM1710 as the bacteria and certain amounts of worm compost was used as fertilizer. The study was designed according to randomized block design with three replicates. The applications were a control, only Rhodobacter (B: 10mL/pod), only solid vermicompost (V: 8%) and three different amounts vermicompost together with a constant bacterial amount (V1B: 4%, V2B: 8%, V3B: 12%) and they were applied to the root area of the plants. Harvesting was done after 40-45 days. The chlorophyll and protein contents of fresh plant rinsed with distilled water were determined after harvest. Among the agro-morphological traits of the Malabar spinach, plant length, leaf length, side branching, plant wet and dry weights increased by all the applications (B, V, V1B, V2B and V3B) compared to the control. There was no significant increase in the leaf number; however, bacteria and vermicompost applications increased the side branching of the plant. The protein amount is not different among the applications but significantly higher than the control. The brightness value L is higher than control in all the applications, the highest L was observed by VB3. The (a) value indicating the green shade decreased by all the applications except for the B application. The (b) value had positive values by all applications showing that the yellow shade is dominant in this plant. The addition of free-living nitrogen fixing bacteria and the vermicompost revealed to increase the quality parameters of the Malabar spinach significantly, especially the organic compound. This product contributed as energy source for bacteria activity.

KEYWORDS:
Agro-morphological traits, Basella alba L., Rhodobacter capsulatus, vermicompost

INTRODUCTION

Malabar spinach (Basella alba L.) is a member of Basellaceae family. It is a fast growing, perennial, creeping plant which has high tolerance to elevated temperatures [1, 2, 3]. It is well-known as Climber, Malabar, Ceylon, Indian and Grapevine spinach [4, 5, 6]. It is naturally grown in Tropical Asia while the origin is thought to be India and Indonesia [7]. This plant can easily be cultivated in suitable climate and soil [8]. The creeping stem, leaves and young flower buds can be consumed as a vegetable. It is produced as substitute for the real spinach (Spinacea oleracea L.) and also an ethno medical plant. This leafy vegetable contains high amounts of Vitamin A, Vitamin C, flavonoid, saponin, carotenoid and amino acids. Besides, as a functional food, Basella alba L. has androgenic, anti-diabetic, anti-inflammatory, antimicrobial, antioxidant and anti-ulcer properties [3].

Today it is a must to obtain more products from unit area of soil in order to meet the nutritional requirements of the humans. On the other hand, more inorganic fertilizer is required to be applied to obtain more products from unit area of soil. Excessive use of inorganic fertilizers led to pollution of soil, water and other natural resources and serious health problems. The amount of inorganic fertilizers to be used as the main source of plant nutrition in order to increase efficiency [9], obtain highest amount of growth [10,11,12,13] and they have mostly been excessively utilized in olericulture [14, 15].

Rhodobacter capsulatus is a gram negative, photosynthetic purple non-sulfur bacterium that lives in soil and fresh water. It can fix the free nitrogen from the air by the help of the nitrogenase enzyme. This bacterium has been the subject of many researches because of its versatile metabolism, nitrogen fixing and hydrogen producing abili-
ties [16]. The ability of this bacterium to utilize various organic substances made it possible to employ in waste water treatment and remediation of the bottom mud polluted with organic substances. Moreover, it was used in crop development by being a nitrogen fixing bacterium [17, 18]. According to the reference [19], the increasing effect of Rhodobacter sphaeroides, another Rhodobacter species, on the root and shoot lengths, dry and wet weights and chlorophyll content of cucumber suggested that this bacterium would increase the plant growth in agricultural lands. Similarly, the purple non-sulfur bacterium were shown to increase the germination of tomato seeds by 30% and the dry weight of germinated seedlings by almost three times and were suggested to be candidates for bio fertilizers in agriculture [20].

In recent years, the use of vermicompost has been increased all around the world because of being organic fertilizer and a good soil amelioration material. According to [21], vermicompost does not contain any pathogens and heavy metals because of the healthy production methods and the properties of the worms, and therefore is a valuable organic fertilizer that increases the productivity of the soil. Besides being a nutritious source for plants, it has positive effects on the biological, chemical and physical features of the soil. Vermicompost is a worldwide accepted organic compost to be used in organic agriculture [22]. The word vermicompost is used for humus like substance obtained from composting of organic wastes using worms. In short, it means worm compost [21].

The purpose of this research was to identify the effects of vermicompost and bacteria usage on some agro-morphological properties of Malabar spinach which is an exotic vegetable in Turkey.

MATERIALS AND METHODS

A pot experiment was carried out in controlled conditions in 2018. This pot experiment was carried out in Tekirdag Namik Kemal University, Agricultural Faculty, Department of Soil Science and Plant Nutrition. The standard variety of the Malabar spinach Basella alba L. obtained from Zengarden firm, Turkey was used in this study (Figure 1). The experiment was under controlled climatic conditions in the lab. The ambient temperature was 25°C and the humidity was 80% in average that is the temperature preferred by the plant [6]. The seeds were sown to the multiscelled trays as duplicates. Peat (Klasmann-Deilmann Potgrond H, Germany) was used as production medium.

Some Specifications of The Peat. Some specifications of the used peat are: 160-260 mg/kg N, 180-280 mg/kg P2O5, 200-150 mg/kg K2O, 80-150 mg/kg Mg, pH: 6, organic matter 70 % and 35 % C. The seedlings were planted as 1 plant/pot in 5L of pots after 25-30 days of sowing. The experiment was designed according to randomized block design with 3 replicates. The applications in the trial were as follows: 1 control, only bacteria (B: 10mL/pod) only solid vermicompost (V: 8%), 3 different doses of vermicompost while keeping constant level of bacteria (V1: 4%; V2: 8%; V3: 12%). The solid vermicompost used in this study was obtained from a private vermicompost producing company in Suleymampasa, Tekirdag, Turkey, and was applied to the root zone.

FIGURE 1
General view from post-harvest Malabar spinach’s

Some Specifications of the Vermicompost. The pH of the vermicompost used in this study was 8.1 and it contained 65.6% humic acid, 216 mg/kg Zn, 65.6% organic matter, 21.1% humidity, 1.6% P2O5, total N was 2.1% and soluble K2O was 1.5%, and the C/N and soluble salt ratios were 14.4 and 6.5 mmos/cm respectively. These specifications show that this vermicompost was one of the ideal ones produced in Turkey, the Turkish Republic Ministry of Agriculture and Forestry regulations. It was produced by the worm species Eisenia fetida fed mostly with cattle dung. The average organic matter of similar vermicompost produced in Turkey was reported to be 51% [23].

Bacteria and Medium. Rhodobacter capsulatus DSM1710 obtained from German Collection of Microorganisms and Cell Cultures was used in this study. It was grown photosynthetically in a rich medium MPYE containing bactopeptone and yeast extract. The bacteria were collected by centrifugation in the log phase of growth. Cells were washed twice with sterile distilled water and 10 mL suspension containing 25x10⁷ bacteria were applied to the root area of the plants according to the experimental design.
**Plant Sampling and Analyses.** The Malabar spinach plants were harvested 40-45 days after planting. The indications for color were taken by using a Hunter Lab D25LT Color Measurement device with a large measurement range suitable for non-uniform materials. The color parameters were referred as brightness (L) and color coordinates of (a) and (b) [24, 25]. The measurements were carried out on randomly chosen leaves from each plant in three replicates. Plant length (cm), leaf length (cm), and leaf width (cm) were measured with a ruler and leaf and side branch numbers were counted [26]. The fresh weight of the plants was measured immediately after harvesting. Dry weights were obtained after washing with distilled water and after drying at 65 °C for 48 hours [27]. The total protein content was estimated in dry material by Kjeldahl method [28]. In addition, no pesticides applications were applied throughout the study.

**Statistical Analysis.** The results of the experiment were evaluated by the use of SPSS 21 statistics software. ANOVA variance analysis and Duncan multiple comparison tests were conducted on the research results.

**RESULTS AND DISCUSSION**

Influence of bacteria and vermicompost applications on the some agro-morphological properties of Malabar spinach are given in Table 1 and Figure 2.

According to the Table 1, among the agro-morphological properties of the Malabar spinach, plant length, leaf length, side branching, plant fresh and dry weights increased by all the applications of treatments compared to the control. However, there was no significant increase in the leaf number, and only sole bacteria and vermicompost applications increased significantly the side branching of the plant.

Rhizobacteria have plant growth promoter effects in plants [29, 30, 31, 32]. They may change the microbial composition of the rhizosphere on behalf of the beneficial microorganisms directly by producing growth hormones or indirectly by changing the amount of the mineral nutrients [33].

Bacteria promote plant growth especially in early period of plant growth and this gives more positive results in the plants whose leaves are consumed [34]. It was shown that the use of mineral nitrogen fertilizer alone negatively affected sugar beet; however, sugar beet quality was improved with inoculation of bacteria [35].

The bacteria supporting the plant growth can increase the plant growth by producing plant growth hormones [36]. A research on *Abelmoschus esculentus* showed the positive influence of vermicompost and bacteria applications on plant morphological traits [37]. The amount of protein in our study were all very similar and all-together higher in the vermicompost and bacteria applied groups compared to the control (Table 1). Bacteria applications increase the protein levels in the plants [32]. It was also found that *Rhodobacter* species fix significant amounts of nitrogen [38]. The nitrogen fixed by *R. capsulatus* in the present study is considered to be effective on the nitrogen and protein contents.

**TABLE 1**

<table>
<thead>
<tr>
<th>Agro-morphological property</th>
<th>Control</th>
<th>B</th>
<th>V</th>
<th>V1+B</th>
<th>V2+B</th>
<th>V3+B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf height (cm)</strong></td>
<td>120.00±0.5b</td>
<td>182.00±11.9a</td>
<td>167.66±14.44a</td>
<td>163.66±20.66a</td>
<td>183.34±7.26a</td>
<td>153.00±12.28ab</td>
</tr>
<tr>
<td><strong>Leaf width (cm)</strong></td>
<td>10.22±0.67b</td>
<td>12.77±0.44a</td>
<td>13.83±0.78a</td>
<td>14.23±0.61a</td>
<td>14.11±0.40a</td>
<td>13.44±0.48a</td>
</tr>
<tr>
<td><strong>Leaf number (piece)</strong></td>
<td>7.94±0.30c</td>
<td>10.10±0.24bc</td>
<td>10.17±0.28bc</td>
<td>13.23±1.89a</td>
<td>10.28±0.89bc</td>
<td>11.22±0.58ab</td>
</tr>
<tr>
<td><strong>Number of near shoot (piece)</strong></td>
<td>39±0.57ns</td>
<td>50.6±6.35ns</td>
<td>65±16.50ns</td>
<td>48±12.66ns</td>
<td>70.3±9.49ns</td>
<td>59.6±9.69ns</td>
</tr>
<tr>
<td><strong>Protein (%)</strong></td>
<td>8.0±0.57c</td>
<td>17.66±1.85a</td>
<td>17.33±1.76a</td>
<td>9.6±3.84b</td>
<td>18.33±0.88a</td>
<td>9.6±2.40ab</td>
</tr>
<tr>
<td><strong>Fresh weight (g)</strong></td>
<td>19.39±0.08b</td>
<td>27.97±0.83a</td>
<td>25.24±2.34a</td>
<td>27.47±0.96a</td>
<td>27.94±1.73a</td>
<td>26.7±0.34a</td>
</tr>
<tr>
<td><strong>Dry matter yield (g)</strong></td>
<td>40.48±0.64b</td>
<td>66.0±6.65ab</td>
<td>73.83±15.38a</td>
<td>61.66±11.49a</td>
<td>77.33±5.36a</td>
<td>68.8±6.93ab</td>
</tr>
</tbody>
</table>

B: 10 ml/pod *Rhodobacter*; V: 8% Vermicompost; V1+B: (4% Vermicompost + 10 ml Bacteria); V2+B: (8% Vermicompost + 10ml Bacteria); V3+B: (12% Vermicompost+10ml Bacteria). * The values are mean of three replications; ** each parameter was evaluated individually and values in the same column with different letters are statistically significant at the level of 5%. ns: non-significant.
Employing bacteria was shown to increase the effectiveness of vermicompost and also the protein content [39]. The combination of vermicompost, vermiwash, microorganisms *Rhizobium* and *Azospirillum* gave the highest protein content (4.62 mg/g) in *Abelmoschus esculentus* [37].

According to Table 1, the brightness value L increased in all the applications of vermicompost compared to the control and bacteria application treatments. The (a) value showing the green shade of the plants had negative values in all vermicompost applications, namely the plant is in a green state. However, this value being higher in all the applications compared to control shows that the greenness of the plant decreased compared to the control. The bacteria only application was the closest to the control condition. The (b) value, on the other hand, had positive values in all applications and they are all larger than the control. This means the yellow shade is dominant in this plant. The combination of vermicompost, vermiwash, microorganisms *Rhizobium* and *Azospirillum* also resulted in the highest chlorophyll-a and chlorophyll-b contents in *Abelmoschus esculentus* [37].

The nitrogen fixing bacteria and organic compost applications revealed to increase the quality parameters of the Malabar spinach significantly taking the agro-morphological traits into account. The pure cultures of microorganisms fail because of many reasons [32]. The pure cultures usually do not reach high inoculum concentration suitable for growth, staying alive and adapting to the soil conditions. In order to reveal the bacteria interactions, an organic matter should be applied to soils as energy source.

This study showed that the vermicompost and bacteria applications have the potential to be used in sustainable plant growing of the Malabar spinach. The inoculation of bacteria together with the vermicompost may have caused the bacteria to obtain the nutrients more efficiently and be protected from competition.

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The authors declare that there is no conflict of interest.

**REFERENCES**


THE ATTITUDES OF LOCAL PEOPLE TOWARDS HYDROPOWER PLANT: A CASE STUDY FROM TURKEY

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ABSTRACT

The aim of this study is to determine the attitudes of the population living in Yusufeli district of Artvin province to hydropower plants and the socio-demographic and intellectual factors affecting the hydropower plants' usefulness to the region. The main material of the study is the survey data with 140 people. The factors affecting the usefulness of hydropower plants to the region were analyzed by logistic regression method. In the study, the average age of individuals is 50.18, 45.0% of individuals are planning to migrate and 35.7% think that hydropower plants are beneficial to the region. According to the results of the regression analysis; as income level increases, a positive relationship was found between the individuals with primary school degree, believing that Turkey's dependence on foreign energy would be reduced by hydropower plants and a sufficient amount of water would be left for the protection of rivers and the idea that hydropower plants are beneficial to the region. According to these results, the fact that the policymakers provide new agricultural lands that substitute for flooded agricultural land to farmers in regions where hydropower plants will be installed can ensure that farmers can continue agricultural production and thus contribute to the reduction of migration in the region.

KEYWORDS:
People, attitudes, hydropower plant, logistic regression, Turkey

INTRODUCTION

Energy is an important factor in the economic development of countries. Therefore, energy consumption is considered as an indicator of the level of development of countries. The rapid increase in the world population together with the urbanization and industrialization caused the demand for energy to increase rapidly. Most of the energy produced in the world is still produced from fossil fuels: coal, oil and natural gas [1]. However, the rapid depletion of fossil fuels, global warming, climate change, reduction of greenhouse gases and uncertainties in oil prices have led the countries all over the world to use renewable energy sources. [2-4].

Because hydropower plants are an important renewable energy source [5] and provide efficient, reliable and relatively low cost of electricity production, they are considered an important electricity production option [6, 7]. However, the rapid development of large hydropower plants in recent years, especially in developing countries, has led to debates on economic [8], social [9, 10] and environmental issues [11-13]. Although there is some debate, hydropower plants are important in order to provide the energy needed, especially in developing countries, and it is one of the most frequently used energy production methods in many countries around the world.

In 2017, 25.0% of the world's electricity generation (24,345 TWh) is produced from renewable energy sources and this ratio is estimated to increase to 31.4% in 2040. It is estimated that the electricity generated from renewable energy sources will increase by 2.8% annually between 2015-2040. While 71.0% of electricity produced from renewable energy sources in the world was produced from hydropower plants in 2015, it is estimated that this ratio will decrease to 53.0% in 2040 due to limiting the installation of new medium and large-scale hydropower plants due to access to water resources and environmental concerns in many countries [14].

In Turkey, as it is in the world, there is an increasing trend towards renewable energy sources. The most important reason for Turkey is high electrical energy production from fossil sources and also the importation of these resources. The increases in oil and natural gas prices cause the electrical energy obtained from fossil fuels become quite costly for Turkey. In Turkey, of the total electricity generation in 2018, 31.0% was obtained from natural gas, 38.0% coal, 21.0% hydropower plant, 7.0% wind, 2.0% geothermal and 2.0% other sources [15].

Turkey is a country with a high hydropower potential thanks to its topographic structure and appropriate river flows [16, 17]. Turkey's hydropower potential is of approximately 433 billion kWh/year, technically viable potential is of 216 billion
kWh/year and the economic hydropower energy potential is of 164 billion kWh/year [18]. Turkey's theoretical hydropower potential is 1% of the world theoretical potential and the economic potential is 16% of Europe's economic potential. There are 636 hydropower plants in operation by the end of June 2018 in Turkey. Their total installed capacity is of 27,912 MW, and it corresponds to Turkey’s 32% of total installed capacity. 58.2 billion kWh electricity was generated from hydropower in 2017 [19].

Turkey's hydropower potential varies significantly by region. The hydropower energy potential of Southeast Anatolia, Eastern Anatolia and Eastern Black Sea regions is quite high thanks to both the presence of rivers and the topographic structure. Yusufeli district of Artvin province where the study was conducted is located in the Çoruh River Basin in the Eastern Black Sea region. Çoruh River Basin includes Borçka, Artvin Center and Yusufeli districts and many villages connected to Artvin and it is the basin with the highest economically usable energy potential (45%) [20]. As a result of research conducted in Çoruh River Basin which ranks the third in Turkey, its potential was noticed and 10 large dams were planned to be built on the basin. 5 of the planned dams are located within the boundaries of Artvin province [21]. There are a total of 25 hydropower plants operating in the province of Artvin, with an installed capacity of 1,728 MW and an annual electricity production of 3,975 GWh [22].

Yusufeli District, where the research was conducted, is located in an area where the Eastern Black Sea and Eastern Anatolia culture are approaching to each other. The main source of the revenue for people is agriculture. Expropriation process was performed in the remaining areas of Yusufeli dam’s reservoir area. Most of the expropriated areas are composed of agricultural land and settlements. The dam lake, which will be formed as a result of the construction of Yusufeli Dam and Hydropower Plant, will leave Yusufeli district center and three villages completely under water. In addition, 16 villages and/or their lands will be partially flooded. The individuals who lose their economic income due to the fact that the agricultural areas will be inundated, have to migrate to any districts within the boundaries of Artvin or to different cities with the expropriation price. There are not many studies on the socio-economic effects of hydropower plants in Yusufeli district of Artvin province. For this reason, it is very important to reveal the attitude and thoughts of the people of Yusufeli about hydropower plants. The aim of this study is to determine the attitudes of the population living in Yusufeli district of Artvin province to hydropower plants and the socio-demographic and intellectual factors affecting the hydropower plants’ usefulness to the region.

MATERIALS AND METHODS

The main material of the study is the survey data of 140 people in Yusufeli district of Artvin province. In the study, 90% confidence interval and 5% error margin were used in the calculation of sample volume. The surveys were conducted in February and March 2018. Proportional sampling method was used to determine the sample volume [23, 24].

\[
Np(1-p) \\
\frac{(N-1)\sigma_p^2}{p(1-p)}
\]

In the research, the socio-demographic characteristics of the individuals who participated in the survey were presented as frequency tables and the attitudes of the individuals related to the hydropower plants were determined by using the 5-point Likert scale. The basic approach on the Likert scale is based on the fact that individual area given judgments on the subject investigated and a concentration is reached on these judgments. In the research, individuals were asked various statements related to hydropower plants and the level of participation of individuals in these statements was tried to be determined.

Factors affecting the usefulness of hydropower plants to the region were analyzed using the limited dependent variable regression model "Logit estimation method". In cases where the dependent variable is binary (0, 1), commonly used analysis methods are Logistic Regression (LR), Logit, Probit and Linear Probability Models [25]. In logistic regression model, the fact that the normality assumption requirement is not required and it is easy to interpret increases the usage of the model. In the study, whether the individuals who took part in the survey think that the hydropower plants are beneficial to the region or not was determined as the limited dependent variable. Those who think they are beneficial are coded with “1” and who think that they are not beneficial are coded with “0”. Logit model is formulated as follows [26].

\[
P_0 = E(Y = 1|X) = \frac{1}{1+e^{-[\alpha+\beta X]}}
\]

\[
P_1 = E(Y = 1|X) = \frac{1}{1+e^{-[\alpha+\beta X]}} = \frac{1}{1+e^{-[\alpha+\beta X]}}
\]

RESULTS AND DISCUSSION

The socio-demographic characteristics of the individuals participating in the survey are given in Table 1. The average age of the individuals participating in the survey was 50.18, the average household size was 4.51 and the average monthly income was determined as 1976 TL. In a similar study on the subject in the region, the average age of the participants was 49.54 and the average household size was 5.79 [7]. In the study, it was determined that 30.73% of the participants were primary school, 34.28% were secondary school, 27.17% were high school and 7.85% were university graduates. In another study conducted in the same region, it was reported
that 31.7% of individuals were high school graduates and 15.0% were university graduates [21]. In the study, it was determined that 10.0% of the individuals were farmers, 37.87% were tradesmen, 15.71% were civil servants and 20.71% were retired. In a similar study conducted in the same region, it was determined that 17.9% of individuals were farmers, 29.4% were tradesmen and 9.0% were civil servants [21], and in a similar study in Greece, it was determined that 6.0% of individuals were farmers, 8.3% were unemployed and 76.9% were workers [5].

The level of participation of the participants in the statements related to hydropower plants is given in Table 2. It was determined that the participants partly agreed on the statements of “Without hydropower plant, rivers flow in vain”, “Hydropower plants have little impact on the environment”, “There is enough water left to protect the rivers”, “Nuclear power plants are better than hydropower plants” and “The contribution of hydropower plants to the region is indisputable.” According to these results, it can be said that the individuals don’t agree on the hydropower plants’ statements about their benefit to the region and the environment. In the study conducted in Greece, it was determined that the participants partly agreed on the statements of “Hydropower plants have very little protective effect on the prevention of floods”, “Construction of hydropower plants reduces fish fauna”, and “Hydropower plants reduce carbon dioxide emissions” [5].

It was determined that the individuals are undecided on the statements of “Environmental impact assessment is carried out in hydropower plant projects, is not it enough”, “Hydropower plants are being built in naturally protected areas”, and “Thanks to hydropower plants, we will get rid of foreign dependency in energy”.

### TABLE 1
Socio-demographic statistics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30</td>
<td>68</td>
<td>50.18</td>
<td>8.159</td>
</tr>
<tr>
<td>Number of people in the family</td>
<td>1</td>
<td>18</td>
<td>4.51</td>
<td>1.965</td>
</tr>
<tr>
<td>Monthly income (TL)</td>
<td>500</td>
<td>4500</td>
<td>1976</td>
<td>847.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary School</td>
<td>43</td>
<td>30.73</td>
</tr>
<tr>
<td>Secondary School</td>
<td>48</td>
<td>34.28</td>
</tr>
<tr>
<td>High School</td>
<td>38</td>
<td>27.17</td>
</tr>
<tr>
<td>College</td>
<td>11</td>
<td>7.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unemployed</td>
<td>11</td>
<td>7.85</td>
</tr>
<tr>
<td>Employee</td>
<td>11</td>
<td>7.85</td>
</tr>
<tr>
<td>Farmer</td>
<td>14</td>
<td>10.00</td>
</tr>
<tr>
<td>Handicraftsman</td>
<td>53</td>
<td>37.87</td>
</tr>
<tr>
<td>Government official</td>
<td>22</td>
<td>15.71</td>
</tr>
<tr>
<td>Retired</td>
<td>29</td>
<td>20.72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Social Insurance</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>8</td>
<td>5.70</td>
</tr>
<tr>
<td>Yes</td>
<td>132</td>
<td>81.30</td>
</tr>
</tbody>
</table>

### TABLE 2
Attitudes of people toward hydropower plant

<table>
<thead>
<tr>
<th>Statement</th>
<th>Mean</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without hydropower plant, rivers flow in vain</td>
<td>2.56</td>
<td>0.85</td>
</tr>
<tr>
<td>Hydropower is a renewable source of energy</td>
<td>3.65</td>
<td>0.75</td>
</tr>
<tr>
<td>Hydropower plants have little impact on the environment</td>
<td>2.51</td>
<td>0.90</td>
</tr>
<tr>
<td>There is enough water left to protect the rivers</td>
<td>2.78</td>
<td>0.88</td>
</tr>
<tr>
<td>Environmental impact assessment is carried out in hydropower plant projects, is not it enough</td>
<td>3.08</td>
<td>0.86</td>
</tr>
<tr>
<td>Thanks to hydropower plants, we will get rid of foreign dependency in energy</td>
<td>3.01</td>
<td>0.94</td>
</tr>
<tr>
<td>Hydropower plants are being built in naturally protected areas</td>
<td>3.25</td>
<td>0.95</td>
</tr>
<tr>
<td>Turkey is rich in water resources and its hydropower potential is high</td>
<td>3.61</td>
<td>0.82</td>
</tr>
<tr>
<td>Nuclear power plants are better than hydropower plants</td>
<td>2.62</td>
<td>1.07</td>
</tr>
<tr>
<td>Wind turbines instead of hydropower power plants should be installed</td>
<td>3.99</td>
<td>0.81</td>
</tr>
<tr>
<td>Hydropower plants force local people to migrate</td>
<td>3.54</td>
<td>0.74</td>
</tr>
<tr>
<td>The contribution of hydropower plants to the region is indisputable</td>
<td>2.87</td>
<td>0.94</td>
</tr>
</tbody>
</table>

TABLE 3
Variable definition and sample means

<table>
<thead>
<tr>
<th>Variables</th>
<th>Definition</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depended Variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydropower plants usefulness for the region (1: Yes; 0: No)</td>
<td></td>
<td>0.357 (0.481)</td>
</tr>
<tr>
<td>Independent Variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous Independent variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of people in the family</td>
<td>NBRF</td>
<td>4.514 (1.965)</td>
</tr>
<tr>
<td>Age</td>
<td>AGE</td>
<td>50.178 (8.158)</td>
</tr>
<tr>
<td>Monthly income (TL)</td>
<td>INCOME</td>
<td>1976 (847.304)</td>
</tr>
<tr>
<td>Binary Independent variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education (Primary school education)</td>
<td>EDU</td>
<td>0.307 (0.463)</td>
</tr>
<tr>
<td>Occupation (Farmer)</td>
<td>OCU</td>
<td>0.100 (0.301)</td>
</tr>
<tr>
<td>Employed in the hydropower plant (Work HPP)</td>
<td>WORK</td>
<td>0.393 (0.490)</td>
</tr>
<tr>
<td>Intend to migrate</td>
<td>MIGRATE</td>
<td>0.450 (0.499)</td>
</tr>
<tr>
<td>Without hydropower plant, rivers flow in vain</td>
<td>WTHHPP</td>
<td>0.471 (0.501)</td>
</tr>
<tr>
<td>There is enough water left to protect the rivers</td>
<td>EWPR</td>
<td>0.621 (0.487)</td>
</tr>
<tr>
<td>Environmental impact assessment is carried out in hydropower plant projects, is not it enough</td>
<td>EIA</td>
<td>0.779 (0.417)</td>
</tr>
<tr>
<td>Thanks to hydropower plants, we will get rid of foreign dependency in energy</td>
<td>THPDE</td>
<td>0.693 (0.463)</td>
</tr>
<tr>
<td>Nuclear power plants are better than hydropower plants</td>
<td>NPP</td>
<td>0.500 (0.502)</td>
</tr>
<tr>
<td>Having livestock</td>
<td>LIVESTOCK</td>
<td>0.614 (0.489)</td>
</tr>
<tr>
<td>Sample size</td>
<td></td>
<td>140</td>
</tr>
</tbody>
</table>

Note: Standard deviation are in parentheses.

TABLE 4
Binomial Logit model estimation results

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>Z</th>
<th>P-value</th>
<th>Marginal Effect</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-4.043</td>
<td>-2.100</td>
<td>0.0357</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>NBRF</td>
<td>-0.080</td>
<td>-0.518</td>
<td>0.6047</td>
<td>-0.010</td>
<td>1.178</td>
</tr>
<tr>
<td>AGE</td>
<td>-0.019</td>
<td>-0.659</td>
<td>0.5102</td>
<td>-0.003</td>
<td>1.160</td>
</tr>
<tr>
<td>INCOME</td>
<td>0.0008**</td>
<td>2.496</td>
<td>0.0125</td>
<td>0.0001</td>
<td>1.151</td>
</tr>
<tr>
<td>EDU</td>
<td>0.993*</td>
<td>1.689</td>
<td>0.0912</td>
<td>0.126</td>
<td>1.107</td>
</tr>
<tr>
<td>OCU</td>
<td>-2.149**</td>
<td>-2.092</td>
<td>0.0364</td>
<td>-0.244</td>
<td>1.057</td>
</tr>
<tr>
<td>WORK</td>
<td>-0.385</td>
<td>-0.724</td>
<td>0.4691</td>
<td>-0.498</td>
<td>1.145</td>
</tr>
<tr>
<td>MIGRATE</td>
<td>-1.590***</td>
<td>-2.804</td>
<td>0.0051</td>
<td>-0.219</td>
<td>1.290</td>
</tr>
<tr>
<td>WTHHPP</td>
<td>1.068*</td>
<td>1.896</td>
<td>0.0579</td>
<td>0.145</td>
<td>1.440</td>
</tr>
<tr>
<td>EWPR</td>
<td>1.256**</td>
<td>2.271</td>
<td>0.0232</td>
<td>0.173</td>
<td>1.203</td>
</tr>
<tr>
<td>EIA</td>
<td>1.232*</td>
<td>1.799</td>
<td>0.0720</td>
<td>0.157</td>
<td>1.090</td>
</tr>
<tr>
<td>THPDE</td>
<td>2.373***</td>
<td>3.219</td>
<td>0.0013</td>
<td>0.302</td>
<td>1.228</td>
</tr>
<tr>
<td>NPP</td>
<td>-0.363</td>
<td>-0.691</td>
<td>0.4897</td>
<td>-0.047</td>
<td>1.139</td>
</tr>
<tr>
<td>LIVESTOCK</td>
<td>-0.626</td>
<td>-1.215</td>
<td>0.2244</td>
<td>-0.082</td>
<td>1.130</td>
</tr>
</tbody>
</table>

McFadden R-Squared: 0.386  Log likelihood: -56.058  Res. Log likelihood: -91.246  X2 (13): 70.376***

Statistical significance *** at the 1% level; ** at the 5% level; * at the 10% level.

It was determined that the individuals significantly agreed on the statements of “Hydropower is a renewable source of energy”, “Turkey is rich in water resources and its hydropower potential is high”, “Wind turbines instead of hydropower plants should be installed”, and “Hydropower plants force local people to migrate”. In the study conducted in Greece, it was determined that the participants significantly agreed on the statements of “Construction of hydropower plants should have a more stringent regulation”, “Hydropower plant construction provides job opportunities for people in the region” [5].

Definitions of variables used in the model and descriptive statistics of variables are given in Table 3. In the study, it was determined that 39.3% of the individuals participated in the survey are working for the construction or operation of hydropower plants, 45.0% of them think to migrate and 35.7% of them believe that hydropower plants are beneficial to the region.

In the study, logistic regression analysis results performed in order to determine the factors effecting the hydropower plants’ usefulness to the region are given in Table 4. The model was found statistically significant and the validity ratio of the independent variables used in the model was calculated as 79.3%.

In the study, a positive relationship was found between the income level of individuals and the thought that hydropower plants are beneficial to the region. According to this result, as individuals’ income level increases, the probability of the thought that hydropower plants are beneficial to the region is increases. The increase in the monthly income of individuals by 1000 TL increases the probability of the
thought that hydropower plants to be beneficial to the region by 1%.

In the study, it is more likely for primary school graduates to think that hydropower plants are beneficial to the region when compared to individuals with secondary or more education level. In other words, those with a high education level are less likely to think that hydropower plants are beneficial to the region. This result can be said to meet expectations, because as the level of education increases, individuals’ perceptions about environment and natural life and their sensitivity to environmental events increase. In a similar study conducted in the region, it was determined that as the individuals’ education level increases, they are more likely to be against the dam construction in their regions [7, 27].

In the study, it is 24.4% less likely for farmers to think that hydropower plants are beneficial to the region when compared to other professions, because farmers are most adversely affected (their lands are submerged, they lose their lands due to expropriation and they are forced to migrate from their villages, etc.) by the hydropower plants. Therefore, it can be said that the result of the study is consistent with the expectations.

In the study, it is 21.9% less likely for the individuals planning to migrate to think that hydropower plants are beneficial to the region. In other words, those who are planning to migrate do not think that hydropower plants are beneficial. The migration phenomenon in the district that has been emigrating for years due to various reasons has an important place in the memory of the local people [21]. It can be said that the individuals who are planning to migrate in the region are the individuals who are experiencing economic problems. The livelihood of a significant majority of families in the region is based on agriculture. Due to the construction of dams and hydropower plants in the region, many agricultural lands remain under water and agricultural production is not possible. This situation is one of the important factors triggering migration in the district.

In the study, for the individuals who think without hydropower plants, the rivers flow in vain, a sufficient amount of water is left for the protection of rivers, the environmental impact assessment report is sufficient for the installation of hydropower plants and thanks to hydropower plants, Turkey will get rid of dependence on foreign energy, the probability of the idea that hydropower plants are beneficial to the region is 14.5%, 17.3%, 15.7% and 30.2% higher respectively when compared to the ones who don’t think so. Since all of the aforementioned considerations are generally indicative of the positive aspects of hydropower plants, the fact that some believe that hydropower plants are beneficial to the region complies with expectations. Here, the fact that with the electricity generated by hydropower plants, Turkey’s dependence on foreign energy will be reduced is the factor having the greatest impact.

CONCLUSIONS

In the study, the attitudes of the population living in Yusufeli district of Artvin province to hydropower plants and the socio-demographic and intellectual factors affecting the hydropower plants’ usefulness to the region were determined. In the study, one third (1/3) of individuals believe that hydropower plants are beneficial to the region, in other words, two thirds of the population (2/3) think that hydropower plants are not beneficial to the region. According to this result, a significant majority of the individuals participated in the survey do not find hydropower plants in the region useful.

In the study, it was found that individuals largely disagree on positive statements related to hydropower plants like “Without hydropower plants, rivers flow in vain”, “Hydropower plants have little impact on the environment”, and “The contribution of hydropower plants to the region is indisputable”, but it was found that individuals largely agree on negative statements about hydropower plants like “Hydropower plants force local people to migrate”. These results show that the respondents have a negative attitude towards the hydropower plants in the district. In the study, it was determined that farmers and the individuals who are planning to migrate from the district the think that hydropower plants are not useful to the region.

According to the results obtained from the research, it is important to take ideas and thoughts of the people who live in the places where hydropower plants will be installed and to inform them about the possible risks and negative sides of hydropower plants and to eliminate these risks and negative sides by public authority to change the attitudes of the people to hydropower plants in a positive way. The fact that the results obtained from the research are taken into consideration by the policy makers for the new hydropower plants can contribute to the reduction of social problems.

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Adem Aksoy for his contributions to the provision of research data.

REFERENCES


Sever, R. and Ulu Kalm, Ö. (2010) Some thoughts of Arttvin community about dams that have been built/have been building in Arttvin. Eastern Geographical Review. 23, 65-79.


PEROXIDASE FROM KIWANO (CUCUMIS METULIFERUS): BIOCHEMICAL CHARACTERIZATION AND PURIFICATION BY USING THREE-PHASE PARTITIONING

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2Sakarya University, Faculty of Arts and Sciences, Chemistry, Sakarya, Turkey

ABSTRACT

Peroxidase (POD) was extracted from kiwano (Cucumis metuliferus) and purified for the first time using three-phase partitioning (TPP). The POD was purified 4.47-fold with 138.78% recovery of enzyme activity. In the sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis, the POD showed a molecular weight of approximately 38 kDa. The optimum pH and temperature were found to be in the range of 4.0-7.5 and 30°C-40°C, respectively, for substrates. The maximum substrate specificity was observed using caffeic acid (Km = 0.03 mM) as the substrate. Seven inhibitors were investigated, of which KCN was the strongest inhibitor. Tests were performed for identifying various metal ions, of which Fe2+ was the most effective inhibitor and Hg2+ was the strongest activator. All tested organic solvents inhibited the POD activity. The optimum ionic strength of POD was found in 4 mM NaCl. The POD had a high salt tolerance with an enhancement of approximately 12%-20% enzyme activity after 2 h incubation in NaCl. Enzyme storage stability led to decrease in the POD activity by 84% at room temperature after 400 h, 83% at +4°C after 60 days, and 73% at −20°C after 135 days. To our knowledge, the present study is the first to report the purification and characterization of the POD extracted from kiwano, which could be useful in potential applications.

KEYWORDS:
Enzyme kinetics, kiwano, peroxidase, three-phase partitioning

INTRODUCTION

Peroxidase (POD) is a heme-containing enzyme, which is a member of oxidoreductases (E.C.1.11.1.7); and it catalyzes the oxidation of various substrates comprising organic and inorganic structures in the presence of hydrogen peroxide (H2O2) [1]. It is mostly found in the cell wall and is widely distributed in the plant [2]. Peroxidases play a role in essential plant processes such as plant hormone regulation, lignin biosynthesis, and defense mechanisms [3]. Peroxidases are involved in numerous industrial applications, some of which have been reported in various areas such as analytical, environmental, and medical fields. Some of these applications include the synthesis of numerous aromatic compounds, the removal of phenolic compounds from wastewaters, the removal of peroxides from food and other industrial wastes, biomarkers in clinical diagnoses, and for the decolorization of industrial dye [4, 5].

Horseradish (Armoracia rusticana P.Gaertn., B.Mey. & Scherb.) has been frequently used as the primary commercial source of the POD over the years. It has preferred due to wide substrate specificity, low pH, and thermal stability features [6].

Kiwano, also known as the African horned cucumber or jelly melon, belongs to the Cucurbitaceae family and is one of the plant sources of POD. It is native to Africa and South Eastern America and has also been grown in the tropical regions of California, Chile, Canada, Australia, and New Zealand [7]. Kiwano contains several beneficial nutrients including sodium, phosphorus, magnesium, calcium, potassium, iron, vitamin C, and vitamin E [8]. In addition, kiwano contains several beneficial secondary metabolites including alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids, and terpenoids [9, 10]. The health benefits of kiwano stem from these nutrients; furthermore, kiwano reportedly contains antiviral [11], antimicrobial [12], anti-diabetic [10], anti-ulcer [13], anti-protozoan [14], and hematological effects [15].

Kiwano has the potential of becoming a novel source of POD, but there still exists a paucity of knowledge on its exploitation. It is necessary to find an alternative source for commercial production of POD and, therefore, there is a need for research to find novel alternative POD sources that can be easily purified, have wide substrate specificities, and show better stability to extreme environmental conditions [16]. In recent years, peroxidases have been purified through various methods and they have been characterized from many plant sources such as black gram [17], avocado [18], and wheat [19]. The aim of the present study was to partially purify kiwano using the three-phase partitioning (TPP) method, to characterize it; investigate the effects of inhibitors, metal
ions and organic solvents on it; and to determine its optimum ionic strength, salt tolerance, and storage stability.

The aim of the present study is to purify partially kiwano by three-phase partitioning method, characterize it, investigate the effects of inhibitors, metal ions and organic solvents on it and determine its optimum ionic strength, salt tolerance, and storage stability.

**MATERIALS AND METHODS**

**Plant materials and chemicals.** Fresh kiwano fruits were purchased from a local market Istanbul, Turkey. Chemicals of interest were preferred in the analytical grade. Enzyme assay and the determination of protein content were performed using Shimatzu UV 2401 PC UV-vis spectrophotometer.

**Extraction of POD.** Excluding the outer shell, kiwano fruits were cut into small pieces. The crude enzyme extract was prepared by mixing 10 g of sample with 30 mL of 0.1 M phosphate buffer (pH 7.0) containing 0.5% (w/v) polyvinylpyrrolidone and 0.2 mM ascorbic acid. For extracting POD, the mixture was stirred at room temperature for 5 min using a blender. Next, the homogenate was filtered through three layers of cheesecloth and centrifuged at 5000 x g for 15 min at 4°C. The supernatant was collected for further assays.

**Three-phase partitioning.** TPP is a novel technique that aims to purify proteins by adding ammonium sulfate and t-butanol to the extract. Protein solutions are separated into the following three phases: lower aqueous, middle, and upper phases. Polar components such as saccharides are located in the lower aqueous phase, while the middle phase is enriched with protein and the upper phase contains pigments, lipids and enzyme inhibitors. According to the procedure by Ozer et al. [20], the protein content in the previously assigned POD extracted from kiwano was saturated with 50% (w/v) (NH₄)₂SO₄ at 25°C and gently vortexed to dissolve it. The pH of the solution was maintained at 7.0 and then added t-butanol was added in the ratio of 1:1.5 (v/v). The solution was gently vortexed and then incubated for 1 h at 25°C. The POD-rich middle and lower aqueous phases were carefully collected with a pipette. The middle phase was dissolved in 0.1 M phosphate buffer, pH 7.0. The activity of POD in these phases was measured and the quantity of total protein was determined by the Lowry procedure, using bovine serum albumin as standard [21]. According to the result of the first TPP application, the middle phase contained the highest POD content. Therefore, we decided to apply a second TPP application to the middle phase following the same procedure (total 85% (NH₄)₂SO₄ and 1:1.5 t-butanol). At the end of the two TPP applications, the middle phase was dialyzed overnight against 0.1 M phosphate buffer, pH 7.0 at 4°C. The activity and protein content of POD were thus determined.

**Native and sodium dodecyl sulfate polyacrylamide gel electrophoresis.** The method by Laemmli [22] was used for polyacrylamide gel electrophoresis (PAGE) of the POD. Polyacrylamide gel (12%) was used for native and sodium dodecyl sulfate (SDS) PAGE. After employing SDS-PAGE, the gel was dyed with Coomassie Brilliant Blue R-250. In native-PAGE, the gel was incubated in 30 mM 4-methylcatechol solution for 1 h.

**POD activity assay.** In the POD activity assay, the reaction solution (3 mL) consisted of 100 µL of the crude enzyme solution, 2690 µL appropriate buffer (0.1 M), 60 µL H₂O₂ (1 mM), and 150 µL substrate (3 mM). The absorbance of the mixture was read at 420 nm during 60 s at room temperature. In all experiments, various substrates, consisting of 4-methylcatechol, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), guaiacol, caffeic acid, o-dianisidine, o-phenylenediamine, pyrogallol, catechol, and H₂O₂ were used.

**Optimum pH.** The effect of pH (3.0 - 9.5) on POD activity was measured in 0.1 M buffers of citrate buffer (3.0 - 6.0), phosphate buffer (6.0 - 8.0), and Tris-HCl (pH 8.0 - 9.5) at standard assay conditions, using all substrates, separately. The residual activities at different pH values were compared with those obtained at the optimal pH (%).

**Optimum temperature.** The optimum temperature was determined at a temperature range of 0 °C - 100°C. A water bath was used for high temperatures and an ice bath for low temperatures. The mixture containing the 0.1 M buffer at appropriate optimum pH for each substrate and POD extract was heated or cooled. The temperature was monitored using a stainless steel stem digital thermometer. After reaching a temperature equilibrium, enzyme activity was measured under standard assay conditions. The remaining activities at different temperature values were compared with those obtained at the optimal temperature (%).

**Substrate specificity and POD kinetics.** Substrate specificity and POD kinetics were investigated by determining the activity of different substrate concentrations, which were ranging from 0.01 to 20 mM under optimal conditions, at a fixed concentration of H₂O₂ or vice versa. Michaelis-Menten constant (Kₘ) and maximum velocity (Vₘₕₐₓ) values were determined for POD reactions with each of these substrates as per the Lineweaver-Burk graph [23].
### TABLE 1
Overall purification of the POD extracted from kiwano through TPP.

<table>
<thead>
<tr>
<th>Step</th>
<th>Activity (U/mL)</th>
<th>Protein (mg/mL)</th>
<th>Specific Activity (U/mg)</th>
<th>Activity Yield (%)</th>
<th>Fold purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>444.8</td>
<td>1.17</td>
<td>381.72</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>First TPP (lower phase)</td>
<td>7.3</td>
<td>0.0065</td>
<td>1116.11</td>
<td>1.71</td>
<td>0.02</td>
</tr>
<tr>
<td>1st TPP (middle phase)</td>
<td>502.2</td>
<td>0.016</td>
<td>3180.84</td>
<td>117.4</td>
<td>0.84</td>
</tr>
<tr>
<td>2nd TPP (middle phase)</td>
<td>657.2</td>
<td>0.90</td>
<td>734.10</td>
<td>147.75</td>
<td>1.92</td>
</tr>
<tr>
<td>Dialysis (middle phase)</td>
<td>617.3</td>
<td>0.36</td>
<td>1705.43</td>
<td>138.78</td>
<td>4.47</td>
</tr>
</tbody>
</table>

Effect of some inhibitors. A wide range of inhibitors, sodium azide (NaN₃; 1mM), thiourea (1 mM), KCl (0.001 mM), L-cysteine (0.5 mM), EDTA (0.1 mM), Triton X-100 (0.1%, v/v), and L-glutathione (1 mM), were subjected to experiments for determining their inhibitory effects on POD extracted from kiwano. Each inhibitor was added to the reaction mixture after which it was gently vortexed and incubated for 60 min at room temperature. The enzyme activity was thereafter measured under standard enzyme assay. The percentage of the remaining enzyme activity samples was calculated under the assumption that the activity of the control sample is at 100%.

Effect of some metal ions. The effects of metal ions (Fe³⁺, Fe²⁺, Cu²⁺, Zn²⁺, Mg²⁺, Hg²⁺, Ba²⁺, Ca²⁺, Li⁺, Co²⁺, Mn²⁺, Pb²⁺, Sn²⁺, Na⁺, K⁺, Ni²⁺, Cd²⁺, Al³⁺) were verified at different concentrations (1 mM, 5 mM, and 10 mM). Each metal ion was added to the reaction mixture at these concentrations and incubated for 1 h at 4°C. The enzyme activities of all samples were measured under standard assay conditions. The percentage relative activity of samples was calculated under the assumption that the activity of the control sample is at 100%.

Effect of organic solvent. Eight organic solvents [ethanol, methanol, dimethyl sulfoxide (DMSO), acetone, butanol, isopropanol, chloroform, and ethyl acetate] were used in this experiment. The reaction mixture that was exposed to organic solvents was incubated within a shaker for 32 h at room temperature. The enzyme activity was measured under standard assay conditions at the end of the 2nd, 6th, and 32nd h. The control sample was without any organic solvent and was assumed to be at 100%, and with that reference, the percentage relative activities of other samples were calculated.

Ionic strength. The effects of varying NaCl solutions in the concentration range of 1 - 5 mM on POD were investigated. The enzyme activity was measured using standard enzyme assay. The control sample without NaCl was assumed to be at 100%, and with that reference the relative enzyme activities of other samples were calculated.

Salt tolerance. The enzyme solution was incubated with NaCl solutions (changing the concentration of range between 1-5 mM) for 2 h at room temperature. The enzyme activities were measured under standard assay conditions. The percentage relative enzyme activities of samples were calculated by assuming that the control sample activity was at 100%.

Storage stability. The enzyme solution was stored at room temperature, 4°C and −20°C separately for 135 days at most. The residual activities of samples at these temperatures were calculated by measuring their enzyme activities at certain time intervals.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA) and Excel (Microsoft Co, Redmond, WA, USA). All results were expressed as means with their standard deviations (SDs).

RESULTS AND DISCUSSION

POD purification and determination of molecular weight. In this study, the POD was first isolated from kiwano and then purified using ammonium sulfate fractionation, TPP, and dialysis. Various conventional procedures consisting of many techniques were used for the purification of the POD. These are complex, expensive, and time-consuming methods [24]. However, TPP is easy, cheap, rapid, and comprises only one step of purification. Thus, TPP was preferred for the purification of the POD extracted from kiwano. In this study, the POD in the middle phase was purified 1.92-fold with a 147.75% recovery of the enzyme activity by consecutively applying the TPP method to the middle phase twice consecutively. After dialysis, the POD was purified 4.47-fold with 138.78% total recovery of the enzyme activity. The optimum parameters of purification were 85% (w/v) total (NH₄)₂SO₄ saturation (pH 7.0), and the ratio of crude extract to t-butanol of 1:1.5 (v/v) at room temperature. The overall purification results of the POD extracted from kiwano are presented in Table 1. It has been reported that POD from *Amsonia orientalis* Decne. was purified 12.5-fold with 162% recovery of the enzyme activity in the presence of 20% (w/v) (NH₄)₂SO₄ saturation at pH 6.0 and 1:1 (v/v) ratio of crude extract to t-
butanol using TPP [25]. The maximum purity of 18.20-fold with 93.96% recovery of enzyme activity from orange peels POD was obtained using TPP applied with 50% ammonium sulfate saturation at pH 6 and temperature 30°C in a ratio of 1:1.5 (v/v) crude extract: t-butanol [26]. POD from bitter gourd waste was purified 4.89-fold with 177% recovery of enzyme activity by TPP [27]. PODs from various sources have been purified by using the TPP technique and have obtained different purification folds and yields. POD from kiwano was purified for the first time using the TPP method which was applied twice and then the purification was enhanced with dialysis.

The POD isolated from rosemary leaves had a maximum activity in pH 6.0 [29]. In general, maximum enzyme activity was observed in the pH range of 4.0 - 7.5. However, the enzyme activity decreased significantly at the pH of lower than 4.0 and higher than 7.5.

**Determination of optimum temperature.**

The optimum temperature was recorded in the range of 30°C - 40°C for different substrates (Table 2). Belcarz et al. [30] recorded 40°C as the optimum temperature of spring cabbage POD and Aghelan et al. [29] recorded 40°C as the optimum temperature of POD for guaiacol obtained from rosemary leaves. The optimum temperature of the POD enzyme obtained from different sources has been recorded in a similar range.

**Substrate specificity and enzyme kinetics.**

The effect of substrates; namely 4-methylcatechol, ABTS, guaiacol, caffeic acid, o-dianisidine, o-phenylenediamine, pyrogallol, and catechol on POD activity was determined by changing the concentration of the substrate and keeping the concentration of a second substrate (H2O2) constant. Km values for substrates were as follows: 1.63, 0.52, 2.02, 0.03, 0.24, 1.46, 1.47, and 5.23 mM (Table 2). Km and Vmax of H2O2 were measured by changing the H2O2 concentration and keeping the substrate concentrations constant. The Km values for H2O2 toward substrates were 1.144 mM, 0.01 mM, 0.254 mM, 0.524 mM, 1.644 mM, 1.463 mM, 0.15mM and 0.363 mM (Table 3). Thus, POD extracted from kiwano exhibited maximum substrate affinity toward caffeic acid followed by o-dianisidine, ABTS, 4-methylcatechol, o-phenylenediamine, pyrogallol, guaiacol, and catechol in that order (Table 2). The affinity of the POD extracted from kiwano for the 4-methylcatechol substrate (Km = 1.63 mM) was higher than POD extracted from cauliflower buds (Km = 8.19 mM) [31]. The POD extracted from kiwano (Km = 0.52 mM) exhibits lower affinity for the ABTS substrate compared with POD extracted from spring cabbage (Km = 0.0377 mM) [30]. Furthermore, the POD extracted from kiwano exhibits higher affinity than POD extracted from oil palm leaf (Km = 1 mM) [32]. The POD extracted from kiwano for the guaiacol substrate (Km = 2.02 mM) was higher than POD obtained from spring cabbage (Km = 6.41 mM) [30], oil palm leaf (Km = 3.96 mM) [32], and rosemary leaves (Km = 28.8 mM) [29]. The POD extracted from kiwano had a higher affinity for caffeic acid substrate (Km = 0.03 mM) than POD obtained from avocado (Km = 0.1673 mM) [18]. The POD extracted from kiwano for o-dianisidine substrate (Km = 0.24 mM) had similar affinity as compared with POD obtained from spring cabbage (Km = 0.357 mM) [30] and avocado (Km = 0.200 mM) [18]. The POD extracted from kiwano showed higher affinity for o-phenylenediamine substrate (Km = 1.46 mM) than the
affinities shown by POD from morning glory (Km = 2.02 mM) [33] and C. jambhiri cv. adalia (Km = 2.85 mM) [34]. The POD extracted from kiwano for pyrogallol substrate (Km = 1.47 mM) had lower affinity as compared to POD obtained from oil palm leaf (Km = 0.84 mM) [32]. Studies have reported that POD extracted from kiwano had significantly higher affinity than POD obtained from C. jambhiri cv. adalia (Km = 23 mM) [34] and wheat grass (Km = 2.5 mM) [35]. The POD extracted from kiwano showed higher affinity for catechol (Km = 5.23 mM) than the affinities shown by POD from ABTS (Km = 125 mM) [34] and wheat grass (Km = 18.2 mM) [35].

Low Km value for a substrate means high affinity for the enzyme active site and vice versa. The differences in Km of POD for the same substrate may stem from the small variations in the tertiary structure of the enzyme active site.

Effects of some inhibitors on the POD extracted from kiwano. The results of the inhibition effect are shown in Table 4. It was established that KCN strongly inhibited the POD extracted from kiwano and the residual activity was 13.3% at 0.001 mM concentration. The next strongest inhibitory effect was exhibited at L-cysteine with 12.49% residual activity followed by thiourea (45.29%), L-glutathione (47.37%), EDTA (59.61%), and NaN3 (97.95%). In previous literature, the residual activities of avocado POD inhibited by L-cysteine (1 mM), EDTA (1 mM), and Triton X-100 (0.1%, v/v) were recorded as 7.20%, 85.13%, and 83.59%, respectively [18]. Martinez et al. [36] reported that L-glutathione (1 mM), NaN3 (1 mM), and L-cysteine (1 mM) inhibited the POD from strawberry fruits with the residual activity being 55.9%, 70.6%, and 89.9% respectively. The residual activity of extracellular POD from wounded wheat roots inhibited by KCN (0.1 mM) was reported to be 1% by Minibayeva et al. [37]. The activity of POD from jackfruit activity was enhanced by EDTA but inhibited by L-glutathione and L-cysteine [24].

**Effects of some metal ions on the kiwano POD.** The effect of metal ions is shown in Figure 2. As per the results, Mg²⁺, Pb²⁺, and Sn²⁺ inhibited the activity of the POD extracted from kiwano in all concentrations. Fe³⁺ and Zn²⁺ inhibited at concentrations above 1 mM, and Ba²⁺, Al³⁺, and Cd²⁺ inhibited at concentrations above 5 mM, whereas, Fe²⁺, Mn²⁺, Ca²⁺, Cu²⁺, Hg²⁺, Na⁺, K⁺, Co²⁺, Li⁺, and Ni²⁺ activated the activity of the POD extracted from kiwano. Lai et al. [35] reported that POD enzyme activity from some plant sources was activated by some metal ions such as Ca²⁺, Mn²⁺, and Na⁺. Mall et al. [38] reported that Mn²⁺ and Hg²⁺ inhibited POD activation, while Co²⁺, Ca²⁺, and Mg²⁺ activated the POD from a Citrus medica L. leaf. Patel et al. [33] demonstrated that the POD from morning glory was inhibited by Pb²⁺ and Ni²⁺, while Cai et al. [16] reported that POD from J. curcas L. leaves was inhibited by Fe²⁺. Furthermore, Mohamed et al. [34] demonstrated that POD from C. jambhiri cv. adalia was activated by Ba²⁺. It was also reported by Cai et al. [16] that the activity of POD from J. curcas leaves activated by Cd²⁺ at a concentration of 1 mM, but it also inhibited by Cd²⁺ at a concentration above 10 mM. The activity of POD from jackfruit was enhanced by K⁺, Zn²⁺, and Ba²⁺ and inhibited by Ca²⁺, and Cu²⁺ [24].

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Km (mM)</th>
<th>Vmax (U min⁻¹)</th>
<th>Optimum pH</th>
<th>Optimum Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-methylcatechol</td>
<td>1.63 ± 0.4295</td>
<td>0.007 ± 0.0017</td>
<td>7.2</td>
<td>40</td>
</tr>
<tr>
<td>ABTS</td>
<td>0.52 ± 0.0758</td>
<td>0.102 ± 0.0134</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>2.02 ± 0.0705</td>
<td>0.013 ± 0.0019</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.03 ± 0.0031</td>
<td>0.003 ± 0.0001</td>
<td>6.5</td>
<td>30</td>
</tr>
<tr>
<td>o-dianisidine</td>
<td>0.24 ± 0.0208</td>
<td>0.047 ± 0.0052</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>o-phenylenediamine</td>
<td>1.46 ± 0.0330</td>
<td>0.041 ± 0.0024</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>1.47 ± 0.0639</td>
<td>0.010 ± 0.0002</td>
<td>7.5</td>
<td>40</td>
</tr>
<tr>
<td>Catechol</td>
<td>5.23 ± 0.1700</td>
<td>0.010 ± 0.0003</td>
<td>6</td>
<td>40</td>
</tr>
</tbody>
</table>

±value is standard deviation. All experiments were conducted in triplicate.

**TABLE 3**

<table>
<thead>
<tr>
<th>Substrates (concentration const.)</th>
<th>Km (mM)</th>
<th>Vmax (U min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-methylcatechol</td>
<td>1.144 ± 0.0281</td>
<td>0.011 ± 0.0003</td>
</tr>
<tr>
<td>ABTS</td>
<td>0.010 ± 0.0622</td>
<td>0.126 ± 0.0081</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>0.254 ± 0.0091</td>
<td>0.008 ± 0.0013</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.524 ± 0.0127</td>
<td>0.008 ± 0.0045</td>
</tr>
<tr>
<td>o-dianisidine</td>
<td>1.644 ± 0.1148</td>
<td>0.105 ± 0.0070</td>
</tr>
<tr>
<td>o-phenylenediamine</td>
<td>1.463 ± 0.0583</td>
<td>0.053 ± 0.0334</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.150 ± 0.0306</td>
<td>0.002 ± 0.0002</td>
</tr>
<tr>
<td>Catechol</td>
<td>0.363 ± 0.0223</td>
<td>0.007 ± 0.0003</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD of three replicates.
**TABLE 4**

Effect of some inhibitors on the activity of the POD extracted from kiwano.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>Residual activity (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaN₃</td>
<td>1</td>
<td>97.95 ± 0.25</td>
</tr>
<tr>
<td>Thiourea</td>
<td>1</td>
<td>45.29 ± 1.89</td>
</tr>
<tr>
<td>KCN</td>
<td>0.001</td>
<td>13.30 ± 1.70</td>
</tr>
<tr>
<td>L- cysteine</td>
<td>0.5</td>
<td>12.49 ± 0.82</td>
</tr>
<tr>
<td>EDTA</td>
<td>1</td>
<td>59.61 ± 0.19</td>
</tr>
<tr>
<td>Triton X-100 (v/v)²</td>
<td>1</td>
<td>49.75 ± 0.65</td>
</tr>
<tr>
<td>L- glutathione</td>
<td>1</td>
<td>47.37 ± 1.76</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SD of three replicates. * no inhibitor

**TABLE 5**

Effect of some organic solvents on the activity of the POD extracted from kiwano.

<table>
<thead>
<tr>
<th>Residual activity (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd h</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
<td>DMSO</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Ethanol</td>
</tr>
<tr>
<td>Butanol</td>
</tr>
<tr>
<td>Isopropanol</td>
</tr>
<tr>
<td>Chloroform</td>
</tr>
<tr>
<td>Ethyl acetate</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SD of three replicates.

**Effects of some organic solvents on the POD extracted from kiwano.** All solvents reduced the enzyme activity of the POD extracted from kiwano (Table 5). The solvents which had the least effect on the enzyme activity were ethanol, methanol, and DMSO with 13%, 23%, and 23% residual activity respectively, at the end of the 32nd h. The enzyme activity remained more stable in these solvents compared with the measurements recorded in the 2nd, 6th, and 32nd h. In addition, acetone and isopropanol, which had approximately 3% and 6% residual activity at the end of the 32nd h, significantly decreased the enzyme activity in all the recorded measurements. In contrast, butanol, chloroform, and ethyl acetate completely inhibited the enzyme activity after 2nd h. Previous literature shows that after the 24 h incubation, POD from morning glory had 76%, 85%, 82%, 80%, and 81% residual activity in the presence of 30% DMSO, 70% methanol, 65% ethanol, 45% isopropanol, and 30% butanol, respectively [33]. The remaining activity of the kiwano POD extracted
from kiwano was lower in the presence of organic solvents when compared with results reported by Patel et al. [33]. This means that the activity of the POD extracted from kiwano shows less resistance toward organic solvents. This resistance toward organic solvent is beneficial for industrial applications. Thus, because the POD extracted from kiwano shows low resistance toward organic solvents, it cannot be recommended for industrial applications.

Optimum ionic strength and determination of salt tolerance. The result of the optimum ionic strength of POD extracted from kiwano is shown in Figure 3A. The high concentration of NaCl significantly increased the enzyme activity of the POD extracted from kiwano. The optimum concentration of NaCl was 4 mM with a 40% increase in POD activity. The optimum concentration of NaCl for the POD from J. curcas leaves was established to be 2.5 M with 44% increase of enzyme activity [16]. The POD extracted from B. sativus L. had the maximum ionic strength at 1 M of Na2HPO4 concentration [39].

The salt tolerance of POD extracted from kiwano is shown in Figure 3B. Activity enhancement of the POD extracted from kiwano of approximately 12% - 20% maintained after 2 h incubation, suggesting high salt tolerance of POD extracted from kiwano. Cai et al. [16] reported that the POD from J. curcas leaves retained 88% activity after 2 h incubation with 4 M NaCl. The enzyme activity of POD extracted from kiwano increased to some extent in conditions containing NaCl but failed to increase in NaCl-free conditions. Furthermore, it was established that POD activity remains stable after incubation in NaCl for 2 h.

Determination of storage stability. POD extracted from kiwano was left at room temperature and it retained 16% residual activity after 17 days. The enzyme maintained 17% residual activity after 60 days at 4°C. The enzyme had 27% residual activity after 135 days at −20°C. Previous literature shows that POD from spring cabbage remained fully active during 4 weeks of storage at 4°C [30]. Cai et al. [16] established that POD from J. curcas leaves remained intact under −80°C and 4°C conditions for during 180 days. Indeed, J. curcas leaves have been maintained at room temperature for 14 days and they retained 93% residual activity. The results of the present research demonstrated that the storage stability of the POD extracted from kiwano is when compared with the documented results from previous research. The results of the present research, therefore, indicate that kiwano has a low resistance to spoilage.

CONCLUSIONS

POD is an industrially significant enzyme because it has a wide range of biochemical reactions. Thus, novel peroxidases having different characterizations are useful sources for potential applications. To our knowledge, this study is the first to report for the partial purification and characterization of the POD extracted from kiwano. The knowledge obtained from this study may be beneficial for the purification of POD from different sources and for utilizing POD in potential applications.

ACKNOWLEDGEMENTS

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REFERENCES


CARDIOPULMONARY EXERCISE TESTING AT SILICOSIS: A MODALITY TO EVALUATE WORKING PERFORMANCE

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ABSTRACT

In an examination of the relationship between silicosis and lung function, most of the studies focus on pulmonary function tests (PFT). However, no enough data can be found about cardiopulmonary exercise testing (CPET) of silicosis patients. In this study, we applied CPET to silicosis patients; as well as pulmonary function tests to see whether silicosis effects exercise capacity.

Fifty-two silicosis patients, diagnosed at an occupational disease hospital, were included in our study when they admitted to a university hospital occupational disease clinic for follow-up. Informed consent was provided from the patients. CPET and PFT were performed to the patients in addition to chest X-ray and high resolution tomography (HRCT). Statistical analyses were achieved by SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). Mean ± Standard deviation and median were used for descriptive statistics. Kruskal-Wallis Test was used to compare groups, because parametric test assumption was not provided for continuous variables. Post-hoc analysis were performed to find the source of difference. Correlation between continuous variables was analysed by Spearman’s correlation coefficient. P value <0.05 was accepted as statistically significant.

Peak VO2, VAT, maximum load values did not reveal significant difference between ILO category 1, 2 and 3 silicosis patients. Respiratory exchange ratio (RER) differency found close to the significance between ILO categories (p=0.057). Increasing ILO categories revealed increased peak RER values at decreased maximum load (category 3 versus 2; p=0.050, r=0.635 and category 3 versus 1; p=0.023, r= - 0.529). FEV1/FVC found decreased at category 3 (p=0.488). After adjustment for smoking, decreased FEV1/FVC at category 3 found significant (p=0.025).

Smoking is one of the important factors that can confound the association between silicosis and lung function. After adjustment for smoking, only FEV1/FVC was decreased with disease progression. CPET revealed no any difference between silicosis categories. According to the literature, during early categories of silicosis, pulmonary function loss is limited. Hence, cardiopulmonary exercise testing might be one of the modalities to evaluate workforce loss at silicosis patients.

KEYWORDS:
Silicosis, CPET, Exercise

INTRODUCTION

Silicosis is a form of pneumoconiosis caused by inhalation of silicon dioxide (silica) crystals, which are commonly found in the structure of the Earth’s crust. It results from chronic inhalation of substances like quartz, cristobalite, and tridymite [1]. Silicosis is more common in some occupational locations and fields, including quarries, quartz mills, sandblasting areas, mines, foundries, the glass industry, the ceramics industry, porcelain crafting, stained glass production, cement production, and tile and brick production [1]. Workers in the tile and ceramics industries are at risk of developing silicosis, lung cancer, chronic obstructive pulmonary disease (COPD), and some extra-pulmonary diseases, as their occupational exposure to dusts like crystalline silica, mica, kaolin, quartz, and tridymite is common [2, 3].

From a literature search, it can be seen that prolonged exposure to high levels of dust causes pulmonary diseases like pulmonary fibrosis, emphysema, airflow obstruction, and reduced lung function as aspects of silicosis [4, 5]. Moreover, in the literature, there is a consensus on the view that a statistically significant relationship exists between cumulative respirable dust, lung function loss, and bronchitis symptoms in both smokers and nonsmokers [6]. It is also known that reduced lung function is associated with exposure to low-level concrete dust containing silica [7]. In studies conducted on South African gold miners, it was shown that smoking and silica dust exposure have a synergistic effect on COPD mortality [8]. Cytotoxic effects, activation of oxidant production in pulmonary phagocytes and alveolar macrophages, release of mediators from alveolar macrophages and alveolar epithelial cells, and secretion of growth factors from alveolar macrophages and alveolar epithelial cells can be considered factors influencing the disease pathogenesis [1].
radiological category of the disease may not be significantly correlated with the clinical severity and functional loss until the advanced stages of the disease [1, 9].

Many chronic diseases that affect lung function primarily present during exercise. Tests during rest may be insufficient to detect defects in the function of the organ system during exertion. Exercise tests have long been used for both diagnostic testing and evaluation of functional capacity [10].

Although the severity of the disease has been defined radiologically in silicosis patients, there are various difficulties in determining the disease-related loss of workforce. The pulmonary function test (PFT), cardiopulmonary exercise test (CPET), and dynamic tests based on functional evaluation are widely used for determining the loss to the workforce due to environmental and occupational diseases. In the literature, there are contradictory results on the relationship between the radiological category of the disease and PFTs [11, 14]. A limited number of studies have evaluated the CPET, which can objectively identify functional loss in the evaluation of loss of workforce in silicosis. In our study, we aimed to investigate the changes occurring in the CPET and PFT results in patients with silicosis to determine the functional loss caused by the disease.

MATERIALS AND METHODS

Fifty-two patients who had previously been diagnosed with silicosis in the occupational diseases hospital and applied to the occupational diseases outpatient clinic of the university hospital for follow-up were included in the study. CPETs and PFTs were performed for follow-up and functional evaluation. Informed consent was provided by each patient.

Exercise testing was conducted in accordance with the American College of Sports Medicine guidelines [15]. Electrocardiograms and pulse oximetry were continuously monitored during the test. The heart rate and blood pressure were recorded during the final 30 seconds of each stage. Expired air was analyzed breath by breath for O2 and CO2 using a mass spectrometer.

The following variables were calculated and averaged sequentially every 15 seconds during the exercise: the oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio (RER), ventilatory anaerobic threshold (VAT), maximum load (Load-Max) heart rate, and oxygen saturation (SaO2). All the instruments were calibrated before each test.

Statistical analyses were carried out using SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). The mean ± standard deviation and median were used for descriptive statistics. The Kruskal–Wallis test was used for comparing groups, as the parametric test assumption was not provided for continuous variables. Post hoc analyses were performed to find the source of difference. Correlations between continuous variables were analyzed using Spearman’s correlation coefficient. A p-value < 0.05 was accepted as statistically significant.

RESULTS

The mean age of the 52 patients included in the study was 38.5 years. The radiological categories of silicosis according to International Labor Organization (ILO) standards were found to be 61.5%, 21.2%, and 17.3% for categories 1, 2, and 3, respectively. The patients mainly comprised ceramics factory employees. Their occupational groups are shown in Table 1.

Most of the patients (40.4%) worked in foundry departments (Table 2). The patients’ smoking habits are shown in Table 3. No significant relationship was found between the department where the laborers worked and the disease severity.

<table>
<thead>
<tr>
<th>TABLE 1 Occupational Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Ceramics Factory</td>
</tr>
<tr>
<td>Denim Grinding</td>
</tr>
<tr>
<td>Brick/Tile Factory</td>
</tr>
<tr>
<td>Mine Worker</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 2 Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Foundry</td>
</tr>
<tr>
<td>Quality Control</td>
</tr>
<tr>
<td>Retouching/Felting</td>
</tr>
<tr>
<td>Bakery</td>
</tr>
<tr>
<td>Glazing</td>
</tr>
<tr>
<td>Design</td>
</tr>
<tr>
<td>Denim Grinding</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 3 Smoking Habits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Smoker</td>
</tr>
<tr>
<td>Ex-smoker</td>
</tr>
<tr>
<td>Nonsmoker</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

The RER values determined in the patients according to the silicosis categories are given in Table 4. In post hoc analysis, the peak RER values were significantly lower in category 3 patients compared with category 1 (p = 0.023) and category 2 patients (p = 0.050; Table 5).
## TABLE 4
### Peak RER Values According to the ILO Categories

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.01</td>
<td>32</td>
<td>0.03</td>
<td>1.01</td>
<td>0.97</td>
<td>1.07</td>
<td>0.057</td>
</tr>
<tr>
<td>2</td>
<td>1.03</td>
<td>11</td>
<td>0.04</td>
<td>1.04</td>
<td>0.98</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.14</td>
<td>9</td>
<td>0.10</td>
<td>1.12</td>
<td>1.06</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1.04</td>
<td>52</td>
<td>0.07</td>
<td>1.04</td>
<td>0.97</td>
<td>1.33</td>
<td></td>
</tr>
</tbody>
</table>

## TABLE 5
### Peak RER Values According to the ILO Categories: Post Hoc Analysis

<table>
<thead>
<tr>
<th>ILO Category</th>
<th>ILO Category</th>
<th>Mean Difference (ILO)</th>
<th>Std. Error</th>
<th>P</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>-0.017</td>
<td>0.016</td>
<td>0.675</td>
<td>-0.061 - 0.027</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.126*</td>
<td>0.036</td>
<td>0.023</td>
<td>0.235 - 0.018</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.017</td>
<td>0.016</td>
<td>0.675</td>
<td>-0.027 - 0.061</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.109*</td>
<td>0.039</td>
<td>0.050</td>
<td>-0.219 - 0.000</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.126*</td>
<td>0.036</td>
<td>0.023</td>
<td>0.018 - 0.235</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.109*</td>
<td>0.039</td>
<td>0.050</td>
<td>-0.001 - 0.219</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

## TABLE 6
### Peak VO2 during CPET

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.29</td>
<td>32</td>
<td>0.38</td>
<td>2.22</td>
<td>1.74</td>
<td>3.04</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.19</td>
<td>11</td>
<td>0.07</td>
<td>2.21</td>
<td>2.12</td>
<td>2.29</td>
<td>0.206</td>
</tr>
<tr>
<td>3</td>
<td>1.79</td>
<td>9</td>
<td>0.51</td>
<td>1.68</td>
<td>1.31</td>
<td>2.62</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2.18</td>
<td>52</td>
<td>0.40</td>
<td>2.20</td>
<td>1.31</td>
<td>3.04</td>
<td></td>
</tr>
</tbody>
</table>

## TABLE 7
### Maximum Load during CPET

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>157.37</td>
<td>32</td>
<td>23.14</td>
<td>146.50</td>
<td>130</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>141.09</td>
<td>11</td>
<td>6.62</td>
<td>144</td>
<td>133</td>
<td>148</td>
<td>0.319</td>
</tr>
<tr>
<td>3</td>
<td>117.55</td>
<td>9</td>
<td>31.12</td>
<td>100</td>
<td>96</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>147.03</td>
<td>52</td>
<td>26.72</td>
<td>145</td>
<td>96</td>
<td>191</td>
<td></td>
</tr>
</tbody>
</table>

## TABLE 8
### Predicted FVC Values According to the ILO Categories

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88.12</td>
<td>32</td>
<td>13.93</td>
<td>91.5</td>
<td>65</td>
<td>107</td>
<td>0.145</td>
</tr>
<tr>
<td>2</td>
<td>92.63</td>
<td>11</td>
<td>10.05</td>
<td>98</td>
<td>77</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>75.66</td>
<td>9</td>
<td>16.23</td>
<td>65</td>
<td>58</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>86.92</td>
<td>52</td>
<td>14.47</td>
<td>91.5</td>
<td>58</td>
<td>107</td>
<td></td>
</tr>
</tbody>
</table>

## TABLE 9
### FEVI/FVC Values According to the ILO Categories

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85.00</td>
<td>32</td>
<td>3.40</td>
<td>85</td>
<td>79</td>
<td>90</td>
<td>0.488</td>
</tr>
<tr>
<td>2</td>
<td>87.36</td>
<td>11</td>
<td>4.27</td>
<td>90</td>
<td>82</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>74.00</td>
<td>9</td>
<td>8.35</td>
<td>76</td>
<td>64</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>83.59</td>
<td>52</td>
<td>6.49</td>
<td>84</td>
<td>64</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

## TABLE 10
### Smoking Habits in Relation to the Disease Categories

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Smoking</th>
<th>N</th>
<th>%</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N</td>
<td>6</td>
<td>18</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>n%</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>%</td>
<td>27.3</td>
<td>45.5</td>
<td>27.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>%</td>
<td>9</td>
<td>29</td>
<td>14</td>
<td>52</td>
</tr>
</tbody>
</table>
In our study, as the ILO category of the disease progressed, the peak VO2 and Load-Max values decreased during the CPET. However, no statistically significant difference was found between the groups (Tables 6 and 7).

A decrease in the predicted Forced Vital Capacity (FVC) was found in the respiratory function test parameters as the disease severity increased (Table 8; \( p = 0.145 \)). The Predicted Forced Expiratory Volume (FEV1) and FEV1/FVC values (Table 9) did not differ significantly between the groups (\( p = 0.307 \) and \( p = 0.488 \), respectively). After adjusting for smoking, the decreased FEV1/FVC in category 3 was found to be significant (\( p = 0.025 \)).

The rate of smoking and duration of smoking increased as the category of silicosis increased (\( p = 0.590 \) and \( p = 0.160 \), respectively; Tables 10 and 11). There were no patients who had never smoked with category 3 silicosis (Table 10).

### Table 11

<table>
<thead>
<tr>
<th>ILO Stage</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.84</td>
<td>32</td>
<td>6.73</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13.72</td>
<td>11</td>
<td>15.60</td>
<td>0.160</td>
</tr>
<tr>
<td>3</td>
<td>20.33</td>
<td>9</td>
<td>6.40</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12.48</td>
<td>52</td>
<td>9.86</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

The ratio of carbon dioxide output/oxygen uptake (VCO2/O2) is called the gas exchange ratio or RER; this can be used as a rough index of metabolic events [16]. The RER values determined in the patients according to the silicosis categories are given in Table 4. In the post hoc analysis, the peak RER values were significantly lower in stage 3 patients (\( p = 0.050 \)) than those in stages 1 and 2 (\( p = 0.023 \) and \( p = 0.025 \), respectively). As the stage of silicosis progresses, the metabolic load during exercise may increase.

The VAT can be used as an indicator for daily living activities; that the work below this level shows daily activities. It helps to distinguish cardiac and other (pulmonary, etc.) causes of exercise limitation [17, 18]. However, in our study, there was no significant difference in the VAT values according to the severity of silicosis (\( p = 0.289 \)).

In our study, there was no significant relationship between the FVC values and silicosis categories. Contradictory results on FVC have been reported in the literature. Several studies have shown no decrease in FVC in silicosis [19, 25]. In contrast, according to another group of studies, there is a decrease in FVC with silicosis category 2–3 and progressive massive fibrosis (PMF) [26-28]. According to Teculescu et al. [26], reduced FEV1/FVC ratios were found for category 1 and above. In addition, Begin et al. [29] showed reduced FEV1/FVC ratios in category 1 patients. Some studies have shown decreased FEV1 associated with B or C kind of PMF [29, 30]. In our study, there was no significant difference in the FEV1 values.

According to a review [9], reduced lung function parameters can mostly be associated with category 3 and PMF silicosis. Regardless of the mechanism, in category 1 silicosis, lung function is preserved, while silicosis category 2 shows a slight decrease in lung function. The most prominent loss in lung function is found in category 3 and PMF.

In our study, the smoking rate among the workers was found to be high. The PFT and CPET may be affected by smoking. There were no nonsmokers in the category 3 silicosis group; moreover, the smoking rate (Table 10; \( p = 0.590 \)) and duration (Table 11; \( p = 0.160 \)) were found to increase as the disease severity increased. As a result, smoking could be a confounding factor with increasing disease severity.

### Conclusion

In the functional evaluation of silicosis patients and the assessment for loss of workforce, as a single test modality, the CPET does not give enough detailed information; however, it can be used in combination with other tests, such as the PFT and carbon monoxide diffusion testing. With the development of imaging techniques, a new and more detailed disease staging of silicosis compatible with high-resolution computed tomography (HRCT) may correlate better with functional loss in terms of predicting the loss of workforce. Further studies are required with larger sample sizes and combined functional tests for evaluating the loss of workforce in silicosis more accurately.

### References


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DETERMINATION OF RESERVOIR SEDIMENTATION WITH BATHYMETRIC SURVEY:
A CASE STUDY OF OBRUK DAM LAKE

presented in the 19th International MESAEP Symposium, Rome-Italy from October 04-06, 2017

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1Hitit University, Corum, Turkey
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ABSTRACT

The sediments that transported in different ways to dam reservoirs decrease water storage capacity and shortens the reservoir lifetime. This forces the manager of water resources to monitor changes in water floor topography, and determine the sediment amount and sedimentation rate. This is very important in order to maintain current operations, make correct planning for future needs, take necessary precautions to prevent having a shorter lifetime than the projected durations and prolong the dams’ lifetime. Although there are several different methods for determining the amount and rate of sediments, the most commonly used one is the bathymetric survey. In this study, in order to determine sedimentation of dams, a bathymetric survey was conducted in a part of the Obruk Dam Lake located in the Çorum province of Turkey with the most recent geodetic equipment. The obtained results from the bathymetric survey are considered as initial results for the upcoming measurements to monitor the changes in sediment amount and sedimentation rate in the selected part of the Obruk Dam. The results imply that; the bathymetric surveying method is a powerful tool to estimate reservoir sedimentation.

KEYWORDS:
Water management, sedimentation, bathymetric survey, reservoir, dam.

INTRODUCTION

A major part of the Earth is covered with water and it is vital for life. Phrases like ‘water is life’, ‘water is civilization’ and ‘there is no life without water’ emphasize the importance of water in our life. Due to this importance, structures have been built to collect and transfer water from one place to another since the beginning of civilizations. Especially, in the latest century, the number and the capacity of water constructions has increased in line with the increase of population and development in agriculture, industry, technology and living standards.

Nowadays, there have been several million dams and ponds built throughout the world for various purposes including irrigation in agriculture, water supply, power generation, discharge regulation or flood control [1]. Global climate change and disrupted precipitation have further increased the risk of erosion in certain areas of the Earth. Due to increased erosion, more sediments reach river beds and these materials transported by the rivers pose a threat to areas such as lakes, ponds, dams, gulfs, and coasts. The accumulation of sediments in these water-covered areas lead to a significant change in the water floor topography. In our country, sediments that are transferred by these rivers are about four times more than the world average and seventeen times more than the Europe average [2]. This situation also adversely affects sea transportation in places like bays, bay ports, and straits. Besides, it leads to image and odor pollution after a certain level.

Within the context which was briefly mentioned above, sediment accumulation should be monitored periodically with appropriate methods. According to the results obtained from these studies, protective and corrective measures should be taken if necessary. In this frame, one of the monitoring methods to be applied is the precise bathymetric survey. By performing the bathymetric survey periodically, changes in the water floor topography can be monitored, and the amount of sediment transported and the rate of accumulation can be calculated.

In this study, the initial studies and obtained results in order to determine the number of sediments carried on the part of the reservoir of the Obruk Dam Lake, of Çorum City in Turkey are discussed. For this purpose, a bathymetric survey was carried out by using the most recent geodetic equipment. The surveying procedure and the obtained results are given.
MONITORING OF SEDIMENTATION METHODS

Dams are built for many different purposes, part of their reservoir volume is used to store water, while another part is reserved for storage of the sediment that is expected to come from the dam basin. It should be kept in mind that; the reservoir lifetime is limited. The capacity of the dams’ reservoir can be divided into three portions as [3];
- Dead Storage Volume: volume below the lowest outlet level, which cannot be removed,
- Active (or Live) Storage Volume: volume between the lowest outlet level and normal surface level,
- The Flood Control Storage Volume: volume between the normal level and maximum surface level.

Sediments are weathered rock material that is transported, suspended or deposited by flowing water [4]. All constituents of the parent rock material (silt and clays, sand, gravel, and mud) are usually found in the sediment and transported by water from the place of origin to the place of deposition. In watercourses, sediment is the alluvial material carried in suspension or as bed load. Sedimentation is defined as the process of settling and depositing by the gravity of suspended matter in water. The flow of water and sediment into the reservoir cause reservoir sedimentation [5]. The section used to store the sediment from the river basin is called ‘the dead storage volume’ of the dam and the water intake facilities are built on top of this dead volume.

In dam reservoirs, soil particle accumulation can decrease the water storage capacity, water use potential and shorten reservoir lifetime by causing changes in the reservoir topography. Other negative results of reservoir sedimentation are the reduction of flood attenuation, changes in water quality and damages of valves as well as the channels [6]. Changes in existing biogeochemical and ecological cycles are other important disadvantages of reservoir sedimentation [7]. Especially small reservoirs are more affected by these storage losses since the maximum water depth is usually only a few meters and the sediment layer, which accumulates a few decimetres on the bottom of the reservoir, and causes a relatively large decrease in water volume. On average, the yield of sediments and solutes of rivers in the world is equivalent to a drop of 3 cm below the earth’s surface every 1000 years [8]. Sedimentation leads to many issues such as the operation and maintenance of engineering infrastructures, the economic feasibility of the project and environmental issues through social dimension. It is known that an average 0.5-1% of the volume capacities of a large and small reservoir is lost every year in the world due to sedimentation [9].

The dams that are filled before their economic life become unusable for the purpose of their construction. The conservation and effective management of water resources, which have a major prerequisite for human life, has great importance for sustainable resource management [10]. Providing the protection and conservation of the reservoir consistently is important to ensure that quantity and quality are sustainable to meet the demands of the growing population. Reduction in the storage capacity of the reservoir leads to the use of the reservoir below its intended yield. The useful life of the reservoir should be terminated when the storage capacity drops to 20% of the design capacity [6]. Thus, bathymetric survey or lake sediment retrieval should be performed in order to monitor the changes in the reservoir topography by measuring the thickness of the layer of accumulated soil particles, and calculating siltation rates [11]. Proper management of the reservoir requires that current reservoir volumes and sedimentation rates must be determined as accurately as possible [12].

There are several methods to estimate sediment accumulation. Generally, they can be categorized into two groups as indirect and direct methods. Indirect methods are the measurements of suspended sediment fluxes and sediment traps or estimating runoff/sediment yield. Direct methods are bathymetric surveys and sediment coring of accumulated sediments. The bathymetric approach among them is based on a comparison of the measured reservoir topography at two different time periods. First one is at the time of the construction of the dam if available with enough accuracy and the second one is at the time of the survey [11].

The information about reservoir sedimentation survey with bathymetric surveying method is given in [3, 6, 7, 9, 13, 14, 15, 16, 17, 18, 19, 20, 21].

MATERIALS AND METHODS

Description of the Study Area. The Obruk Dam was constructed on Kızılirmak, located North West of Çorum City, in Turkey (Figure 1). It has been operating since 2009. It was built with an economic life of 50 years, mainly for energy production and irrigation of thousands of hectares of the nearby highly efficient Osmancık Lowland. In the designing stage of the dam, the intake structure was placed according to the dead storage volume determined by taking into consideration the sediment amount that can occur during this period.

The clay core semi-permeable inlay body of the dam has a height of 67 meters from the riverbed and 125 meters from the foundation. The installed capacity of the Obruk Hydroelectric Power Plant is 202.8 MW and the annual energy generation is 515 million kWh [22]. Some main technical specifications of the dam are given in Table 1 [23].
Min. operating level (height) 506 m
Max. operating level (height) 510 m
Min. operating level volume 524,510,000 m³
Max. operating level volume 661,110,000 m³
Dam volume 12,830,000 m³
Water Surface Area 50.2 km²
Irrigation Area 7,179 hectares

Bathymetric Surveying. In order to determine the sedimentation rate, a precise bathymetric survey was carried out applying modern methods and approaches by using GNSS and echo sounder in the vicinity of the crest of the dam body. Detailed information on the bathymetric and its corresponding shoreline measurements that were used to determine the amount of sedimentation on the Obruk Dam are given in the following chapters.

i-) Computer-aided Automatic Data Acquisition System (CADAS). The system was used for the bathymetric measurements within the scope of the study. Such systems mainly consist of the following components:
- Hydrographic Survey Software,
- Depth Measurement System (Manual Systems, Acoustic Single or Multi-Beam Systems, Airborne LIDAR, etc.),
- Positioning System (Conventional Measurement Technique, GNSS, and others),
- Orientation Sensor (Gyroscope, Dual Antenna GNSS System, etc.),
- Compensator/Sensor (Integrated system for Attitude Control (heave/pitch/roll), Sound Velocity Profiler, CTD Profiler, etc.),
- Bottom Characteristics (Side Scan Sonar, Video Acquisition System, etc.),
- Subsea Sensors, Unmanned Vehicle,
- Obstacle-detection System,
- Survey Boat/Vessel/Platform,
- Computer (Desktop, Laptop, Hand-held, etc.).

Hydrographic surveys are carried out mainly by measuring depth and determination of coordinates of the corresponding measured point. However, in some cases, other components that are mentioned above can be used depending on the aim of the project. In hydrographic surveying, which have many different aspects from land mapping, intensive study is required from the beginning of the measurements. In these studies, a huge amount of data is gathered and processing is needed to be performed. Most of all these processes are done more easily, conveniently and accurately with an appropriate hydrographic survey and evaluation software. In this bathymetric survey, a hydrographic software, Geometius BathySurvey, was used. Navigation and all the data collection, processing, and storage process were carried out using this software. It provides the ease of use, cost-effectiveness and accurate manner for bathymetric surveying. The BathySurvey hydrographic software package is capable of communicating via serial port with the echo sounder and takes the corresponding coordinates of the measured point from a GNSS receiver. More detailed and latest information about the software can be found in [24].

ii-) Depth Measurement. To determine water floor topography of the Obruk Dam reservoir, a bathymetric survey was conducted on June 8, 2017, using a single-beam echo sounder in conjunction with a GNSS receiver using Geometius BathySurvey hydrographic software package. Bathymetric surveying and trajectory of the survey vessel on Google Earth are presented in Figure 2.

Acoustic Sounding Method is still the most commonly used depth measuring method in many different hydrographic survey projects from shallow water to oceans with acoustic sounding instruments (so-called echo sounder). The depth measurements were carried out with OHMEX SonarMite echo sounder and a transducer mounted on the side of the vessel. The SonarMite is operating at a frequency of 235 kHz with an accuracy of ± 2.5 cm (RMS).

The specifications of the used echo sounder are listed in Table 2 [25].
### Technical specifications of the used echo sounder.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transducer Frequency</td>
<td>235 kHz Active Transducer</td>
</tr>
<tr>
<td>Depth Range</td>
<td>0.30m to 75.00m (Software limited)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>± 0.025m (RMS)</td>
</tr>
<tr>
<td>Sound Velocity Range</td>
<td>1400 to 1600 m/sec</td>
</tr>
<tr>
<td>Data Output Range</td>
<td>2 Hz</td>
</tr>
<tr>
<td>Ultrasonic Ping Rate</td>
<td>3 to 6 Hz (Depth-dependent)</td>
</tr>
<tr>
<td>Overall Dimensions</td>
<td>100 w x 220 h x 45 d (mm)</td>
</tr>
<tr>
<td>Weight</td>
<td>0.75Kg</td>
</tr>
</tbody>
</table>

The bathymetric survey was conducted on predetermined survey lines, which were defined at about 15 m apart from each other as displayed via the hydrographic software. The survey lines were configured to be perpendicular to the shoreline. During the bathymetric survey, about 18,500 points covering approximately 120 hectares of the water surface were surveyed. The maximum depth of about 58 m was measured. The major disadvantages of this sounding procedure are that depths between surveying lines were omitted and in order to obtain the 3D topography of the water floor, the gaps between the survey lines were filled by interpolation [26].

The acoustic sounding method stands for depth determination by measuring the elapsed time of an acoustic pulse that travels to the water floor and returns back. The sound velocity is variable and varies as a function of depth, temperature, salinity, and pressure. Thus, it should be determined as accurately as possible to make accurate depth measurement. There are several different instruments and methods to determine the sound velocity. One of the commonly used calibration methods which is called ‘bar check’ was performed before starting the bathymetric surveying. In this method, a metal bar was lowered to the transducer. The hydrographic survey software used in this study provides the operator to determine the speed of the sound from the acoustic signal in the water by using the bar check wizard very easily and accurately. This tool provides to determine not only sound velocity but also the transducer depth (i.e. draft). The current sound velocity was determined as 1466 m/sec and entered to the echo sounder.

iii-) Positioning. The vessel was equipped with Trimble R10 GNSS receivers for geo-referencing of the corresponding measured sounding point. The coordinates of each point were determined with Real Time-Precise Point Positioning (RT-PPP) GNSS technique using The Trimble CenterPoint RTX (Real Time eXtended) commercial positioning service. The CenterPoint RTX is a proprietary Real Time-Precise Point Positioning (RT-PPP) technology which is an interesting recent development. A new commercial real-time positioning product known as Trimble RTX has been released in the US, claiming to bridge the gap between PPP and Network RTK GNSS. It provides high accuracy, i.e., a few cm level, real-time kinematic positioning almost anywhere in the world without the use of local, regional, or global base station required like in the Network RTK. In this system, base station and connection to CORS-like RTK networks requirement are also eliminated.

A global CORS network (similar to the IGS’s) allows for the generation of precise orbit and clock information for GNSS satellites in real-time. The RTX satellite corrections are generated with data from the Trimble’s worldwide tracking network, consisting of approximately 100 reference stations globally distributed. While the system has been initially introduced for supporting GPS and GLONASS satellites, nowadays it supports all navigation satellite systems including GPS, GLONASS, Galileo, BeiDou and QZSS. It is claimed that this technique is capable of providing real-time positioning with less than 4-centimeter accuracy in horizontal (with 95%) and initialization times less than 30
### TABLE 3
Accuracy performance of the Trimble R10 GNSS receiver [28].

<table>
<thead>
<tr>
<th>Surveying Mode</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-Time Kinematic Surveying</td>
<td></td>
</tr>
<tr>
<td>Single Baseline &lt;30 km</td>
<td>8 mm + 1 ppm RMS</td>
</tr>
<tr>
<td>Horizontal</td>
<td>8 mm + 0.5 ppm RMS</td>
</tr>
<tr>
<td>Vertical</td>
<td>15 mm + 1 ppm RMS</td>
</tr>
<tr>
<td>Network RTK</td>
<td>15 mm + 0.5 ppm RMS</td>
</tr>
<tr>
<td>Vertical</td>
<td></td>
</tr>
<tr>
<td>Horizontal</td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td></td>
</tr>
<tr>
<td>Horizontal</td>
<td></td>
</tr>
<tr>
<td>Trimble RTX (Satellite and Cellular/Internet (IP))</td>
<td></td>
</tr>
<tr>
<td>CenterPoint RTX</td>
<td>4 cm RMS</td>
</tr>
<tr>
<td>Horizontal</td>
<td>9 cm RMS</td>
</tr>
<tr>
<td>Vertical</td>
<td>2 cm RMS</td>
</tr>
<tr>
<td>Operating range (inland)</td>
<td>In select regions</td>
</tr>
<tr>
<td>CenterPoint RTX Fast</td>
<td>5 cm RMS</td>
</tr>
<tr>
<td>Horizontal</td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td></td>
</tr>
<tr>
<td>Operating range (inland)</td>
<td></td>
</tr>
</tbody>
</table>

minutes globally. The necessary satellite orbit and clock correction information are broadcasted to the rover by an L-band satellite downlink from a geostationary satellite, similar to the use of SBAS satellites for a wide-area and by IP/Cellular worldwide. The error sources such as receiver clock, atmospheric delays are modeled at the rover location [27].

The positioning accuracy performance of used multi-constellation and multi-frequency GNSS receivers are given in Table 3.

Depth and GNSS position data were received simultaneously by the Geometius BathSurvey hydrographic surveying package and stored for future evaluation, while necessary operations for navigation were being performed. In this context, when the survey was performing, the real-time GNSS-derived coordinates and depth were displayed on the screen of the computer. Additionally, the vessel operator can move on the pre-defined survey lines by looking at the desktop computer or hand-held monitor. This provides the vessel operator ability to view the current location of the vessel and observe whether it was separated from the pre-defined survey lines or not.

iv-) Tide Gauge. The level of water surface on the day of measurement was determined from the staff gauge station established on the shore of Obruk Dam. For this purpose, a steel staff with a dimension of 100 cm length was used.

The height of the staff’s zero point was determined from the nearest known geodetic control point using spirit leveling. Staff readings were repeated at 15-minute intervals while surveying was continuing.

v-) Survey Vessel. A wooden vessel for recreational purposes was used for the measurements. It has a dimension of 12.5 m length and 3 m width with the capacity of 12 to 20 person (Figure 2, left).

Shoreline Surveying with an Unmanned Aerial Vehicle (UAV) Producing a Bathymetric Map. Although the boundary of the bathymetric survey is restricted to the shoreline, the surface topography must be determined up to a certain bandwidth as the continuation of the water floor topography of the shoreline. According to the map and map production regulations applied for all kinds of dam survey projects in Turkey, the limit of hydrographic measurements to be applied for the dams is determined as the dam crest level. Within this context, while the topographic structure of the water floor of the existing reservoir is being determined by bathymetric surveying, shorelines or 3D mappings for the region above the existing water level up to the crest level are determined by using different measurement techniques as conventional terrestrial, space-based GNSS measurement, photogrammetric and remote-sensing techniques [19]. Conventional surveys became less preferred and leave their place to GNSS systems due to the difficulties such as the influence of weather conditions on the measurements, the necessity of difficult field works and the restriction of measurement to certain times of the day. However, it is difficult or even impossible in some cases to implement this method because of the densely wooded and difficult topographical structures of the reservoir area and the difficulties of measuring along the shoreline.

In recent years, Unmanned Aerial Vehicles (UAVs) have been widely used in many different areas. UAVs provide fast, accurate and economical solutions for the creation of higher resolution maps in smaller areas. UAVs offer significant advantages during geo-data collection especially in difficult (harsh) environments like dam watershed. Using UAV is a low-cost alternative to the classical manned aerial photography or complementary solution to terrestrial data acquisition. UAVs increase data acquisition speed while reducing the cost [29].
In this study, 3D mapping of the mentioned land band between the existing water level and crest level was conducted by using a ‘DJI Phantom 3 Pro’ UAV. It has a weight of 1,280 grams and can reach a maximum speed of 16 m/s. The 12 MP camera also has an embedded GNSS receiver that can receive data from GPS and GLONASS satellites. In practice, vertical aerial photographs were taken from 100 m above the average surface having 75% side-overlap and 70% forward-overlap. In the study, 10 ground control points having highly accurate known coordinates from GNSS surveying were used.

Photos taken with the UAV were evaluated with ‘Pix4D Mapper Pro’ software and 3D point cloud, orthophotos and Digital Surface Models (DSM) of the study area were produced (Figure 3).

The bathymetric and UAV-based land measurement data were merged and 3D model of the study area have been obtained. From there, a high-resolution digital map of the study area was produced (Figure 4).

**Determination of Accumulated Sediment in Dam Reservoir.** Although an accurately produced map can be used for several tasks, it will especially be used as a reference surface to calculate the volume of sediment. The volume (amount) of sedimentation accumulated in the lake can be calculated by comparing the first (initial) bathymetric survey (resolution at least 1:1000) with the following ones. Construction of this dam was started in 1996 and the water has been holding since 2007. A large-scale map belongs to the period of the construction of the dam having 3D data to be used for sediment calculation in the studies could not be obtained. Latest large-scale topographic maps for the Obruk Dam basin were produced in 1977 with 1:1000 scale by using conventional terrestrial surveying technique. Due to their production technique, they were not considered as the initial maps for the sediment calculation which is deposited in the dam reservoir. Furthermore, a bathymetric survey was not carried out after water holding; thus the latest topographical maps produced for the dam belong to 1977. It was estimated that these maps, produced about 30 years ago before holding water, could not be used as an initial map for a reliable and accurate sediment calculation and the bathymetric map generated from this study will be used as the initial map for the further studies. When the second survey is done, the evolution of the reservoir capacity over time could be calculated easily by comparing both of these water floor topographies. The second survey time is suggested at least 10 years later to detect significant
changes [11]. The resulting volume will give the quantity of accumulated sediment. Moreover, annual storage capacity loss rates can be calculated. In the case of coming across with the situation of finding a sediment action greater than anticipated in the study, the necessary precautions will be taken and feedback will be given to decision makers for sustainable management and use of this important water basin.

CONCLUSION

In this study, a bathymetric survey was conducted for determining the reservoir sedimentation which should be carefully monitored in order to be able to effectively manage a sustainable perspective and protect the existing water resources in our dams. For this purpose, a certain part of the Obruk Dam reservoir has been measured by using the bathymetric surveying method, which is the most common sediment monitoring method. In the study, Trimble RTX CenterPoint Positioning service, which allows seamless positioning and the UAV that currently has limited use in this area were used as well as commonly used geodetic equipment. Herewith, a method using the most recent technological geodetic equipment that allows sedimentation monitoring to be done quickly, conveniently and with high accuracy is given in the application.

The changes in water floor topography of water environments and the rates of sedimentation must be determined periodically in order to store more water, to take precautions for prolonging the operating life, and to plan for possible projections of the dams. This issue plays a crucial role in maintaining current operations and planning for future needs in dam management operations. These studies and outcomes are undoubtedly significant for the protection of storage capacities and efficient operation of the dams. It should be kept in mind that all water resources are essential for all humanity, not just for us. Therefore, all measures should be taken to ensure that water resources are protected effectively and managed from a sustainable perspective. In this context, the most important information and decision sources are bathymetric maps with current, and sufficient accuracy and they should be produced with high accuracy using the latest up-to-date technology. It should be noted that, depending on new advances in technologies for surveying equipment and approaches like the one introduced and used in this study will make the surveying easy and more economical while reducing time, workload and calculation of reservoir sedimentation.

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MECHANICAL ANALYSIS ABOUT HARD PARTICLES MOVING IN A SOFT TUBE FRACTURE

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ABSTRACT

The downward movement of solid particles in the deformable channel is ubiquitous in nature, such as snakes devouring eggs and sediment particles going through soft tube fissures. This study takes the phenomenon of snakes devouring eggs as an example to conduct a mechanical analysis. The study obtains the force acting on the eggs in a channel, and builds the force model of the eggs in horizontal direction inside the snake's body. The model is also the mechanical model of the migrating non-deformable solid in a deformable channel. It can be obtained that the main cause of the egg's movement inside the lumen is that the pressure of the wall on the front end of the egg is greater than the sum of the pressure of the wall on the rear end and the friction of the middle section. The study contributes to the understanding of non-deformable solids' downward migration in the tube cavity. The analysis process can provide reference for the migration of underground sediments through rock and soil tube fissures in hydraulic engineering.

KEYWORDS:
Solid particles, deformable channels, mechanical mechanism, mechanical model

INTRODUCTION

During the process of rock formation, the rock is perhaps cracked, and the sediment deposition occurs crack with the water evaporation [1]. As a result of continuous water erosion, in the course of time, many caves and tube is formed in sandstone and mudstone, as shown in Fig. 1A. Many linear holes is made in straw stone by water and soils sedimentation, as shown in Fig. 1B.

In this erosion process of some particles moving along the tube, fracture and cave is generated by water and groundwater drive. The particle presses the two sides' plane or the tube wall. If the soil and the rock are very soft at this time, while the sediment particles are very hard, it will appear that the soil particles squeeze both the rock/soils sides. In this case, stress analysis of the sediment particles in the fissures needs to be studied. It is helpful to understand and solve the plugging and dredge the dam bottom fracture.

One hard particle force in a soft fracture is well analyzed and derived in the research [2], and author cleverly connected the hard particle force with the joint roughness coefficient (JRC) of fissure. That research is about the hard particle force pressed by two planes of a soft fracture. But in fact, many particles move in a tube, as shown in Fig. 1A and B. This research aim is the mechanical analysis about hard particles moving in a fracture shown in Fig. 1C. While the research in Sun [2] is simplified into Fig. 1D. This research supplies the Sun [2] research.

In future, the sediment and sand is used to plug the rock fracture against leaking [3]. Hard sediment particles in fracture and crack of soft soils is very like the egg moving in snake body, as shown in Fig. 2. So the eggs are analyzed in this paper to understand and study the hard sediment particles moving in soils fracture and a tube-fracture.

The swallowing of food and movement of food inside the organism are complex processes. The most common and typical process is a snake devouring the egg and the movement of the egg inside the snake’s body, as shown in Fig. 2.

Regarding the movement of food inside the organism, one belief is that the creep and contraction of the esophagus wall push food to the stomach. The transmission of peristalsis relies on the pumping effect of the container [4]. Another statement is that the lumen wall is affected by the contraction waves which do not exceed the static boundary [5]. In this case, complex biomechanical behaviors are involved, including the relaxation time of the lumen wall. The cycle is expressed as [6]. The mathematical reasoning in these studies is extremely complex as well.

One of the important contents of these studies is the rheological food, either viscous or non-viscous. Many studies are concerned with the mixtures of solids and fluids or minced food, such as stirred cream, egg tarts and tomato paste [7]. The studies on the whole block of non-deformable solid food are few. There are no explanations available
(A) Honeycomb sandstone. (B) Straw stone with a linear hole. (C) The calculation diagram of this paper. A hard ball moves in a soft tube. The radius of the ball is bigger than that of the tube, so the tube are deformed. (D) The calculation diagram in reference [2]. There is an angle between the two planes. A hard ball in the middle of two soft planes, and the planes are deformed.

FIGURE 1

Hard particles moving in a fracture and squeezing the soils and rock

(A) Honeycomb sandstone. (B) Straw stone with a linear hole. (C) The calculation diagram of this paper. A hard ball moves in a soft tube. The radius of the ball is bigger than that of the tube, so the tube are deformed. (D) The calculation diagram in reference [2]. There is an angle between the two planes. A hard ball in the middle of two soft planes, and the planes are deformed.

(B) Straw stone with a linear hole. (C) The calculation diagram of this paper. A hard ball moves in a soft tube. The radius of the ball is bigger than that of the tube, so the tube are deformed. (D) The calculation diagram in reference [2]. There is an angle between the two planes. A hard ball in the middle of two soft planes, and the planes are deformed.

FIGURE 2

Process of the snake devouring the egg

(A) Honeycomb sandstone. (B) Straw stone with a linear hole. (C) The calculation diagram of this paper. A hard ball moves in a soft tube. The radius of the ball is bigger than that of the tube, so the tube are deformed. (D) The calculation diagram in reference [2]. There is an angle between the two planes. A hard ball in the middle of two soft planes, and the planes are deformed.

In the process of snakes devouring the eggs, the diameters of most eggs are greater than that of lumens inside the snake's body. Because of the non-deformability of eggs, the transmission of peristalsis walls only plays the role of lubricating the friction between the lumen walls and eggs at this time. It has nothing to do with the downward movement of eggs.

In the course of study on the biological materials, researchers have carried out numerous mechanical researches and applications [8-11]. This study simplifies the contraction process of the lumen wall in the mechanical analysis, and attempts to explain the moving process of food in the single lumen by investigating the mechanical mechanism.

It is significant to do mechanical analysis on the downward movement of the eggs in the snake's body, because it will facilitate the understanding of the stressing situation of the egg in the snake's body. Apart from this, it can also be applied in other areas. The eggshell is solid, and its downward movement through the lumen of the snake's body has great similarities with the solid sediment particles passing through the underground rock and soil fissures in hydraulic engineering. The study in this aspect can provide reference to rainfall [12] seepage [13] in
Meaning of symbols in Fig. 3:
n_1, the squeeze exerted by the snake's body / lumen on the front end of the egg, Pa;  
n_3, the squeeze exerted by the snake's body / lumen on the rear end of the egg, Pa;  
f_2, the squeeze exerted by the snake's body / lumen on the middle of the egg, Pa;  
r, the radius of the snake's body / lumen, m;  
R represents the radii of the front and rear ends of the egg respectively, m;  
F_1 is the horizontal component of the force caused by n_1, Pa;  
F_2 is the friction on the middle of the egg caused by f_2, Pa;  
F_3 is the horizontal component of the force caused by n_3, Pa.

Meaning of symbols in Fig. 4:

Fig. A stands the cross section of the snake body and Fig. B is the section of the Fig. A;  
R is the egg’s radius;  
f_2, n_1, n_3 are the similar in the Fig. 3;  
f_3 is the transverse tensile of the snake body because the egg squeezing effect.

Fault, groundwater transportation [14], fissure expansion [1] and the stressing analysis of the migrating underground sediment [3, 15-18] within the rock and soil. In hydraulic engineering, it is helpful to understand the dam sediment leak stoppage, dam stability [19] and groundwater fissure channels unclogging.

**MECHANICAL MODEL**

The devoured egg enters the mouth under the squeeze of the lower jaw, and is further pushed into the esophagus. Then it moves downward through the lumen. Stressing analysis of the snake devouring the egg is simplified as shown in Fig. 3.

The mechanical analysis on the cross-section of the snake's body is shown in Fig. 4.
The body of the snake and egg squeeze each other. Under the squeezing of eggs, the diameter of snake's body becomes bigger, and the toroidal perimeter increases. \( k \) denotes the coefficient of elasticity. According to Hooker's law,

\[
f_1 = k \frac{2 \pi R - 2 \pi r}{2 \pi r}
\]

Therefore,

\[
f_1 = k \frac{R-r}{r}
\]

As shown in Fig. 4B, the force reaches a balance in the semi-circle horizontal direction,

\[
2 f_1 = 2 \int_0^{\pi/2} f_2 R \cos \beta d \beta
\]

Hence, the following can be obtained:

\[
f_2 = k \frac{R-r}{Rr}
\]

(1)

According to Fig. 3, there is

\[
F_x = \int_x^{x_1} \mu f_2 2 \pi R dx
\]

\[
= 2 \pi \mu k \int_x^{x_1} \frac{R-r}{r} dx
\]

Where \( \mu \) denotes the coefficient of friction.

Under the action of \( n_1 \) and \( n_2 \) on the egg, the backward and forward horizontal components (denoted by \( F_1 \) and \( F_3 \) respectively) are produced. As shown in Fig. 3, \( n_1 \) and \( n_3 \) can be determined in the same way as the solving of \( f_1 \). The difference is that the values of radius \( R \) are different, corresponding to \( R \cos \alpha_1 \), \( R \cos \alpha_3 \), and \( R \), respectively.

\[
F_1 = \int_0^{\alpha_{\text{max}}} n_1 2 \pi R^2 \sin \alpha_1 R \cos \alpha_1 d \alpha_1
\]

(3)

\[
F_3 = \int_0^{\alpha_{\text{max}}} n_3 2 \pi R^2 \sin \alpha_3 R \cos \alpha_3 d \alpha_3
\]

(4)

Where \( n_1 \) is the pressure on the front part, i.e. \( f_2 \) when radius \( R = R \cos \alpha_1 \).

After substituting \( R = R \cos \alpha_1 \) into Eq. (1), the following is obtained,

\[
n_1 = f_2 \left. \right|_R = R \cos \alpha_1 = k \frac{R \cos \alpha_1 - r_1}{R \cos \alpha_1}
\]

(5)

\[
n_3 = f_2 \left. \right|_R = R \cos \alpha_3 = k \frac{R \cos \alpha_3 - r_3}{R \cos \alpha_3}
\]

(6)

Where \( r_1 \) and \( r_3 \) denote the radii of the front part and the rear part of the snake's body / lumen, respectively.

Furthermore, Eq. (5) and (6) are substituted into Eq. (3) and (4), then

\[
F_1 = 2 \pi R k \int_0^{\alpha_{\text{max}}} \frac{R \cos \alpha_1 - r_1}{r_1} \sin \alpha_1 d \alpha_1
\]

(7)

\[
F_3 = 2 \pi R k \int_0^{\alpha_{\text{max}}} \frac{R \cos \alpha_3 - r_3}{r_1} \sin \alpha_3 d \alpha_3
\]

(8)

The pressure on the egg in the horizontal direction is,

\[
W = F_1 - F_2 - F_3
\]

(9)

Combining Eq. (2), (7), (8) and (9) yields the mechanical model of the egg inside the snake's body in the horizontal direction. It is also the mechanical model of the migration of a non-deformable solid in a deformable channel.

\[
\begin{align*}
F_1 &= 2 \pi R k \int_0^{\alpha_{\text{max}}} \frac{R \cos \alpha_1 - r_1}{r_1} \sin \alpha_1 d \alpha_1 \\
F_2 &= 2 \pi \mu k \int_x^{x_1} \frac{R-r}{r} dx
\end{align*}
\]

(10)

In the equation above, \( W > 0 \) means that the egg move backward; \( W = 0 \) means that the egg reach an equilibrium state; \( W < 0 \) means that the left side is smaller than the right side, i.e. The egg is moving forward; \( L \) denotes the length of the middle part of the egg.

\[
\cos (\alpha_{\text{max}}) = \frac{r}{R}, x_1 = x + L
\]

**CALCULATION EXAMPLE**

The snake's body is thick in the middle and thin at the two ends. Consequently, along the direction of body length, the diameter of the snake's body varies, which can be represented by a function of \( x \) in terms of radius \( R \). Hence, the stress acting on the eggs also changes as it moves inside the snake's body. The changes of the diameter of the snake's body with the body length are found as follows: the length of the lumen is 200cm; the maximum diameter of 6 cm occurs at 100cm, which is at the middle of the snake's body; the minimum occurs at the ends, being 3cm. The curve of the radius is

\[
r = 3 - 0.00015(x - 100)^2
\]

Where \( r \)'s unit is cm, \( x \) denotes the distance to the snake's head in the unit of cm; the maximum radius is 3cm, the minimum radius is 1.5cm, and the value of the elastic coefficient \( k \) does not influence the result of \( W \). The friction coefficient \( \mu = 0.001 \), the radius of the egg \( R = 3 \)cm, and the total length of the egg is 8cm. Therefore, \( L = 8 - 2R = 8 - 2 \times 3 = 2 \)cm. This is substituted into Eq. (10).

\[
W = 2 \pi R k \int_0^{\alpha_{\text{max}}} \frac{R \cos \alpha_1 - r_1}{r_1} \sin \alpha_1 d \alpha_1
\]

(11)

Where,
By substituting Eq. (12) and (13) into (11), the following would be derived,

\[ W = 2 \pi R k \int_0^{\alpha_{1-\text{max}}} \left[ \frac{R \cos \alpha}{3 - 0.00015(x - R \sin \alpha_{1-\text{max}} - 100)^2} \right] \sin \alpha \, d\alpha \]

\[ -2 \pi R k \int_0^{\alpha_{2-\text{max}}} \left[ \frac{R \cos \alpha}{3 - 0.00015(x - 100)^2} \right] \sin \alpha \, d\alpha \]

Where \( \alpha_{1-\text{max}} \) satisfies such conditions.

At the location of \( x - R \sin \alpha_{1} \), the lumen does not stretch, and the lumen radius \( r = R \cos \alpha_{1} \), becomes,

\[ 3 - 0.00015(x - R \sin \alpha_{1} - 100)^2 = R \cos \alpha_{1} \]

At this time, \( \alpha_{1} = \alpha_{1-\text{max}} \).

\( \alpha_{2-\text{max}} \) satisfies a condition that at the location \( x + L + R \sin \alpha_{3} \), the lumen does not stretch, and the lumen radius \( r = R \cos \alpha_{3} \), as shown in the following equation,

\[ 3 - 0.00015(x + L + R \sin \alpha_{3} - 100)^2 = R \cos \alpha_{3} \]

\( \alpha_{3} = \alpha_{3-\text{max}} \) would be derived.

The numerical calculation is carried out with the following equations.

\[
\begin{align*}
F_1 &= -10000 \int \left( \frac{R \sin \alpha_{1-\text{max}} + 100 - x}{(100 - x)^2 - 20000} \right) + \\
&\quad \frac{100}{3\sqrt{2}} \frac{R \sin \alpha_{1-\text{max}} + 100 - x - 100\sqrt{2}}{R \sin \alpha_{1-\text{max}} + 100 - x + 100\sqrt{2}} \\
&\quad + \cos \alpha_{1-\text{max}} - 1
\end{align*}
\]

\[
\begin{align*}
F_2 &= -\mu L - \frac{100}{3\sqrt{2}} \mu R \ln \left( \frac{x + L + 100 - 100\sqrt{2}}{x + L + 100 + 100\sqrt{2}} \right) \\
&\quad \frac{100}{3} \left( \frac{R \sin \alpha_{3-\text{max}} + x + L - 100}{(x + L - 100)^2 - 20000} \right) \\
&\quad + \frac{R \cos \alpha_{3-\text{max}} - R \cos \alpha_{3-\text{max}} - (x + L - 100)}{3\sqrt{2}} \ln \left( \frac{R \sin \alpha_{3-\text{max}} + x + L - 100 + 100\sqrt{2}}{R \sin \alpha_{3-\text{max}} + x + L - 100 - 100\sqrt{2}} \right)
\end{align*}
\]

\[
\begin{align*}
F_3 &= -10000 \int \left( \frac{R \sin \alpha_{3-\text{max}} + x + L - 100}{(x + L - 100)^2 - 20000} \right) \\
&\quad + \frac{100}{3\sqrt{2}} \frac{R \sin \alpha_{3-\text{max}} + x + L - 100 + 100\sqrt{2}}{R \sin \alpha_{3-\text{max}} + x + L - 100 - 100\sqrt{2}} \\
&\quad + \frac{R \cos \alpha_{3-\text{max}} - R \cos \alpha_{3-\text{max}} - (x + L - 100)}{3\sqrt{2}} \ln \left( \frac{R \sin \alpha_{3-\text{max}} + x + L - 100 + 100\sqrt{2}}{R \sin \alpha_{3-\text{max}} + x + L - 100 - 100\sqrt{2}} \right)
\end{align*}
\]

**TABLE 1**

<table>
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<th>( F_1/(2\pi k) )</th>
<th>( F_2/(2\pi k) )</th>
<th>( F_3/(2\pi k) )</th>
<th>( W/(2\pi k) = F_1/(2\pi k) - F_2/(2\pi k) = F_1/(2\pi k) - F_3/(2\pi k) )</th>
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<td>8.17E-06</td>
<td>1.4E-05</td>
<td>1.87E-05</td>
</tr>
</tbody>
</table>

**FIGURE 5**

Pressures in the Length Direction
The related parameters are substituted into Eq. (14), (15) and (16). Within the range of [0, 100] along the length direction of the lumen, the force is acting on the lumen along the horizontal direction. The calculated results are shown in Table 1.

The length stands the distance from the head of the snake. \( r \) is the radius of the snake. \( \alpha \) is the \( \alpha_{\text{max}} \) or \( \alpha_{\text{min}} \). \( n \) stands the \( n_1 \) or \( n_2 \). The ellipse stands the egg and the black fan on the ellipse stands the pressure \( n_1 \) and \( n_2 \). \( r, n, \alpha, F \) and \( W \) decrease along the snake’s body. \( W \) is more than zero, so the egg can continue to move in the snake’s body, but the move trend decreases along the snake’s body.

DISCUSSION

Within the length range of [0, 100], the friction inside the lumen is small, and differs greatly from the horizontal forces acting on the front and rear ends. The front end of the egg is squeezed horizontally, the middle suffered the friction, and the rear end is squeezed horizontally. The horizontal force acting on the front of the egg, \( F_1 \), is larger than the sum of the horizontal squeeze acting on the back of egg \( F_2 \) and the friction on the middle section \( F_3 \). Due to the resultant force, the egg move backward through the lumen. Along the length direction, the difference reduces, and tends towards 0. This is because the diameter of the lumen inside the snake’s body increases gradually from the front to the middle. The lumen is squeezed by the egg, and consequently, the tensile deformation decreases. The above reasons also explain that the egg will move from the front to the rear inside the snake’s body after swallowing. Besides, the peristalsis of the lumen also facilitates the occurrence of this phenomenon.

When the egg just enters the snake's mouth, the front end is not subject to the squeezing by the lumen. The snake would have to push the egg by using the lower jaw. The pressure on the front end of the eggs is larger than the sum of the pressure on the rear end and the friction on the middle section, which is the main cause of the movement of the egg inside the cavity.

In this study, the mechanical process of the downward movement of the egg inside the snake has been analyzed. Furthermore, the mechanical model of the downward movement is built, and the exact calculation example is presented. The results lead to better understand the transporting of a non-deformable solid in a cavity. The analysis process provides a reference for the calculation of the transportation of ground sediment [15-17] through the fissure of rock/soil body at oil drilling.

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EXPOSURE TO ENVIRONMENTAL CONTAMINATION IN WOMEN LIVING IN NAPLES AND CASERTA PROVINCES (SOUTH ITALY):
HAIR TRACE METALS ANALYSIS, FIRST DATA

presented in the 19th International MESAEP Symposium, Rome-Italy from October 04-06, 2017

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ABSTRACT

The aim of this study was to evaluate the Sn, Cd, Pb, Se, Ba, Cr, Cu, Ni, Be, Al, V, Fe, Mn, Sb concentrations in women’s hair childbearing age (20-30 years) along a litoral area, which falls into two provinces of Campania Region: Naples and Caserta. This area is subject to a high human impact. In addition, since the hair allows us to evaluate the different inputs linked both endogenous contaminations (deposition through the blood) and exogenous (deposition of airborne particulate matter and/or cosmetic products used), it was also possible to evaluate them. Preliminary data have shown a not uniform accumulation. An endogenous contamination for Cd, Pb, Cu, Ni, Be, Al, V, Fe, Mn, Sb; an exogenous for Cr; and a mixed one for Se and Sn were detected. Through Kruskal-Wallis (p) test significance coefficient, the data showed a clear and significant accumulation of Sn, Cd, Se, Ni, Al, V and Sb.

KEYWORDS:
Hair, trace metals, environmental contamination

INTRODUCTION

Three decades of illegal practices of waste dumping and consequent environmental abuse have made the Campania Region a unique case in the context of waste related health outcomes. Scientific evidence is supported by a significant increase in cancer mortality and malformation occurrence in specific areas of the Campania Region, where improper waste management and illegal waste trafficking have been repeatedly documented [1]. For more than two decades the illegal and/or inappropriate treatment and disposal of urban and industrial waste has contaminated the region’s soil, superficial and underground waters, and atmosphere, threatening every single living organism and being problem remained hidden to the public. Among various toxic substances in the waste dumping, heavy metals are of special concern because of their toxicity, mobility and non-biodegradability.

The effects of environmental exposure to trace metals on human health have been discussed over the last few decades, particularly with regard toxic metals that originate by anthropic activities, in particular through the study of these in blood and in urine. In the last ten years, hair has been used as trace metal biomarkers. In fact, the only data available, in the Campania region, are those related to metals in blood and urine. These are referred to children being a class more exposed to environmental contamination and because several studies reported a number of important pathologies to them. However, it is not so much, what we know about contamination by trace metals in women of childbearing age and the way; they can be transferred into the fetus during pregnancy.

As metals are present in the air, soil, food and water, they can enter into the body through the skin, the respiratory tract and the digestive system, being excess excreted directly through sweat, hair, urine and excrement [2]. The use of non-conventional matrices, such as hair, in toxicological-environmental analyses, is linked to the possibility of increasing the range in which the investigated substance is detectable in that matrix, in addition to the non-invasive sampling. In fact, this range stays only a few hours in the blood, a few days in the urine and months in the hair.

In addition, the xenobiotics deposition (organic substances, metals, etc.) in this tissue can occur both through endogenous and exogenous mechanisms.
Sweat glands and direct contact of environmental contaminants with the hair outer surface determine exogenous deposition. Endogenous deposition, instead, is due to the bloodstream and the sebaceous glands.

The various substances transported by the blood flow inside the hair matrix through the papilla with a mechanism that has not been clearly clarified yet. In particular, the keratinic sulfuryl groups sequester inorganic elements, such as metals. This union takes place continuously during hair growth. Any changes in their content in the fluids are thus regularly recorded in the length direction. The study of these phenomena could provide information both from the diagnostic and toxicological point of view, and from the evaluation of nutritional and/or pathological conditions.

Hair reflects the total bodily accumulation of the elements better than biological fluids; in fact, the concentration of these is higher (e.g. an order of magnitude), than the ones found in the blood, serum and urine. In contrast, hair, significantly susceptible to exogenous contamination (powders, detergents, cosmetic treatments, etc.), is characterized by the remarkable biological variability (sex, age, ethnic origin, eating habits) that do not allow an easy identification of "normal" reference values. In this regard, the aim of this study was to evaluate Sn, Cd, Pb, Se, Ba, Cr, Cu, Ni, Be, Al, V, Fe, Mn, Sb concentrations in women's hair fertile age (20-30 years) coming from the Domitian coast Flegreo and Agro Aversano, which falls into two provinces of Campania region, Naples and Caserta. This area, according to the data provided by the Regional Environmental Protection Agency of Campania [3], is subject to a high man-made impact.

Moreover, as this matrix allows us to evaluate the different inputs (endogenous and exogenous) we can also evaluate both of them.

MATERIALS AND METHODS

Study area. The enrollment of subjects occurred by Azienda Ospedaliera S. Anna and S. Sebastiano of Caserta and Azienda Ospedaliera Moscati of Avellino is relative, in this first project phase, to the area that is part of the Domitian coast Flegreo and Agro Aversano. In 1998, Agroaversano and Litorale Domitorio, the two areas in Campania most affected by illegal dumping and burning, were included in the national priority list of reclamation sites. Since that moment, not so much has been done to clean up the contaminated areas [4].

The perimeter includes the territory of 75 municipalities in the provinces of Naples and Caserta (Fig. 1).

For this area, the Regional Environmental Protection Agency of Campania (ARPAC) has carried out investigations to assess soil, groundwater and water contamination status. The results showed an exceeding of the limits set for Be, Ni, Pb, Cu, Sn, V, Sb, Cd, Se and Zn and for Zn, Mn, Al, Ni, Cr, Se and Fe, in Naples province for soils and waters respectively [4]. As far as the limited area of the Caserta province, the percentages of exceeding concern Be, Sn and Zn in the soils and only Mn in waters.

Experimental Procedure. Sampling. Samples were collected from female in fertile age that had carried out therapeutic abortion with diagnosis of fetal malformation (34 subjects) considered exposed (E). Women's enrollment and hair sampling was carried out through the collaboration with Azienda Ospedaliera S. Anna and S. Sebastiano of Caserta and Azienda Ospedaliera Moscati of Avellino. The individual's number, examined for each area, Naples and Caserta province, are respectively 19 and 15. All subjects were interviewed to obtain detailed information on their family, dietary habits, lifestyle and personal medical history. Patients read and accepted the written informed consent approved by the local Ethical Committee. Biological samples were obtained according to the local existing legislation on privacy protection (D.M. n.675, 13/12/96) and the Declaration of Helsinki. Personal data were recorded and analyzed in an anonymous format.

Hair samples (3-4 cm long) were collected from the scalp using a pair of sterilized stainless steel scissors washed with ethanol, a neutral solvent, to remove external contamination, if any, and dried [5]. Besides, hair samples were divided into two portions along the horizontal axis and vertically cut with stainless steel scissors, to evaluate endogenous and exogenous (unwashed hair) input.

All hair samples, about two grams, were sealed in plastic bags prior to analysis.

Samples Pretreatment. Samples pre-washed with non-ionic detergent (CERAMOL 311) were soaked in deionized water for 10 min. This was followed by soaking in acetone to remove external contamination followed by washing with deionized water. Unwashed hair did not undergo treatment. Subsequently, all samples (washed and unwashed) were dried in an oven at 60 °C to constant dry weight and to kept in a desiccator and to store for later mineralization [6, 7].

Chemical Analysis. To evaluate the total Sn, Cd, Pb, Se, Ba, Cr, Cu, Ni, Be, Al, V, Fe, Mn and Sb concentration, hair samples were mineralised using a combination of hydrogen peroxide and nitric acid (H2O2 50% v/v: HNO3 65% v/v = 1:3) in a microwave oven (Milestone—mls 1200—Microwave Laboratory Systems). After digestion, the solutions were diluted by deionised water to a final volume of 50 mL. The concentration of each element was measured by atomic adsorption spectrometry.
(SpectrAA 20 Varian) via graphite furnace and flame. Accuracy was checked by concurrent analysis of standards (Resource © by PSP Volume 26 – No. 1/2017, pages 475-482 Fresenius Environmental Bulletin 477 Technology Corporation. Laramie, WY) and the recovery was in a range 90–110% for each element.

**Statistics.** All the analyses were performed in triplicate for each hair sample and expressed as mean ± SD. Correlations between parameters were calculated using the simple Pearson correlation coefficient.

Metal accumulation was calculated like:

$$MA \, (\%) = \frac{(M_{\text{HW}} \times 100)}{M_{\text{HNW}}}$$

where $M_{\text{HW}}$ was metal concentration found in washed hair and $M_{\text{HNW}}$ was metal concentration found in unwashed hair.

The results of the analytical determinations were treated with a series of statistical tests to determine the type of data distribution and the attribution of a toxicological significance. Outliers have been identified by Box and Whisker Plot approach. After identified, outliers have been eliminated from the following statistical elaboration.

The application of the Kolmogorov-Smirnov test revealed that most of the elements were characterized by non-normal numerical distributions, consequently, for the final processing of results, a non-parametric test was performed for unmatched data such as Kruskal-Wallis test. The non-parametric test applied has a discriminatory capacity of the actual differences between the data population groups (parameter p). In this case, the test has provided an overview of the existence or not of a significant exposure to the elements considered, to which individuals are subjected in the study area.

Considering that 50% values indicated a mixed contribution, it should be noted: (a) an endogenous contamination for Cd, Pb, Ba, Cu, Ni, Be, Al, V, Fe, Mn, Sb; (b) Exogenous for Cr; (c) mixed for Se and Sn (Fig.3).

Factors related to environmental pollution and, consequently, to the absorption of toxic metals, found in cigarette smoke (Cd), in paints or paintings (Pb), in cosmetics (Pb, Be), in hydrogenated oils (Ni), in deodorant components (Al), in the cooking utensils and pots (Cu and Al), are the cause of their accumulation in the tissues.

Naturally, the large presence of metals in the environment of the study area also depends on the presence of industrial plants (chemical, pharmaceutical, cement, purification plants), composting sites, waste treatment and storage facilities, legal landfills, sites of abandoning waste. Vanadium industrial/environmental sources, for example, include: mineral treatment, phosphate fertilizers, oil and coal combustion, steel production and chemicals used to fix dyes and printing. The study area has an industrial agglomeration and various sources of metal emission with an important relapse of them also in soil and water. In these two matrices, in fact, it has been exceeded the limits set imposed for Be, Ni, Pb, Cu, Sn, V, Sb, Cd, Se, Zn and Mn [6, 7]. Moreover, metal toxicity is also enhanced by mineral deficiencies becoming increasingly frequent with the use of refined foods, industrially prepared and cultivated in poor mineral soil [7].

Besides, from the analysis of the significance levels obtained (p) by Kruskal-Wallis test, there was a clear difference between the two matrices, washed (W) and not washed (NW), for most metals, and in particular for Sn, Pb, Se, Ba, Cr, Cu, Ni and Fe (Tab.1).

There were no law limits set with respect to metal concentrations in hair and so, geometric averages have been compared to reference values (RV) [8].

Now, RVs are becoming more importance and space in the scientific and normative world, in fact they appear to function as a means to evaluate the absorption of the contaminant by human. They have been widely recognized as useful tools (a) to observe who exhibits a contaminant level higher than the RV; (b) to identify possible sources and ways of absorption of the contaminant; (c) to monitor the occurrence of possible effects on human health. The specific purposes of RVs are: 1) to explore health-status; 2) to study significant individual physiological changes; 3) to study the effects of the environment on man; 4) to monitor the patient / individual over time; 5) to assess the effectiveness of individual means of protection; 6) to help to diagnose; 7) to experiment and select therapies, etc.

From the analysis of the obtained significance levels (p), there was a clear and significant

**RESULTS AND DISCUSSION**

Preliminary data on the concentrations of trace metals found in the hair of female in fertile age resident in the provinces of Naples and Caserta showed a non-uniform accumulation.
accumulation of Sn, Cd, Se, Ni, Al, V and Sb. In the soils of the study area, all these metals, except for Al, showed an overcoming of the imposed limits set by [6]. In addition the enrollment took place, at the first stage, in the provinces of Naples and Caserta, figures 4 and 5 also showed the percentage of the average accumulation for each metal in washed hair samples compared to not washed for both the province of Naples (Fig. 3 left) and Caserta (Fig. 3 right).

Considering a mixed contribution for values of 50%, it is observed a more exogenous contamination only for Cr (% washed between 42 and 44%) for the subjects enrolled in the province of Naples and Caserta. Table 2 and 3 showed geometric averages for each metal in both matrices in Naples (Tab. 2) and Caserta province (Tab. 2) compared to the reference values (RV).

A significant difference between the two matrices (NW and W) for all metals except Cd, Be, Al, Mn and Sb and a Sn, Cd, Se, Ni, Al, V and Sb accumulation for subjects coming from provinces of Naples and Caserta were showed.

Being both areas affected by similar emission sources, the accumulations of most metals appeared the same. The only differences were related to a greater accumulation of metals (Sn, Cd, Se, Ni, Al, V and Sb) for subjects resident in the province of Naples than Caserta.

All these metals showed percentages exceeding the limits imposed for soils in the study area [6].

The higher levels of these elements could also be linked to the fact that, due to the very mild climate conditions, most of the population in this area spends more time in outdoor activities than those residents in areas where climatic conditions are more rigid and people privilege indoor activities [9].

Moreover, in both subjects residing in the two provinces, high Al and Se levels have been highlighted. Considering the wide distribution of Al in nature, exposure to it occurs unequivocally through direct inhalation of atmospheric particulate matter or through food [10] and water, particularly that with low pH [11]. Excluding drinking water, which generally has a pH above 6.5, small particles of food and soil should be Al’s main sources for women living in the provinces of Naples and Caserta.

Unlike aluminum, selenium is an essential nutrient. An important beneficial effect of selenium is related to its role in antioxidant enzymes, selenium-proteins, such as glutathione, peroxidase and isoenzymes. Experiments have shown that this element may confer some protective effect on toxic metals [12-15], and may perform a prevention of cancer [16, 17].

Comparing the data obtained by a biomonitoring study using hair, in four Italian cities (Arezzo, Vicenza, Valenza and Rome), it has been observed higher concentrations of Cd and Cr, while lower Pb and Cu and Ni concentrations [8].

**TABLE 1**

<table>
<thead>
<tr>
<th>Geometric average</th>
<th>RV</th>
<th>Kruskal-Wallis test (W/NW)</th>
<th>Kruskal-Wallis test (W/W)</th>
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<tr>
<td>Sn</td>
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<tr>
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</tr>
<tr>
<td>Pb</td>
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<td>0</td>
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<tr>
<td>Se</td>
<td>18.25</td>
<td>36.38</td>
<td>7.11</td>
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<tr>
<td>Ba</td>
<td>0.32</td>
<td>0.33</td>
<td>0.75</td>
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<tr>
<td>Cr</td>
<td>0.42</td>
<td>0.96</td>
<td>0.49</td>
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<td>Cu</td>
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<tr>
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<td>0.000</td>
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<td>0.11</td>
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<tr>
<td>Sn</td>
<td>1.53</td>
<td>1.62</td>
<td>0.095</td>
</tr>
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</table>

* obtained using the max range value
The correlations tested in the several individuals resident in the provinces of Naples and Caserta (tab.3) showed that most metals were correlated with each other, highlighting for a common origin.

**CONCLUSION**

From these early results it has been shown that hair is a tissue that can absorb potentially toxic minerals. It has appeared, in particular, that the contamination sources for the study area were multiple. In fact, they were linked to both the...
lifestyles of each individual and the industrial/environmental inputs that affect this territory. Comparisons between different individuals from both provinces (Naples and Caserta) showed similar accumulation of metals in hair, as both areas are affected by similar emission sources.

The Environmental Protection Agency (EPA), has included in the analysis of trace metals, storage tissues, such as hair, in addition to blood tests, because defense mechanisms remove the toxins from the serum, to deposit them in the tissues.

However, an integrated study of the exposure measurement to environmental contaminants through their quantification in hair samples associated with their analysis in blood, serum and urine, in different environmental matrices and also in food, would represent an indispensable tool for assessing the possible influence of environmental determinants on human health, given the innumerable variables that the environment itself has.

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EVALUATION OF TRACE ELEMENTS CONTENT IN BUFFALO MOZZARELLA PRODUCED IN ITALIAN DAIRY FARMS: FIRST DATA

presented in the 19th International MESAEP Symposium, Rome-Italy from October 04-06, 2017

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ABSTRACT

The aim of this work was to assay some traces concentrations in buffalo mozzarella and buffalo milk sampled in two dairy farms of Caserta province (Campania Region, Italy). The data showed that: (a) metals concentrations in buffalo milk were under law limits set; (b) Cu, Pb and Cr concentrations in buffalo mozzarella appeared similar to those found in buffalo milk, while Ni and Al concentrations were significantly lower; (c) Pb and Cd concentrations, the only present in the European Union Commission Regulation (EU) (1881/2006), were under law limits.

KEYWORDS:
Buffalo mozzarella, trace metals, dairy farms

INTRODUCTION

In higher organisms, the trace elements intake occurs mainly through the respiratory system or through the food chain; it is the case of many dangerous elements or compounds such as dioxins, pesticides, metals and metalloids. Moreover, these compounds generally have an anthropogenic origin, and therefore their concentration in the environment grows with increased urbanization, agricultural activity and industrial emissions. In this scenario, controlling the levels of these elements in foods is not only an important aspect of food quality, but also an indirect monitoring system of human activities to assess impacts on soil, water and air [1-8]. In particular, direct correlations between the metal concentrations in air or soil and the increase in human emissions have been observed in the literature, not only on space-time scale, but also for short periods and small areas [9-10].

Trace metals in milk and dairy products derive from several factors such as environmental conditions and even possible contamination during the various stages of production processes.

Southern Italy, which have the 73% of buffalo farms, is an important source of income for rural areas and in particular for Campania Region, which has a large number of them (254,030). Buffalo milk is entirely used for cheese production, especially mozzarella. The buffalo mozzarella is a dairy product traditionally designed in Campania, especially in the provinces of Caserta and Salerno, although the production of this typical cheese is also carried out in other parts of Campania.

Currently, no data is available regarding contamination levels of heavy metals in buffalo mozzarella; therefore the purpose of this work was to detect the concentrations of some heavy metals in the buffalo mozzarella sampled in two dairy farms in the province of Caserta. Besides, it has been possible to test the same metals in milk and feeding of lactating buffaloes as well as in buffalo drinking water in order to assess possible accumulation paths.

MATERIALS AND METHODS

Buffalo dairy farms. Table 1 and 2 showes the main characteristics of the two buffalo dairy farms studied and the buffalo feeding types in the two sampling time.

Chemical Analysis. All samples (buffalo mozzarella, buffalo milk and feed) were lyophilized and then they were ground to powder by a Fritsch pulverisette 6 with an agate mortar, to prevent element contamination.

To evaluate the total Ni, Cu, Cd, Cr, Pb and V concentration, samples were mineralized using a combination of hydrogen peroxide and nitric acid (H2O2 50% v/v : HNO3 65% v/v = 1:3) in a microwave oven (Milestone—mls 1200—Microwave Laboratory Systems). After digestion, the solutions were diluted by deionized water to a final volume of 50 mL. The concentration of each element was measured by atomic adsorption spectrometry (SpectAA 20 Varian) via graphite furnace. Accuracy was
checked by concurrent analysis of standards (Resource © by PSP Volume 26 – No. 1/2017, pages 475–482 Fresenius Environmental Bulletin 477 Technology Corporation, Laramie, WY) and the recovery was in a range 90–110% for each element.

The water samples were analyzed directly by atomic adsorption spectrometry (SpectrAA 20 Varian).

Statistics. All measurements were performed in triplicate for each sample ± SD. The significance of differences was tested by analysis of variance (ANOVA) followed by a Tukey test (MINITAB INC 13).

**TABLE 1**

<table>
<thead>
<tr>
<th>Dairy farm</th>
<th>Feeding type</th>
<th>Water used</th>
<th>Milk produced per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>hay</td>
<td>Water well</td>
<td>33.5 kg</td>
</tr>
<tr>
<td>B</td>
<td>hay</td>
<td>Water well</td>
<td>41.3 kg</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Type of feed</th>
<th>Fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td></td>
</tr>
<tr>
<td>Oatmeal</td>
<td></td>
</tr>
<tr>
<td>Oat hay</td>
<td></td>
</tr>
<tr>
<td>Triticale</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

No significant differences were found between the two sampling seasons (April and September 2016) in the drinking water for buffalo taken from the two dairy farms (A and B) (Fig. 1).

V content for both dairy farms was similar, while significantly higher concentrations of Cu, Cr and Cd were found in water from dairy farm B.

Besides, the relative content of each metal in the drinking water was lower than the limits imposed by the Italian decree [11] (Fig. 1).

As regards the feed, it should be noted that the ones used by dairy farm A in April and September were not the same. They were, in fact, related to their seasonality and market availability (Tab. 2). For dairy farm B, instead, the feed used was always the same in both sampling seasons (Tab. 2).

By comparing the two dairy farms (A and B), independently of the feed type used, Cd and V concentrations were significantly higher in A, while Cr and Pb were significantly higher in B (Fig. 2); Ni, Cu and Al contents, however, did not show any differences.

Dairy farm A showed different metals accumulation in the two samplings. In particular, in the first sampling, significantly higher concentrations of Pb and Ni in the complementary feed, Cu in Oatmeal, Cd, Cr and V in Oat hay and Pb in the triticale were highlighted. In the second sampling, instead, significantly higher concentrations of Ni in complementary feed, Cu in the fine bran, Pb and V in oatmeal, Cd in corn flour and mixed hay were also highlighted in the fine bran, silty triticale and oatmeal.

On the other hand, dairy farm B showed, in the first sampling, the significantly higher concentrations of Ni, V and Cu in complementary feed and Cd in corn stalks.

Comparing the two dairy farms, regardless of the type of feed used, higher Cd and V concentrations were found in dairy farm A, while Cr and Pb concentrations appeared higher in dairy farm B. For Ni and Cu, instead, there were no significant differences.

As is well-known, environmental contaminants, which can spread both in the air and in drinking water and/or irrigation water, are absorbed by animals through inhalation, percutaneous and oral route [12]. As regards feed, it must be recognized that these are the most important and significant carrier of the contamination. Among the heavy metals, Pb is one of the most dangerous elements for toxicity and for many contamination sources (pastures and forage harvested in proximity to roads with high transit and/or near industries that use metal in their machining cycles, industrial waste, accumulators, and abandoned building materials on pastures). In this study, the dairy farm B showed higher Pb concentrations. In fact, although it was located in an agricultural area, it was near major roads with respect to dairy farm A. In addition, lead, thanks to its wide use in industrial processes, is the most common metal in the atmosphere [13]. This could explain the high concentrations that were found in relatively remote sites from urban and industrial areas.

Copper, an essential trace element for hemopoiesis, for the synthesis of myelin and bone tissue and for certain oxidizing-activity enzymes present in the soil, unlike Pb, is absorbed mainly by the root system of plants and fodder which can thus be dangerous. High concentration, in fact, could be linked to the use of pesticide-contaminated fodder (molluscicides, anti-clotting agents). Other environmental pollutant is Cd. Contamination occurs either by inhalation of dust (typically cadmium oxide) emitted
by specialized industries in extracting Zn, or by intake of contaminated herbs [12].

Lead and Cd contents (the only two feed elements subject to legislation) were also compared to the limits set [14] (10 μg Pb/g and 1 μg Cd/g) showed values that fall within the limits except for the contents of Cd found in mixed hay and corn flour from dairy farm A, where, however, they were exceeded.

Comparing the data obtained with literature, Ni, Cu and Cd concentrations were comparable with those found in 183 feed samples collected in different farms in England and Wales [15]. Lead and Cr concentrations appeared lower and higher respectively when compared to that found by other authors [15].

In literature, Pb concentrations in dairy feed show variable concentrations. High variation makes it certainly difficult to determine a trend in concentration levels between different types of feed, but generally low Pb concentrations have been found in corn grain while the highest in mineral blends. Lead availability in the soil is generally low. In a study on Pb absorption in corn plantations cultivated on soils receiving high levels of Pb through sewage and sludge, it was found that absorption was ~1% compared to the total amount of Pb used [16]. It seems probable that the high variability associated with Pb concentrations in fodder could be related to different sources, such as airborne contamination.

As for Cr, it was present in all feed components and showed higher concentrations than other heavy contaminating metals (i.e., As, Cd and Pb) [17]. In the literature a variable content of Cr in the feed was highlighted, and in particular it has been shown that the mixing process of components in steel containers contains about 18% of Cr can be added to its levels in food and then contaminated [18].

Figure 4 shows trace metals concentrations assayed in buffalo milk coming from both dairy farms during the two sampling time.

The trace metals content was always significantly higher in April than in September (at least for p <0.05), regardless of the dairy farms considered, except Cu.

In addition, generally, the significantly higher values of trace metals were tested on dairy farm A except for Cd, where there were no differences between dairy farms except for the second sampling, and for Cu where the dairy farm B showed significantly higher values. Concerning some tested metals, it was possible to compare the data obtained with the limits set [19], which defines the maximum Pb content. Cd content, on the other hand, was compared to concentrations considered physiologically in milk [20].

In all analyzed milk samples, the metal content found, expressed by fresh weight, was all below or close to the limits for both dairy farms and for each sampling (Tab. 3).

These metal concentrations in the milk are clear consequences of the respective concentrations in the forage.

A comparative study of the metals content in buffalo milk and cow’s milk showed higher concentrations of Cr and Pb in buffalo milk samples [21]. Buffalo milk, in fact, has high fat content that would favor the formation of bioactive complexes (lipophilic) resulting in metal retention [22-23].

In many countries, monitoring is carried out to assess the level of these pollutants in milk. Some Greek researchers have analyzed the lead concentration in two hundred samples of cow milk, goat and sheep taken from different areas near Athens [24]. In all samples, the presence of this contaminant was revealed, but at concentrations below the limits established by the European Community. In this study, it has also been shown that milk from farms located near mining sites or high density traffic areas is the one with the highest concentrations of lead, followed by that of animals living in industrial areas. Less contaminated milk was found in sheep living in rural areas and in cows reared on an island not far from Athens [24].

FIGURE 1
Trace metal concentrations found in buffalo drinking water assayed in the two dairy farms (A and B). The data are reported as an average of 3 field samples and 2 laboratory ± SD. Different letters represent significant differences between the farms at least for P<0.05. At the top, the limits set [12].
A similar study was conducted in Turkey, where 75 samples of raw milk from different geographical areas were analyzed, with the aim of detecting possible contamination of heavy metals. Major lead concentrations were found in samples from industrialized areas, followed by those in high traffic areas and finally from milk taken in rural areas with a statistically significant difference between the various regions. The concentrations found in the milk of industrial areas as well as those of high traffic areas were above the legal limits for Turkey (0.02 mg/kg).

Milk samples were also analyzed in Italy to assess the level of metal contamination. Researchers from the Institute of Hygiene and Preventive Medicine at the University of Genoa have analyzed ten sampling 

FIGURE 2

Trace metal concentrations found in the different feed used for the feeding of lactating buffaloes assayed in the A (left) and B dairy farm (right), in the two different sampling seasons. The data are reported as the average of 3 field samples and 2 laboratory ± SD and expressed in µg/g dw.
types of cow’s milk in the Genoa area and analytical determinations of different metals including Pb, Ni, Cr. Lead concentrations were not determinable because below the instrument's detection limit, while Cr and Ni showed the following values: Cr 0.004-0.012 μg/g (mg/kg), Ni 0.01-0.024 μg/g (mg/kg) [25].

The International Dairy Federation has stated that the normal concentrations of Ni in cow's milk are on average 2.5 μg/kg, with variations ranging between 0.4 and 6 μg/kg, while for chromium the concentrations were 2.5 μg/kg [26].

Figure 4 shows trace metals concentrations tested in buffalo mozzarella from the two dairy farms (A and B) during the two sampling times.

The data are reported as the average of 3 field trials and 2 laboratory ± SD and expressed in μg/g dw.

The mozzarella from dairy farm A showed higher concentrations in April and September. Observing, however, the data on buffalo mozzarella from dairy farm B, there were no statistically significant differences between the sampling. This was probably related to the different type of feeding for dairy farm A in the two samples.

Unfortunately, for buffalo mozzarella, there are no literature data on trace metal content. Studies carried out so far refer to PCB content and bacteriological contamination [27-28].

Regarding the limits set by the legislation, only for Pb it was possible to compare the data recorded with the limits set [1].

In order to compare the data obtained with the legislation, a conversion factor, as provided by the Regulation, was applied, given the ratio of the quantity of milk needed to produce one kilogram of cheese. As a mega gram of buffalo milk produces 240 kg of mozzarella, the data has been re-elaborated applying the conversion factor that provided results below the legal limits. Cadmium content was also compared to concentrations considered physiologically in milk [20] and the data, once processed using the conversion factor, and showed values below the expected limits.

![Trace metal concentrations found in milk and in the buffalo mozzarella assayed in the two dairy farms (A and B). Different letters represent significant differences between the farms and sampling seasons at least for P<0.05. Data are reported as the average of 3 field samples and 2 laboratory ± SD and expressed in μg/g dw.](image-url)
CONCLUSION

In conclusion, trace metals do not contaminate the analyzed buffalo mozzarella. Of course, this is just the first data of a study that involves monitoring of several Campania dairy farms. The data that will be collected in other companies monitored, as expected in this project, will allow us to expand the variability and make the collected data more reliable from the statistical point of view.

There is also a need for continuous monitoring of toxic elements due to the continuous release of exogenous elements into the environment that may be a source of risk in the near future. The results of this study, along with those produced in the future, could contribute to the creation of a maximum tolerance limit for heavy metals in buffalo mozzarella.

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EFFECTS OF TRANSPORTATION PARAMETERS ON CLIMATIC ELEMENTS BETWEEN URBAN AND RURAL AREAS: A CASE STUDY OF ERZURUM

presented in the 19th International MESAEP Symposium, Rome-Italy from October 04-06, 2017

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ABSTRACT

Roads make it possible for urban people to access public services and materials they need. Enlargement of road surfaces impacts climatic elements in urban areas since they heat excessively air depending heavily on the absorption rate of solar radiation due to lower albedo. Erzurum is an important city in Turkey since it is the largest city of East Anatolia Region of the country. Harsh continental climatic characteristics are prevalent and majorly service sector employs more than half of people in the city. The aim of the study is to evaluate the change in mean yearly long term temperature (1960 – 2016) and rainfall (1929 – 2016) values obtained from the station operated and maintained regularly by the Turkish State Meteorological Service (MGM) at the city airport about 7 km bird – eye from the city center by considering some urban parameters, human population, road and building surfaces and the number of motor vehicles.

It was found that human population has been decreasing incessantly in the city until today, but the number of motor – vehicles and the total surface area of buildings and the roads is in an increasing trend. In terms of climatic elements (temperature and rainfall), it is seen that mean yearly temperatures are in increasing trend after especially 1992 and rainfall is in a general decreasing trend in especially recent years.

Such an apparent change in climatic elements in the city is dependent on a dense and quick transformation trend in the city, which is the reflection of the socioeconomic development policies adopted by central government over at least the last ten years based on land use/valuation or rant. In the example of Erzurum, urban climate is getting warmer and drier which is a condition that must urgently be taken under control for human health and better living environment.

KEYWORDS:
urban climate, road surface, temperature, relative humidity

INTRODUCTION

Roads act in a region as circulatory system connecting organs to heart in human body and carry passengers and freights in and to the regions just like veins do. In cities, roads are vitally important parts of urban daily life, which make it possible for people to access public services and materials they need (e.g. accessing markets for salesmen and buyers, workplaces, schools and hospitals etc.). Roads have numerous benefits and great contributions to socioeconomic development in a region / city [1,2].

Type, size, structure and use of roads have considerably changed since the beginning of industrial revolution depending on the changing anthropogenic activities and human needs, which are based strictly on more production and more consumption. Even though roads can serve for economic prosperity and social welfare, they have some costs in both monetary and environmental terms, i.e. investment funds and pollution in every type of natural reserves (air, water, soil) [1].

Impacts of roads on ecosystem goods and services are evaluated in the related to result from chemical contents released by road structures into water, soil and air (decaying them), changes in climate (e.g. increased temperature on the road surface), hydrological process (changing the direction of water flows), modified habitat (changing the distribution of plant species) and habitat quality (changing the structure of animal population) [3]. In addition to these negative effects of roads in natural environment, urban areas may face more severe effects of road surfaces due to the densely built structure of cities as the combined effects of both physical and climatic factors (e.g. street canyons, lower albedo – higher solar radiation, lower wind speeds etc.) [4]. Among the most important signals and requirements of urbanisation and urban lands is the paved / impervious surfaces including roads (asphalt or concrete covered). Of such surfaces asphalt and concrete covered one’s areas are those which cause excessive heat storage and exhibit disadvantageous characteristics for climate [5]. Enlargement of road surfaces is expected to have three types of impacts on climatic
elements in urban areas. Firstly, these surfaces heat excessively the air above them compared to other surfaces since they absorb greater rate of solar radiations due to their lower albedo. Secondly, increasing in the length and surface area of roads means additional motor – vehicles movements which cause again the deposition of higher rate of solar radiation due to exhaust gases involving greenhouse gases and particle and waste heat from exhausts. Thirdly, new road surfaces mean new transformed surfaces (i.e. covered surfaces by buildings and other structures).

As the result of a consistently growing process in every sector beginning from 19th century, road surfaces occupied an important part of cities, which were also growing depending on human population movements based on industrial productions. Such a process is nearly ceased in developed western countries where maybe the last intervention or reformation was witnessed after the Second World War, however; in newly developing countries, the mentioned process is going on very fast.

Turkey is accepted to be among such countries and it has been in a socioeconomic development effort for nearly an age, the last half of which clearly caused enlargement of cities, transformed nature surfaces based on population movements from rural to urban and large metropolitan areas. For example, in 1927 census in the first years of modern republic, 75% of Turkish population lived in rural. When it comes to today, the situation has turned out to be reverse. Such a condition has changed the ratio of urbanized lands in whole territory of the country [6]. As the result of the mentioned process, natural, cultivable or forest lands have been transformed to impervious surfaces, including structured (roads, buildings, pavements etc.) areas especially around cities in order to find places for various activities such as housing, sheltering, producing, depositing and connecting all these activities to each other (roads).

Climate is the long-term means (at least 30 years according to WMO) of instant atmospheric conditions, which are shaped by atmospheric elements like temperature, wind, precipitation, solar radiation etc.

This study is related to the determination of possible changes in two climatic elements, mean yearly temperature and rainfall which may affect and determine the situation related to winter tourism identity of the city of Erzurum, which is a medium – sized unindustrialized Turkish city, by considering some urban parameters, human population, road and building surfaces and the number of motor vehicles.

**MATERIALS AND METHODS**

Erzurum city center is located in the east part of Turkey (39° 55’N; 41° 16’E; Figure 1) at an elevation changing between 1850m and 2100m in the skirts of Palandöken Mountain, an internationally famous ski center. Harsh continental climatic characteristics are prevalent in the city, where temperature can reach up to 36.5°C and down to -37.2°C (Table 1).

Table 1 gives average and total values of some meteorological parameters measured over a period from 1950 to 2015, where long term mean temperature is 5.6°C, annual rainfall is 403.3mm and mean relative humidity is 66.3%. Apart from the figure and table, mean yearly wind speed is 2.7m/sec and prevalent wind direction is ENE in summer and WSW in winter due to frontal systems in the city center. The city is among small – middle sized and unindustrialized Turkish cities, where more than half of the working people are in service (mainly public) sector. Population of the city center is about 417.385 according to Turkish State Statistics Institution in 2016. The first meteorological observation station was established in the city in 1929 and transferred to the city airport, nearly 7 km away, in 1988. From time to time, both in the city center and at the airport two stations were operated simultaneously.

![FIGURE 1](Location of study area.)
Data and Measurement. Since the aim of the study is to evaluate the long term change in mean yearly temperature and rainfall, long term temperature (1960 – 2016) and rainfall (1929 – 2016) values are evaluated over the data obtained from the station (1758m; 39°.57’N-41°.10’E). It is operated and maintained regularly by the Turkish State Meteorological Service (MGM) for the aims of aviation at the city airport about 7 km bird – eye from the city center. It is in the middle of a vast plain covered with grassy plant species. The area shelters no buildings or human activities except for one – storey service buildings around the station (Figure 2).

RESULTS

As can be seen in Figure 3, the human population has decreased in the city consistently from 800 thousand in 2000 to 760 thousand in 2016. In spite of this decreasing trend in human population, the number of motor – vehicles registered officially in the city has been in an increasing trend since 2005. The number of motor – vehicles was close to 60 thousand in 2005 and doubled by reaching nearly 120 thousand, which means one of every six people owns a motor-vehicle. This rate is expected to be higher in the city center since the given rate includes those in rural part of the province. Total surface area of buildings registered yearly is a parameter, which can be supplied by TurkStat (TÜİK), and this parameter is also in a consistent increase beginning from 2002. The mentioned trend for building surface area in the city is valid exactly for the road surfaces, which is a parameter determined using GIS methods. In terms of climatic elements (temperature and rainfall), it is seen that mean yearly temperatures are in increasing trend after especially 1992 and rainfall is in a general decreasing trend in especially recent years.

Results are convenient with those found in previous studies conducted for the same city [7,8].

CONCLUSION

Erzurum is an important city since it is the largest city of East Anatolia Region of the country, founded at one of the highest elevations in the country, slightly more than 2000m. Harsh mountainous, continental climatic characteristics are prevalent in the city and majorly service sector employs more than half of people in the city. This means the city is expected to get harm far less than other Turkish cities because there is no heavy industry in it.

In the example of Erzurum, urban climate is getting warmer and drier which is a condition that must urgently be taken under control for human health and better living environment.
The city harbors have no significant anthropogenic activity except for the construction works for building housing estates, which result in surface transformation in close proximity of the urban core. Such dense and quick transformation trend is the result/reflection of the socioeconomic development policies adopted by central government over at least the last ten years based on land use/valuation or rant.

As the value of land increases, excessive money from overvaluation of land flows to landowners and then other end users, which in turn increases consumption and requires new lands to open for construction to sustain this circulation. Therefore, as the human population decreases in the city, the number of cars and surface area of roads and buildings increase.

Such a wasteful approach to natural resources is an unfaith and against sustainability principles. As can be seen in the changes in the climatic parameters, this approach causes losses not only in natural lands but also pollutes and decays the quality of air.

Changes in climatic elements in the city depend on the factors caused by only people’s daily routine activities e.g. going to work (motor vehicle use, opening new roads and housing areas, domestic heating) in a short period of time even in addition to global circulation.

Based on national economic development policies, nearly all cities of Turkey are expanding and sprawling mainly on natural lands causing larger impervious surfaces and waste heat. In order to reduce or remove such negative effects city planning principles must absolutely be followed and efficient urban green spaces must be considered in all large or small scale projects. All the materials used in buildings especially surface coatings and pavements must be selected from those respectful to climatic conditions e.g. not increasing temperatures.
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EXPLOITATION OF PEANUT AND HAZELNUT SHELLS AS AGRICULTURAL INDUSTRIAL WASTES FOR SOLID BIOFUEL PRODUCTION

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ABSTRACT

In the present study, biopellets produced by peanut and hazelnut shells and the higher and lower heating values (HHV and LHV) were determined and compared to the standards used in the European Union for biomass-produced pellets. The HHV and LHV of the biopellets made of peanut and hazelnut shells have been determined according to the test standards of the International Organization for Standardization (ISO). The HHV of the peanut shell pellets were determined as 21.01 MJ/kg, 20.13 MJ/kg and 18.05 MJ/kg in moisture and ash free (MAF) base, dry base and the original base, respectively. The HHV of the hazelnut shell pellets were determined as 20.59 MJ/kg and 17.92 MJ/kg in dry base and the original base, respectively. The LHV of biopellets made of peanut and hazelnut were determined as 18.93 MJ/kg and 19.39 MJ/kg in dry base, respectively. The HHV of hazelnut shell pellets were 1.09% and 0.95%, respectively, according to ISO 1171 standard.

KEYWORDS: Peanut shell, hazelnut shell, biopellet, heating values

INTRODUCTION

Today, energy systems are generally based on the use of fossil energy resources and the amount of consumption is rapidly increasing day by day as the amount of consumption increases. This increase in fossil fuel consumption has already threatened the future of our world. Today, high levels of greenhouse gases (CO\textsubscript{2}, CH\textsubscript{4}, N\textsubscript{2}O) arise as a result of warming activities of industrial production facilities, motor vehicles and residences, which usually use fossil fuels by conventional methods. The International Energy Agency (IEA) has explained that greenhouse gases (GHGs) cause adverse effects on the environment and human health, such as air pollution and climate change.

That 99.5% of the GHGs in the atmosphere are from fossil fuel sources [1]. Because of the use of fossil fuel resources, one third of the world's natural resources, 12% of the forests, one third of the oceanic biological diversity, and 50% of the freshwater waters are destructed [1]. According to the IEA estimates, the average annual increase in world energy demand between 2008 and 2030 is expected to be 1.6%. At the end of this period, it is expected that total energy demand increase to 45% by 2030 [2]. The IEA states that between 2010 and 2035, 93% of the increase in energy demand will come from developed countries [1].

According to the studies, in 2015, the share of renewable energy in world energy production was 10.3%. This means that about 1.5 Gt of CO\textsubscript{2} emission is prevented. According to the "Global Trends in Renewable Energy Investments 2016" report, prepared annually by the United Nations Environment Program (UNEP), investments in renewable energy sources in 2015 are about $266 billion. The investments made to produce energy from fossil fuels in 2015 are about 130 billion dollars. Accordingly, renewable energy investments in the world for 2015 are more than twice as much as investments aimed at producing energy with fossil fuels. This suggests a structural change in energy production. As a result of these investments made in renewable energy in the world, 134 GW of new energy production was provided in 2015 [1]. Therefore, several studies have established concerns renewable energy generation, and its links with energy conservation, environmental degradation and economic growth in different part of the world [3-7].

The conventional use of biomass for energy continues in several parts of the world, but it has generally been replaced by fossil fuels in large scale power generation, industrial production, and transportation. However, in recent years, biomass utilization has received increasing attention for its role in providing renewable sources of energy, chemicals, and other industrial products, at higher efficiencies and with greater selectivity than previously possible [6]. The agro-industrial wastes with more potential to be used as carbon source, are those generated in the food industry, which contain considerable amounts of fermentable sugars, which by
appropriate saccharification and fermentation can be transformed into many biomaterials [7]. The agro-industry generates large amounts of organic residues, which are an important source of cellulose, hemicellulose, and lignocellulose [6]. One of the effective methods for using agricultural residues as improved solid fuels is pelletizing. In recent years, pelletization has become increasingly prevalent and the use of pellets has become widespread. The evaluation of agricultural wastes in the form of solid fuel as a source of energy is of great importance in Turkey as well as in the whole world. One of the easiest and most effective ways to generate energy from agricultural wastes is to use these wastes as solid fuel. However, the most important problem encountered in using vegetable wastes as solid fuel is the low density of vegetable wastes and the high moisture content. Low density and high moisture content also bring with it transportation and storage problems. For this reason, one of the methods to be applied in order to use vegetable wastes efficiently and easily in order to produce energy is to dry these wastes and to pellet them after grinding.

The pelletization of peanut and hazelnut shells has reduced storage and transport costs and improved combustion characteristics, resulting in a cheap, quality, environmentally friendly, domestic and renewable biomass solid fuel. In this study, it is aimed to produce pellets for use as solid biofuel from the remaining biomass materials. The pelletizing process will facilitate the transport and storage of residues and at the same time reduce shipping costs. Most importantly, the evaluation of such biomass materials through pelletization will provide for the economization of waste and will partially or even reduce countries’ external dependency on energy. Therefore, in the present study, biopellets were produced by peanut and hazelnut shells and the higher and lower heating values (HHV and LHV) were determined and compared to the standards used in the European Union for biomass-produced pellets.

MATERIALS AND METHODS

Pelletization Process. The pellet has a small, cylindrical shape resembling animal feed. Biomass pellets are generally 6–12 mm in diameter and 10–30 mm in length. The process of bringing the material into a smaller size (about 30 mm) under pressure is called pelletizing. Pellets can be made of materials such as wood chips, wood chips, tree bark, agricultural products, grains, nuts, almonds, walnut shells, and even paper. Biological crops such as corn cobs, beet pulp, sunflower spices, dried olives, cherry seeds and soybean can also be used in the production of pellets. The pelletizing process increases the density of the material, reducing transportation, storage and transport costs, and ensures homogeneity in size and shape can be fed automatically to the combustion systems for thermal purposes, thus enabling more efficient use of the material. Agricultural wastes can be used singly or as a solid fuel by mixing. In this case, when the wastes with higher quality and higher thermal value are used together with wastes with lower thermal value, the thermal value of the mixture is increased. The quality of agricultural wastes, in terms of thermal value and ash content, are used as solid fuel in excess amount when they are mixed with agricultural wastes which are high in quantity and low in thermal value. Among the remaining wastes from field crop production, the thermal values of rice paddy and rice paddy are the lowest values (14.65 MJ/kg). The fact that the values of thermal values, humidity, ash and flue-gas emissions of agricultural wastes are better than lignite coal indicates that these wastes are sustainable solid fuel sources that can be used instead of fossil coals [8].

In this study, peanut and hazelnut shells were passed through the mill and made into small powder. Then, the powdered material was dried in the oven to reduce the humidity. The dried material was pelletized by pressing under high pressure. The pressed material was cooled and ready for use at the end of packaging. Roller presses are used in biomass pelletizing machines. Small press (approx. 30 mm) is used in the cylinder presses. For this reason, this type of press is also called pellet press. There are a number of molds arranged in thick steel discs or holes drilled on the ring. The material is forced into the molds by means of 2 or 3 cylinders. The process flow and main components for biomass pelletization is given in Fig. 1. Biomass pellet production consists of following processes; raw materials, screening, drying, cyclone separation, forming granulation, cooling, screening, finished products. At the same time, each part is equipped with strict quality control system to ensure the product quality.

Determination of Heating Values of Biopellets. The composition of hydrocarbon fuels is defined based on the basic elements except for moisture and inorganic components (ash). Elemental analysis is the process of determining the ash percentages of carbon (C), hydrogen (H2), nitrogen (N), sulphur (S), chlorine (Cl) and oxygen (O2) in the gaseous products and after the sample of organic material has completely burned. Elemental analysis devices are devices that determine the quantities of these elements in the structure of the material. Elemental analysis equipment works according to the combustion principle. When a sample material known mass is given to it, it burns it and keeps the gases that are released in the C, N and H2, sometimes in the columns containing S and O2, and gives the ratio between the mass of the first given substance and the masses of the elements held in
the column as a percentage. The samples, which are gasified in the first stage, are sent to a chromatographic column with an inert gas. Here, the samples are burned with O\textsubscript{2} gas. Usually the carrier inert gas is helium (He). The gas mixture is sent to the oxidizing catalyst (CuO) zone in order to perform the quantitative combustion process. Then, in a reduction zone where copper (Cu) is present, C, H\textsubscript{2}, N and S are converted into CO\textsubscript{2}, H\textsubscript{2}O, N\textsubscript{2}, SO\textsubscript{2} gases, respectively. After the gases formed and separated are individually trapped in special holding columns, the amount of each of them is determined by means of a thermoconductive detector (TCD or thermal conductivity detector). An electric signal proportional to the amount of each gas is obtained. This electrical signal then gives the percentage of the elemental composition, for example, proportional to the area of the curve obtained in the spectrum.

The HHV and LHV of the biopellets made of peanut and hazelnut shells have been determined according to the test standards of the International Organization for Standardization (ISO) given in Table 1.

### Table 1: Standards of tests for biopellet characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Standards of tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash (%)</td>
<td>ISO 1171 [10]</td>
</tr>
</tbody>
</table>

**Ash.** The ash (%) of the pellets was determined according to the ISO 1171 standard [10]. This International Standard specifies a method for the determination of the ash of all solid mineral fuels. The test portion is heated in air at a specified rate up to a temperature of 815 °C ± 10 °C and maintained at this temperature until constant in mass. The ash is calculated from the mass of the residue after incineration. Two furnaces may be used, one capable of achieving an adequate zone at a uniform temperature of approximately 500 °C and the second capable of maintaining a temperature of 815 °C ± 10 °C. The biomass fuel used for the determination of ash is the general analysis test sample [10].

**Heating Values.** The HHV and LHV were determined according to the ISO 1928 standard [11]. This International Standard specifies a method for the determination of the higher heating value (HHV) of a solid mineral fuel at constant volume and at the reference temperature 25 °C in a bomb calorimeter calibrated by combustion of certified benzoic acid. The result obtained is the HHV of the analysis sample at constant volume with all the water of the combustion products as liquid water. In practice, fuel is burned at constant (atmospheric) pressure and the water is not condensed but is removed as vapour with the flue gases. Under these conditions, the operative heat of combustion is the lower heating value (LHV) at constant pressure.

**The Higher Heating Value (HHV).** A weighed portion of the analysis sample of the solid fuel is burned in high-pressure O\textsubscript{2} in a bomb calorimeter under specified conditions. The effective heat capacity of the calorimeter is determined in calibration experiments by combustion of certified benzoic acid under similar conditions, accounted for in the certificate. The corrected temperature rise is established from observations of temperature before, during and after the combustion reaction takes place. The duration and frequency of the temperature observations depend on the type of calorimeter used. Water is added to the bomb initially to give a saturated vapour phase prior to combustion, thereby allowing all the water formed, from the H\textsubscript{2} and moisture in the sample, to be regarded as liquid water. The HHV is calculated from the corrected temperature rise and the effective heat capacity of the calorimeter, with allowances made for contributions from ignition energy, combustion of the fuse(s) and for thermal effects from side reactions such as the formation of nitric acid. Furthermore, a correction is applied to account for the different the in energy between the aqueous sulfuric acid formed in the bomb reaction and gaseous Sulfur dioxide, i.e. the required reaction product of Sulfur in the fuel.

**The Lower Heating Value (LHV).** The LHV at constant volume and the LHV at constant pressure of the fuel are obtained by calculation from the HHV at constant volume determined on the analy-
sis sample. The calculation of the LHV at constant volume requires information about the moisture and H₂ contents of the analysis sample. In principle, the calculation of the LHV at constant pressure also requires information about the O₂ and nitrogen (N) contents of the sample.

Preparation of the Sample. The O₂, at a pressure high enough to fill the bomb to 3 MPa, pure with an assay of at least 99.5 %, and free from combustible matter. The solid fuel used for the determination of calorific value shall be the analysis sample ground to pass a test sieve with an aperture of 212 μm. The sample shall be well-mixed and in reasonable moisture equilibrium with the laboratory atmosphere. The moisture content shall either be determined on samples weighed within a few hours of the time that samples are weighed for the determination of calorific value, or the sample shall be kept in a small, effectively closed container until moisture analyses are performed, to allow appropriate corrections for moisture in the analysis sample.

Calorimetric Procedure. The calorimetric determination consists of two separate experiments, combustion of the calibrant (benzoic acid) and combustion of the fuel, both under specified conditions. The calorimetric procedure for the two types of experiment is essentially the same. In fact, the overall similarity is a requirement for proper cancellation of systematic errors caused, for example, by uncontrolled heat leaks not accounted for in the evaluation of the corrected temperature rise. The experiment consists of carrying out quantitatively a combustion reaction (in high-pressure O₂ in the bomb) to defined products of combustion and of measuring the change in temperature caused by the total bomb process. The temperature measurements required for the evaluation of the corrected temperature rise are made during a fore period, a main (= reaction) period. For the adiabatic type calorimeter, the fore and after periods need, in principle, be only as long as required to establish the initial (firing) and final temperatures, respectively.

RESULTS AND DISCUSSIONS

In order to compare the properties of biopolymers made of peanut and hazelnut shells in this study, the standards used in the European Union (EU) for pellets made of biomass material (EN 14961-2) [12] are given in Table 2.

The Higher and Lower Heating Values of Biopellets. The calorific value of a fuel is the quantity of heat produced by its combustion at constant pressure and under normal (standard) conditions (i.e. to 0 °C and under a pressure of 1013 mbar). The combustion process generates water vapor and certain techniques may be used to recover the quantity of heat contained in this water vapor by condensing it. Therefore, are there mainly two types of calorific values:

1) The Higher Heating Value (HHV): When 1 kg of a fuel is burnt, the heat obtained by the complete combustion after the products of the combustion are cooled down to room temperature (usually 15 °C) is called the higher heating value (HHV) of that fuel. The water of combustion is entirely condensed and that the heat contained in the water vapor is recovered. The HHV at constant volume is the absolute value of the specific energy of combustion, in joules, for unit mass of a solid fuel burned in O₂ in a calorimetric bomb under the conditions specified. The products of combustion are assumed to consist of gaseous O₂, N, CO₂ and SO₂, of liquid water (in equilibrium with its vapour) saturated with CO₂ under the conditions of the bomb reaction, and of solid ash, all at the reference temperature. The international reference temperature for thermochemistry of 25 °C is adopted as the reference temperature for calorific values [11].

2) The Lower Heating Value (LHV): When 1 kg of a fuel is completely burned and the products of combustions are not cooled down or the heat carried away the products of combustion is not recovered and the steam produced in this process is not condensed then the heat obtained is known as the lower heating value (LHV). The products of combustion contain the water vapor and that the heat in the water vapor is not recovered. The LHV is the energy that turns on as a result of burning the fuel. The LHV at constant volume is the absolute value of the specific energy of combustion, in joules, for unit mass of the fuel burned in O₂ under conditions of constant volume and such that all the water of the reaction products remains as water vapour (in a hypothetical state at 0.1 MPa), the other products being as for the gross calorific value, all at the reference temperature. The LHV at constant pressure is the absolute value of the specific heat (enthalpy) of combustion, in joules, for unit mass of the fuel burned in O₂ at constant pressure under such conditions that all the water of the reaction products remains as water vapour (at 0.1 MPa), the other products being as for the gross calorific value, all at the reference temperature [11].

The relation between the HHV and LHV is the amount of the LHV can be obtained by subtracting the amount heat carried away by the combustion products especially the heat carried away by the steam. The LHV is about 8–9 % lower than the HHV due to the condensation heat of the water vapor which is released as a result of combustion. The HHV of the fuels is also determined by the proximate analysis depending on the exact combustion state. The LHV is the amount of heat that is
### TABLE 2
The standards used in the European Union (EU) for biopellets [12]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ENplus-A1*</th>
<th>ENplus-A2**</th>
<th>EN-B***</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (mm)</td>
<td>6–8</td>
<td>6–8</td>
<td>6–8</td>
<td>EN 16127</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>3.15&lt;L&lt;40</td>
<td>3.15&lt;L&lt;40</td>
<td>3.15&lt;L&lt;40</td>
<td>EN 16127</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>&lt;0.7</td>
<td>&lt;1.5</td>
<td>&lt;3</td>
<td>EN 14775</td>
</tr>
<tr>
<td>Calorific value (MJ/kg)</td>
<td>16.5&lt;Q&lt;19</td>
<td>16.3&lt;Q&lt;19</td>
<td>16&lt;Q&lt;19</td>
<td>EN 14918</td>
</tr>
</tbody>
</table>

EN Plus A1*: Chemically treated wood residues
EN Plus A2**: Timber wastes, wood crusts from industrial activities, roots.
EN-B***: Forest, afforestation and other pure wood, wood processing industry waste and by-products, used wood residues

released when the H₂ gas is released into the gaseous state, and this value is used in heating applications. It can be calculated based on the HHV and H₂ ratio.

The heating value of the biomass is the amount of energy that is released at the end of full combustion. Compared to many fossil fuels, the calorific value of biomass, especially on a volume basis, is low. The reason for this is that the density is low and the O₂ content is high. As the H₂/C ratio increases, the effective calorific value of the fuel decreases. The calorific value of fresh plant biomass such as plant leaf is very low. The reason is that the H₂/C and O/C ratios are high. Biomass is the material with the highest O₂ content among all hydrocarbon fuels. O₂ does not provide a beneficial contribution to the calorific value, making it difficult for the biomass to turn into liquid fuels.

The HHV and LHV of the pellets produced peanut shell have been determined in three different bases, namely moisture and ash free (MAF) base, and dry base the original base. The pellet samples exclude total moisture and ash forming minerals in MAF base. On the other hand, the heating values of the pellets made of hazelnut shells have been determined in two different bases namely dry base and the original base. The HHV of the peanut and hazelnut biopellets in different bases are given in Fig. 2.

The HHV of the peanut shell pellets were determined as 21.01 MJ/kg, 20.13 MJ/kg and 18.05 MJ/kg in MAF base, dry base and the original base, respectively. The HHV of peanut shell pellets in MAF base was determined to be about 14.1% higher than that of the original base. Similarly, the HHV of peanut shell pellets in dry base was determined to be about 10.3% higher than that of the original base. The HHV of the hazelnut shell pellets were determined as 20.59 MJ/kg and 17.92 MJ/kg in dry base and the original base, respectively. The HHV of hazelnut shell pellets in dry base was determined to be about 2.2% higher than that of the peanut shell pellets.

The LHV of the peanut and hazelnut biopellets in different bases are given in Fig. 3. The LHV of biopellets made of peanut and hazelnut were determined as 18.93 MJ/kg and 19.39 MJ/kg in dry base, respectively. The LHV of hazelnut shell pellets in dry base was determined to be about 2.4% higher than that of the peanut shell pellets. In practice, the LHV is taken into account as the heating value of the fuels. In this case, it can be stated that the pellets made of hazelnut shells have a higher heating value than those made of peanut shells.
It has been reported that the pellet calorific value in the EU standard (EN 14918) must be within the range of 16–19 MJ/kg for biomass pellets (Table 2) [12]. The HHV and LHV of pellets made of peanut and hazelnut shells in all bases are suitable to the EN 14918 standard. It has been reported by Acar et al., [13] that the calorific value of 90% of the lignite coals in Turkey is below 12.56 MJ/kg. In this case, the LHV of peanut and hazelnut shell pellets are higher than the calorific value of lignite in Turkey. On the other hand, the LHV of peanut and hazelnut shell pellets is higher than that of industrial lignite (12.56 MJ/kg), central lignite (8.37 MJ/kg) and Elbistan lignite (4.60 MJ/kg) in Turkey, reported by Ozturk [14].

Ash. The ash remaining after coal or coke has been incinerated in air is derived from inorganic complexes present in the original coal substance and from associated mineral matter. Therefore, the result of the determination is “ash” and not “ash content” as coal does not contain any ash. The amount of sulfur retained in the ash is in part dependent on the conditions of ashing and, in order to obtain values for the ash on a comparable basis, it is necessary to adhere strictly to the conditions specified [10]. Ash is an inorganic residue that remains in the environment as a result of burning. Silica, aluminum, iron and calcium are the major inorganic constituents and may contain magnesium, titanium, sodium and potassium in small amounts. The presence of alkali metals, especially potassium and chlorine, is important, although the ash content of the biomass is usually small. These components lead to serious problems such as agglomeration, pollution and corrosion in gasifiers and boilers [15].

Ash is one of the most researched features of biomass. However, it is the least understood feature. During the combustion of biomass, natural as well as technological, inorganic, organic and liquid ash form simultaneously. The terms inorganic material, mineral matter and ash for biomass do not mean the same. Inorganic material includes solid crystalline, semi-crystalline and amorphous phases in the biomass. The actual mineral matter, as part of the inorganic matter, includes minerals belonging to the minerals and mineral groups in the biomass. Ash is an inorganic waste that is formed after combustion of biomass is completed and is the combination of the original and newly formed inorganic phases produced from the inorganic, organic and fluid components in the biomass. Combustion temperature significantly affects the total ash yield of biomass. When the composition and origins of biomass waste are not taken into account, only ash production is limited information on its own. For this reason, it needs to be interpreted in conjunction with the phenomena of biomass. Despite these limitations, although the ash yield of biomass can be measured routinely, the actual identification of inorganic constituents is a complex method and may not be determined quickly and routinely. The contents of the ash, volatile matter, Ca, Cl, H₂, K, Mg, Mn, O₂ and Si of the biomass or biomass crown are higher than those of the coal or coal ash, the contents are low.

The ash of biomass is generally lower than that of solid fossil fuels. The ash content of the biomass species at the temperature of 550–600 °C varies from 0.1% to 46% relative to the dry basis. The ash content of peat and coal varies (815 °C) to a somewhat narrower range of 4%[9]. It is desirable that the ash content in a good fuel is low. The ash
value of the pellets produced peanut and hazelnut shells are given in Fig. 4. The ash value of the peanut shell pellets were determined to be 4.19%, 4.05% and 3.76% in dry base, air dry base and original base, respectively according to ISO 1171 standard [10]. The ash value of the hazelnut shell pellets were 1.09% and 0.95%, in dry base, air dry base, respectively. The ash value of hazelnut shell pellets was lower than that of peanut shell pellets in dry and the original bases. The ash value of the pellets produced peanut shells was determined to be higher than the ash content (< 3%) specified in the EN 14775 standard (Table 2) [12].

CONCLUSION

Despite the fact that organic wastes are produced every year in all over the world, these wastes are not evaluated in any way and they are disposed of by being burned or disposed of in landfills. It is primarily necessary to evaluate such wastes and bring them to the national economy. One of the most important benefits that can be achieved in the medium and long term in case of widespread application of such practices is to enable the establishment of agricultural based industry which is one of the most important elements of rural development by evaluating the wastes generated as a result of the production of some agricultural products intensively cultivated in regions as biofuels, to provide employment to people living. In the case of establishing pellet production facilities in rural areas from agricultural wastes, it will provide a useful service for rural employment and rural development.

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CONCENTRATION OF OCHRATOXIN A AS A TOXIC XENOBIOTIC IN MATERNAL BREAST MILK FROM TURKEY

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ABSTRACT

Breast milk is very important nutrition for infants in the first six months of human life. However, it may be contaminated with naturally occurring mycotoxins such as ochratoxins. This study was carried out to detect the Ochratoxin A (OTA) levels in maternal breast milk samples collected from 92 healthy lactating mothers from a rural area. Maternal breast milk samples were directly analyzed to detect the level of OTA using by a quantitative enzyme-linked immunosorbent assay (ELISA) method. A semi-quantitative questionnaire was used to determine the dealing with farming or ranching, focused on probable OTA contamination. OTA was detected in 21 of 92 (22.8%) maternal breast milk samples in the range of 10-100 ng/L. According to the European Union Commission Standard, 6 (6.5%) positive maternal breast milk samples indicated higher than the maximum limit of 40 ng/L for OTA. OTA concentrations were significantly correlated with the ranching but there wasn’t any significant correlation noticed among the lactating mothers who were interrelated in farming. The results highlight the exposure of infants and lactating mothers to OTA shows the consumption of foods. It is recommended for more regular study and strategies to reduce OTA exposure in the food chain and maternal breast milk.

KEYWORDS: Maternal breast milk, OTA, Lactating mothers, Ranching, Farming

INTRODUCTION

Maternal breast milk provides complete nutrition for infants which treats infants’ health, immunity and growth. World Health Organization declared that throughout the early six months of lifetime infants should only be fed with breast milk [1]. It may be contaminated with naturally occurring mycotoxins such as ochratoxins. Ochratoxins are secondary metabolites produced by Aspergillus and Penicillium species during the storage of cereals, and other substrates [2]. Ochratoxin A (OTA) was first defined in 1965 by the South African chemists and isolated by D.B. Scott in sorghum grain from Aspergillus ochraceus K-804 strain [3]. The most important species are known as; A. melleus, A. aliciaeus, A. sclerotiorum, A. ostianus, A. sulphureus, P. chrysogenum, P. cyclopium, P. palitans, P. verruculosurtun, P. variable, P. purpurescens, A. petrakii, and P. commune [4]. In tropical countries, OTA is produced generally by Aspergillus species, especially the widespread A. ochraceus, but in temperate climate countries, the main producer of OTA is P. verrucosum [5].

Ochratoxins are pentaketides which made up of dihydro-isocoumarin connected to β-phenylalanine. The most toxic ochratoxin in foods is Ochratoxin A and only the other ochratoxin found in food is Ochratoxin B which is rarely less toxic. OTA is a comparatively heat-stable toxin and survives majority cooking process to some extent, in spite of the reduction in concentration while heating rest on factors such as pH, temperature, moisture and the other components in the food [6]. Ochratoxins are carcinogenic, nephrotoxic, teratogenic, hepatotoxic, genotoxic and immunosuppressive agents that are suspected that the main agent of Balkan endemic nephropathy a chronic kidney disease with tumors in the renal system [3]. Ochratoxin A is a Group 2 carcinogen and has the longest elimination half-life of the other mycotoxins in the classification of International Agency for Research on Cancer [7]. The incidence of Ochratoxin A in maternal breast milk samples was reported from several countries: Turkey [8], Iran [9], Italy [10], Sierra Leone [11]. These studies show significant regional differences. Ochratoxin A had been found in samples, generally in the cool temperate countries of the Northern hemisphere [12]. The reason may be the dietary habit, climate, and humidity.

As the presence of Ochratoxin A in maternal breast milk is undesirable, the aim of this study was to determine the occurrence and the levels of Ochratoxin A in maternal breast milk samples from Şanlıurfa, the city with the highest birth rate in Turkey. The probable correlation between the
Ochratoxin A levels in positive samples and potential risk factors such as age and type of occupation.

MATERIALS AND METHODS

Sampling methods and materials. In the present study, 92 maternal breast milk samples were obtained from houses of healthy lactating mothers located in Şanlıurfa, Turkey. The samples were investigated for the presence and the levels of OTA. The joining criteria were being a healthy lactating mother and exclusively breastfeeding an infant between 1-260 day old. Lactating mothers were briefed about the study purpose. Written notification consents were signed by participants and 10 mL of maternal breast milk samples were collected by self-expression into the sterile glass tubes. Maternal breast milk samples were put inside an icebox, transferred to the laboratory and kept at -20°C until analysis. The study was approved by the Ethics Committee of the Harran University Medicine Faculty Hospital (Approval number: 15/10/19). Lactating mothers’ socio-demographic information such as occupational status, maternal age, infants age were asked by a semi-quantitative questionnaire focused on probable OTA contamination.

Method validation. Validation of the method was verified to ensure the analysis quality prior to analysis of the maternal breast milk samples by detection of the mean variation coefficient, limit of detection (LOD) and recoveries for spiked sample by the calibration curve, which was acquired using seven standard solutions with different concentrations of OTA (0, 5, 10, 20, 50, 100 and 200 ng/L). The mean coefficient variation, LOD and recovery rate of the method were approximately 13%, 0.01 ng/L and 97% for OTA, respectively.

ELISA procedure. OTA levels were detected in maternal breast milk by a quantitative enzyme-linked immunosorbent assay (ELISA) method (Helica Company, Santa Ana, California, USA CAT NO:991OCH01MS-96). The quantitative analysis of OTA in maternal breast milk samples was carried out according to the kit procedure. ELISA kit contains a microtiter plate (12 strips, every 8 wells), precoated with antibodies directed against OTA. According to the kit instructions, 250 µL of milk samples were diluted by 750 µL absolute methanol ratio at 1:4, mixed vigorously and stood at ambient temperature for 5 minutes. The diluted maternal breast milk samples were centrifuged at 3000xg in 5°C for 10 minutes. The supernatants were used to detect the OTA concentration of in the samples. Six standard solution and maternal breast milk samples were dispensed into the bottom of each well and incubated at room temperature for 30 minutes. Than decanted the wells, washed for a total of three washes after each incubation session. Conjugate was added to each antibody coated well and incubated for 30 minutes. After incubation the washing step done again. The required volume of substrate reagent (100 µL per well) added to each well, covered avoid from light and incubated for 10 minutes. After incubation stop solution dispensed to the wells. All steps were done according to the kit instructions. The optical density of milk samples was measured with a microtiter plate reader using 450 nm filter. Finally, the calibration curve was used to detect the OTA concentration in accordance with the milk samples absorption rate.

Statistical analysis. The descriptive statistical analysis was carried out with Minitab version 17 software. Parameters were evaluated by Pearson R Correlation Tests. Level of P<0.05 was accepted statistically significant. Mann-Whitney U Test was performed to estimate the correlation between OTA levels with mother’s age, infant age, farming, and ranching.

RESULTS AND DISCUSSION

Lactating mothers’ and infants’ descriptive data are shown in Table 1. The average age of lactating mothers was 28.5±6.8 years in a range of 18-41 years. The average age of infants was 148±23.5 days in a range of 1-260 days. There was no significant difference between the mothers’ age, ranching, and farming (P<0.05). Among the examined maternal breast milk samples of lactating mothers, 21 out of 92 samples (23%) had mean OTA of 25.42±4.07 with the range of 10-100 ng/L (Table 2).

### Table 1
Descriptive data of lactating mothers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (n)</th>
<th>Percentage (%)</th>
<th>Min</th>
<th>Max</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of infant (day)</td>
<td>92</td>
<td>100</td>
<td>1</td>
<td>260</td>
<td>148±23.5</td>
</tr>
<tr>
<td>Age of mother (year)</td>
<td>92</td>
<td>100</td>
<td>18</td>
<td>41</td>
<td>28.5±6.8</td>
</tr>
<tr>
<td>Ranching</td>
<td>52</td>
<td>56.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farming</td>
<td>40</td>
<td>43.5</td>
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<td></td>
</tr>
</tbody>
</table>
52 (56.52%) and 40 (43.48%) mothers were involved in ranching and farming respectively. Mann-Whitney U nonparametric test results demonstrated that OTA concentrations were significantly correlated with the ranching (P=0.041) (Table 2). The Mann-Whitney U nonparametric test results indicated that there isn’t any significant correlation among OTA concentration and mothers’ age (P>0.05). Concerning OTA concentration, there wasn’t any significant correlation noticed among the lactating mothers who were interrelated in farming (P>0.05). Furthermore, there was no significant correlation was among the OTA concentration and infants’ age (P>0.05). Frequency distribution of OTA in positive maternal breast milk samples also investigated and especially, 15 out of 21 positive samples showed 10-49 ng/L concentration (Table 3).

Healthful properties of maternal breast milk are unique for infants for this reason purity of maternal breast milk is essential for the earlier period of life [13]. Investigation of OTA levels in maternal breast milk provides the level of exposure information in both infant and mother. The JEFCA (1996) has established a tolerable daily intake of 14 ng/kg bw/day [14] and EFSA (2006) proposed a tolerable daily intake of 17 ng/kg bw/day for adults [15]. In general OTA production in the foodstuffs and agricultural products depends on environmental factors like temperature and humidity. Geographical variations of OTA contaminations in maternal breast milk samples may be due to not only dietary habits but also climatic regional diversity in exposure to fungal spores by inhalation [16].

OTA was detectable in 23% of 92 maternal breast milk samples from lactating mothers living in rural area of Şanlıurfa province. Mean OTA level in maternal breast milk samples from this study was 5.8 ng/L. Data for maternal breast milk levels reported from previous studies in several countries (Table 4) revealed that infants’ intake of OTA is varied quite. The present study is the first report on the OTA levels in maternal breast milk samples in Şanlıurfa, Turkey. According to our literature survey, we noticed two studies in Turkey that they reported 100% of 75 breast milk samples had OTA levels between 620.87–13111.30 ng/L and 48.6% of 70 breast milk samples had OTA with a mean level of 140±30 ng/L in Ankara province [8, 17]. Such high findings on OTA prevalence and levels in maternal breast milk samples from Turkey point out differences in dietary OTA exposure of lactating mothers, probably depending on dietary habits, region/place of residence, and sampling time (year), but the results may also have been influenced that lactating mothers living in Şanlıurfa prepare their own winter food under the abundant sunlight. Compared with Egypt, where 75.8% of milk samples had OTA levels in the range of 5.07-45.01 ng/kg, these OTA levels are higher but the percentage is lower than their results [18]. In addition, in Poland, it was reported that 38% positive maternal breast milk samples had 5.8-17 ng/kg OTA range [19] and in Slovakia, 30% positive of maternal breast milk samples contained OTA ranging from 2.3-60.3 ng/kg [20]. Recently Muñoz et al. (2014) reported that OTA was detected in 80% of maternal breast milk samples ranging from 44 to 184 ng/kg [21]. There have been studies on OTA levels in maternal breast milk are low. For example results from Brazil where 4% of maternal milk samples levels were in the range of 11-24 ng/kg [22], from Sari-Iran where 1.47% of maternal breast milk samples had OTA levels in the range of 90-140 ng/kg [13], from Italy where 20 % of maternal breast milk samples had OTA levels in the range of 0.1-12 ng/kg [23] and, from Norway where 21% positive of maternal breast milk samples had OTA levels in the range of 10-182 ng/kg [24]. OTA levels in Sierra Leone were detected 35% positive in 113 milk samples with a range of 200-337 ng/kg where authors signed the exposure of infants is far exceed permissible in developed country’s animal feeds [11]. OTA levels of the present study are far exceeded those recorded in Fars, Iran with the range 1.6-60 ng/kg in 96.6% positive samples [9].

### Table 2

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<th>Positive n (%)</th>
<th>Range (ng/L)</th>
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<td>Mean±SD</td>
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<td>0–100</td>
<td>16.35±7.17</td>
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<tr>
<td>Farming</td>
<td>40</td>
<td>4</td>
<td>0–50</td>
<td>8.8±0.4</td>
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<tr>
<td>Total</td>
<td>92</td>
<td>21</td>
<td>0–100</td>
<td>25.42±4.07</td>
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### Table 3

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<th>10-49 ng/L</th>
<th>50-99 ng/L</th>
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TABLE 4
OTA concentrations of maternal breast milk in some countries worldwide

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<th>Country</th>
<th>Sample collecting year</th>
<th>Positive n (%)</th>
<th>Total</th>
<th>The range of positive samples (ng/L)</th>
<th>Reference</th>
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<td>Italy</td>
<td>1995</td>
<td>22(20%)</td>
<td>111</td>
<td>1-12</td>
<td>Micco et al. [23]</td>
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<tr>
<td>Sierra Leone</td>
<td>1995</td>
<td>40(35%)</td>
<td>113</td>
<td>200-337</td>
<td>Jonsyn et al. [11]</td>
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<tr>
<td>Norway</td>
<td>1998</td>
<td>38(33%)</td>
<td>115</td>
<td>10-130</td>
<td>Skaug et al. [24]</td>
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<tr>
<td>Norway</td>
<td>2001</td>
<td>17(21%)</td>
<td>80</td>
<td>10-182</td>
<td>Skaug et al. [29]</td>
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<tr>
<td>Egypt</td>
<td>2002</td>
<td>43(36%)</td>
<td>120</td>
<td>5.07-45.01</td>
<td>El-Sayed et al. [18]</td>
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<tr>
<td>Egypt</td>
<td>2000</td>
<td>3(30%)</td>
<td>10</td>
<td>-</td>
<td>El-Sayed et al. [28]</td>
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<tr>
<td>Brazil</td>
<td>2005</td>
<td>2(4%)</td>
<td>50</td>
<td>11-24</td>
<td>Navas et al. [22]</td>
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<td>Egypt</td>
<td>2005</td>
<td>26(72%)</td>
<td>50</td>
<td>-</td>
<td>Hassan et al. [27]</td>
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<td>Poland</td>
<td>2006</td>
<td>5(38%)</td>
<td>13</td>
<td>5.17</td>
<td>Postupolski et. [19]</td>
</tr>
<tr>
<td>Slovakia</td>
<td>2008</td>
<td>23(30%)</td>
<td>76</td>
<td>2.3- 60.3</td>
<td>Dostal et al. [20]</td>
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<tr>
<td>Italy</td>
<td>2008</td>
<td>61(74%)</td>
<td>82</td>
<td>5-405</td>
<td>Galvano et al. [10]</td>
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<td>Ankara/Turkey</td>
<td>2010</td>
<td>75(100%)</td>
<td>75</td>
<td>0.6-13.1</td>
<td>Gürbay et al. [8]</td>
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<tr>
<td>Sari/Iran</td>
<td>2013</td>
<td>5(3.7%)</td>
<td>136</td>
<td>5-16.4</td>
<td>Afshar et al. [13]</td>
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<td>Brazil</td>
<td>2012</td>
<td>66(66%)</td>
<td>100</td>
<td>0.8-21</td>
<td>Iha et al. [30]</td>
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<td>Fars/Iran</td>
<td>2014</td>
<td>84(96.6%)</td>
<td>87</td>
<td>7.6-60</td>
<td>Deghan et al. [9]</td>
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<td>Germany</td>
<td>2013</td>
<td>55(90%)</td>
<td>90</td>
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<td>Chile</td>
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<td>40(80%)</td>
<td>50</td>
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<td>34(48.6%)</td>
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<td>Şanlıurfa/Turkey</td>
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<td>21(23%)</td>
<td>92</td>
<td>10-100</td>
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The present study is the first study concerning the presence and the levels of OTA in maternal breast milk samples of lactating mothers in Şanlıurfa province which has the highest birth rate in Turkey. In conclusion, it appears that environmental factors are the main causes for high levels of OTA in maternal breast milk so extensive regular programs should be improved in order to determine and control ochratoxins in not only humans but also foods, therefore, the level of ochratoxins could be minimized and its side effects could be inhibited. Also, furthermore investigations in the world are proposed so that identify humans exposure to ochratoxins. Finally, the present study could be useful to improve regular dietary educational programs pointed at mothers during pregnancy and lactation to minimizing OTA intake by infants. To establish the future pathological effects for human health; more controlled studies are warranted. Comprehensive control programs should be improved to regularly determine and control this toxin in maternal breast milk and food chain so that the quantity of OTA will be decreased and its effects will be avoided.

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