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SILVICULTURAL AND AMELIORATIVE MEASURES IN THE SPECIAL PURPOSE FOREST IN THE SUBURBAN ZONE OF THE CITY OF BELGRADE, SERBIA

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ABSTRACT

The paper presents the findings of a research study into environmental conditions, the state and the quality of a mixed stand of Hungarian oak and Turkey oak (Quercetum frainetto-cerridis Rudski 1945) belonging to the Lipovica forest complex in the area of Belgrade - Serbia. This is a 65-70 years old even-aged coppice stand. According to its position and its functions in the suburban zone of the city, the stand is classified as a special purpose forest. Regarding the orographic conditions, it has old and developed topography on the southern periphery of the Pannonian Basin, the easterly aspect and the mild slope inclination (8-10°). The soil is classified as lessive brown forest soil. The climate has features of the moist subhumid (Cf) climate type. According to the site conditions and the stand phytosociology, it can be typologically classified as a Hungarian oak and Turkey oak forest (Quercetum frainetto-cerridis typicum) on lessive brown soil. The average number of trees is 740 trees per hectare and the average wood volume is 277.8 m³ ha⁻¹. Hungarian oak accounts for 60.1% of trees in the stand mixture and 73.5% of the wood volume. Turkey oak participates with 13.5% in the number of trees and 25.1% in the wood volume. The mean stand diameter is 20.8 cm, with the mean Turkey oak diameter being by 5.1 cm larger than the mean Hungarian oak diameter. The results of the study indicate that selective thinning of low intensity should be proposed. The proposed silvicultural measures are shown using the methods of simulation and visualization. Taking into consideration the age of the stand, the results of the research and the special purpose character of the forest complex, regeneration measures to be applied should include stand conversion with the longest possible regeneration period.

KEYWORDS:
Special purpose forests, Hungarian oak and Turkey oak forests, stand state, visualization of silvicultural operations

INTRODUCTION

The importance of urban and suburban forests is reflected in a number of public functions and benefits that directly improve the quality of city environment [1-5]. These forest complexes belong to the category of special purpose forests and the sub-category of forests of special importance [6]. Their structural characteristics are a good indicator of ecosystem services and goods they provide [7-10]. The complexities of the modern management of special purpose forests in Serbia and its wider region have been shaped by historical circumstanc-ces and profound impacts of harmful human factors [11-13].

All these factors have contributed to the high share of coppice, degraded, devastated, unattended and privately owned forests. Obvious differences in the productivity of coppice and seed forests are, on an annual basis, reflected not only in the significant wood volume loss of coppice stands but also in the significantly reduced bio-ecological stability and lower functional values of these forests.

In Serbia, a significant portion of special purpose forests occurs within the oak belt, i.e. in the forest community of Hungarian oak and Turkey oak. The total area of these forests in Serbia amounts to 504800 ha or 22.41%. These forests are largely located around large or small urban areas or they provide certain public benefits such as protection and regulation, health, tourism and recreation, science and research, military, cultural or historical benefits, etc. The forests of Hungarian and Turkey oak build climax communities, which are in Serbia in the center of the area of distribution. Therefore this type of forest community is a ‘socioecological synonym’ of the country [14]. In that sense, when we talk about the forests of Hungarian oak and Turkey oak, we also present important socioecological characteristics of a large part of Serbia.

So far, only small-scale research has been conducted in these forests because they are mainly privately-owned and their state is mostly unsatisfactory regarding preservation, quality and vitality. It all stresses the actuality and importance of studying silvicultural and ameliorative measures and silvi-cultural problems of the forests that are found
within suburban and urban zones.

The growing stock of the city of Belgrade is characterized by a high share of stands belonging to the xerothermophilic complex of Hungarian oak-Turkey oak and other forest types. The area of state-owned mixed forests of Hungarian oak and Turkey oak and pure Turkey oak forests is 2968.47 ha or about 22% of the total forested area. [15]. Given the position of these forests within the urban and suburban zone of the city, they are classified as special purpose forests. These are mostly coppice-originated forests.

The Lipovica Forest Complex is part of the south-western suburban zone of the city of Belgrade. According to its most important values, it is classified as a special purpose forest. The largest portion of this complex is the site of Hungarian oak and Turkey oak forest community. Therefore, the stands of Hungarian oak and Turkey oak within the Lipovica Forest are truly representative of the living conditions not only of the study area but also of the significant portion of the wider suburban zone of the city of Belgrade. These are coppice-originated, even-aged stands often with an unfaavourable composition in the mixture of the main tree species reaching the end of the rotation [16]. In these stands, Turkey oak proved to be ecologically dominant species, i.e. comparatively stronger than Hungarian oak which was pushed out or reduced to a minimum as an edifying species. Only a small part of the stands in this complex comprise coppice forests of Hungarian oak and Turkey oak in which Hungarian oak dominates, i.e., has a significant share both in the number of trees and in the wood volume.

Taking into account the above, the paper proposes the following hypotheses:

- the present state of the investigated stands within the Lipovica forest complex is not satisfactory and it doesn’t meet the requirements for the sustainable provision of complex public benefits and specific purposes;
- the state of these forests could be significantly improved by applying adequate silvicultural and ameliorative measures, with the main goal of converting the coppice stands into high forests, i.e., achieving the dominant seed origin of the next generation of trees.

**MATERIALS AND METHODS**

**Study area.** The research was carried out in the suburban zone of the city of Belgrade, within the Lipovica forest complex in the period from 2009 to 2016 (Figure 1). The forest complex covers 1234.04 ha, with the mixed forests of Hungarian oak and Turkey oak occupying 613.89 ha. Field data were collected in the permanent sample plots established for that purpose. A series of experimental areas consisting of 4 sample plots was established in a mixed stand of Hungarian oak and Tur-
key oak, compartment 42. A plot covered an area of 5 acres. The stand was selected on the criterion of uniformity of site and stand conditions within the series.

**Research of ecological conditions.** We conducted a detailed spatial analysis of the data related to basic environmental conditions. Geoinformation technology (GIS) was applied to map the topography of the Lipovica forest complex. The map is in a 1:25000 scale in the form of a satellite image taken from Google Earth and referenced in the UTM coordinate system, using the ArcMap 10 software. Furthermore, the soil profile was opened in the sample areas and the soil analysis was conducted in the soil laboratory of the Faculty of Forestry, University of Belgrade. It included the study of soil physical and chemical properties, structure and classification. Climate data processing was performed on the basis of data obtained from the Košutnjak Meteorological Station of the Republic Hydrometeorological Service of Serbia, using the indirect method for determining the water balance and climate - the Thornthwaite method [17]. The following pedoclimatic parameters were determined: humidity index, aridity index, climate index, and classification of the investigated area by climate type and subtype for the period from 1990 to 2009. The most important vegetation characteristics were studied. The stand type was determined according to the classification system applied in Serbia [18].

**The study of the stand state.** The state and the structure of the stand were studied using the biometric analysis by the standard methodology of the Department of Silviculture of the Faculty of Forestry, University of Belgrade. Each sample plot had its outer boundaries determined and recorded using a compass theodolite in order to calculate the surface area. All trees were marked with numbers and the points where two breast diameters are to be measured were marked. Cross diameters at breast height were measured with an accuracy of up to 0.1 mm and the mean diameter of each tree was calculated. The height of all trees was measured using the Siamto clinometer, with an accuracy of up to 0.1 m. Data processing needed for the purposes of biometric analysis was performed on the basis of tree distribution into 5 cm diameter degrees (with the means of 5.0, 10.0, 15.0 cm, etc.). The measured heights were then used to construct height curves which were fitted using the Prodan’s equation:

\[ h = \frac{d^2}{a+bd+cd^2} + 1.30 \]  

(1)

where \( h \) is height and \( d \) is diameter at breast height.

Volume was calculated using Trifunović’s volume tables for coppice forests [19, 20]. Distribution of the number of trees and wood volume per ha is graphically presented by diameter degrees.

**Statistical data processing.** The basic data on the state of the investigated stand are presented using descriptive statistics. The analysis of variance and Duncan’s test were performed in order to test the hypothesis on the homogeneity of the experiment as a whole (stand homogeneity according to the number of trees and wood volume per ha) and to determine the set of means of individual treatments. *Statistica* 6 software was used for the processing of data.

**The stand quality.** The quality of the stand was determined according to the percentage of trees of certain morphological, biological and technical characteristics, with the aim of determining the phenotypic quality of trees and the degree of stand degradation. Crown classes were determined as well as the stem and the crown quality. A three-level classification was applied. All trees were classified as dominant, codominant or suppressed. The following parameters were used to determine the stem quality: stem straightness and stiffness, forging, mechanical injury, health state and clearness. In determining the stem quality, a three-degree classification was applied – Rupert’s modified classification [21]. The following parameters were used to determine the quality of the crown: length, branch diameter, crown spread and form, mechanical injury and diseases. Based on the analysis of the quality of the stand and the degree of degradation, the stand was classified into a particular group of (good, medium, bad and very bad) stands. In order to determine regeneration measures, we singled out final crop trees (future trees) and they were permanently marked. The following step was to mark trees for felling according to the current silvicultural needs. The data on the quality of the stand are presented in relative amounts and systematized by categories of crown classes, stem quality and crown quality.

**Silvicultural and ameliorative measures.** The average data on the stand state, the selected final crop trees and the marked trees are shown in the table. The proposal of silvicultural and ameliorative measures is accompanied with visual representations of the stand before and after the performed silvicultural treatments [22]. The model of the proposed silvicultural measures was developed using visualization and simulation methods in the SVS (*Stand Visualization System*) software.
RESULTS

Ecological and vegetation characteristics of the stand. The stand orographic conditions are presented on Figure 2. The stand is at 170-180 m a.s.l. It has a mild slope of terrain (8-10°) and eastern aspect.

The bedrock consists of carbonate sandsoils with the compact structured quartz.

Based on the analysis of climate characteristics, the calculated elements of the water balance indicate that the investigated area has the moist subhumid (C2) climate. The humidity index ($I_h$) is 13.37, the aridity index ($I_a$) amounts to 19.54 and the climate index ($I_c$) to 2.64. The Climate Index ($I_c$) shows large fluctuations, from 41.06, during 1999 (moderate humid climate – B type), to -49.76, during 2000 (arid climate - E type). The water balance shows that the observed period had the average annual loss of moisture from the soil through evapotranspiration of 608 mm, which means that only 109 mm remained in the soil. Excess moisture occurred in the period from January to April, while in the period from July to September there was a deficit of moisture in the total average amount of 148 mm, which was unfavorable for vegetation. Finally, the analysis of water balance shows that the average temperature and the average amount of rainfall were favorable in the analyzed period. This is indicated by the ratio of actual ($E_r$) to potential evapotranspiration ($E_p$), where $E_p$ is greater than $E_r$ by 19%. The study area has a transitional climate type resulting from the interaction of the subatlantic climate from the west, the sharp continental from
the east and the mild submediterranean from the south. The water balance points to the high humidity of the climate, which means there are sufficient amounts of moisture available to the forest communities throughout the year.

The soil is lessivized, 81-120 cm deep, dry and classified as lessive brown forest soil. The structure of the soil profile is: Olf-AE-E-B-C. The transformation of organic matter is quite good. Humus-accumulating horizon is about 4 cm thick, dark grey in color and fine-grained. A horizon is at a depth of 4 to 14 cm and has marked eluvial zones. This horizon is brownish gray and lighter in colour than the topsoil. It has fine-grained but very unstable aggregates. The eluvial horizon is at a depth of 14 to 32 cm, light brown in color, silty, with light mechanical composition and undefined structure. Illuvial horizon is developed at a depth of 32 to 60 cm. It is brown in color and has a heavy mechanical composition. According to the pH values in water, the soil ranges from highly acidic to slightly acidic. Lessive brown forest soil types are typical of the forests of Hungarian oak and Turkey oak.

The stand is phytosociologically defined as Quercetum frainetto-cerridis Rudski 1945. Based on the study of ecological conditions, the stand is typologically classified as a Hungarian oak and Turkey oak forest (Quercetum frainetto-cerridis typicum) on lessive brown soil. The stand is coppice originated, even-aged and 65-70 years old. The canopy closure is complete (0.7). It has a mixed stand composition. The upper storey is composed of Hungarian oak and Turkey oak trees, while the lower storey includes flowering ash, wild cherry, wild service tree, hornbeam, elm and field maple. The forest litterfall is medium-sized, with a favorable process of humification. Ground vegetation is medium dense. The health state of the stand is good.

![Figure 3](image-url)

**FIGURE 3**

Distribution of the number of trees by diameter degrees

![Figure 4](image-url)

**FIGURE 4**

Distribution of the wood volume by diameter degrees
Stand state. The investigated stand is representative of the mixed stands of Hungarian oak and Turkey oak in the Lipovica forest complex. Hungarian oak is dominant with the share above 50% both in the number of trees and in the wood volume. The average number of trees is 740 trees per hectare, with an average share of Hungarian oak of 445 trees per hectare or 60.1%, while Turkey oak accounts for 100 trees per hectare or 13.5%. The average share of other species is 195 trees per hectare or 26.4%. All trees are distributed in diameter degrees of 5-35 cm, with the maximum percentage of trees in the diameter degree of 25 cm (37.1%). The average stand diameter is 20.8 cm, with Turkey oak having the mean diameter of 28.0 cm and Hungarian oak of 22.9 cm. The average stand diameter of the trees of other species is 6.8 cm. This confirms the above-stated statement that although it has a significantly smaller share, Turkey oak attains larger dimensions than Hungarian oak. The average height is 21.8 m. The average height of Hungarian oak is 22.9 m, while Turkey oak attains the height of 28.0 m. The average height of other tree species is 8.1 m. The average wood volume is 277.8 m³·ha⁻¹, with Turkey oak accounting for 69.9 m³·ha⁻¹ or 25.1% and Hungarian oak for 204.2 m³·ha⁻¹ or 73.5%. The wood volume of other tree species is only 3.8 m³·ha⁻³ or 1.4%. The current volume increment is, on average, 5.48 m³·ha⁻¹, with the average share of Turkey oak amounting to 24.5% or 1.67 m³·ha⁻¹. The share of Hungarian oak averagely amounts to 73.9%, or of 3.79 m³·ha⁻¹. The share of other species is 1.6%, with an average value of 0.02 m³·ha⁻¹. The percentage of increment amounts to an average of 1.97%, while it is 2.39% for Turkey oak, 1.85% for Hungarian oak and 0.52% for other species. The line of tree distribution by diameter degrees (Figure 3) for the whole stand shows that Hungarian oak trees have a clearly expressed culmination in the diameter degrees of 25 cm and 30 cm. A slightly larger number of trees in smaller diameter degrees belong to the trees of other species that build the lower storey of the stand. Turkey oak, which is less represented, builds the upper storey of the stand and the largest number of Turkey oak trees are distributed in the 30 cm diameter degree (35%). However, it can be concluded that Turkey oak hasn’t significantly influenced the structure of the whole stand characterized by the binomial distribution of the number of trees for both species (Turkey oak and Hungarian oak), which is a typical feature of even-aged forests. The line of wood volume distribution (Figure 4) by diameter degrees clearly confirms this statement. The distribution of wood volume by diameter degrees is basically structured by Hungarian oak with a clearly expressed peak in the diameter degree of 25 cm and a share of 44.9%.

Table 1 shows the main results of descriptive statistics for the number of trees and the wood volume per hectare. As expected, the coefficients of variation in the number of trees per ha of Turkey oak and wood volume per ha of Turkey oak and Hungarian oak, respectively, are greater than the coefficient of variation in the number of trees per ha of all species of trees together. However, it can be noted that the coefficient of variation in the number of Hungarian oak trees per ha is lower than the coefficient of variation in the number of trees of all species, which indicate approximately the same proportion of Hungarian oak trees on all sample plots.

The standardized coefficients of distribution of the number of trees and wood volume (coefficient of asymmetry, α₃ and coefficient of curvature, α₄) normally range from -2.0 to 2.0 [23].

According to the presented values of these coefficients for the number of trees and the wood volume of all tree species and individually for Turkey oak and Hungarian oak, it can be concluded that these distributions deviate slightly from the normal distributions.

The analysis of variance shows that the differences in the mean diameters of trees of all species in all sample plots are generally statistically significant at a probability of 95%, given that the value F=3.18 (p=0.0259). There are no statistically significant differences in the mean diameters between Turkey oak and Hungarian oak trees of individual sample plots at a probability of 95%, given that the

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>Σ</th>
<th>Turkey oak</th>
<th>Hungarian oak</th>
<th>Other species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sample plots</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td>740</td>
<td>277.84</td>
<td>100</td>
<td>69.87</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>154.06</td>
<td>16.22</td>
<td>56.57</td>
<td>44.04</td>
</tr>
<tr>
<td>Coefficient of variation [%]</td>
<td>20.82</td>
<td>5.84</td>
<td>56.57</td>
<td>63.03</td>
</tr>
<tr>
<td>Minimum</td>
<td>600.0</td>
<td>255.42</td>
<td>60.0</td>
<td>34.43</td>
</tr>
<tr>
<td>Maximum</td>
<td>960.0</td>
<td>291.77</td>
<td>180.0</td>
<td>133.64</td>
</tr>
<tr>
<td>Variation range</td>
<td>360.0</td>
<td>36.3</td>
<td>120.0</td>
<td>99.21</td>
</tr>
<tr>
<td>Standardized coefficient of variation (α₃)</td>
<td>1.42</td>
<td>-1.20</td>
<td>1.41</td>
<td>1.60</td>
</tr>
<tr>
<td>Standardized coefficient of curvature (α₄)</td>
<td>2.67</td>
<td>0.90</td>
<td>1.50</td>
<td>2.73</td>
</tr>
</tbody>
</table>

Legend: N - number of trees; V - volume
values of F are F=0.76 (p=0.5187) for Hungarian oak F=0.22 (p=0.8801) for Turkey oak.

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tree quality</strong></td>
</tr>
<tr>
<td><strong>%</strong></td>
</tr>
<tr>
<td>Turkey oak</td>
</tr>
<tr>
<td>1 - good</td>
</tr>
<tr>
<td>2 - medium</td>
</tr>
<tr>
<td>3 - bad</td>
</tr>
<tr>
<td>Hungarian oak</td>
</tr>
<tr>
<td>1 - good</td>
</tr>
<tr>
<td>2 - medium</td>
</tr>
<tr>
<td>3 - bad</td>
</tr>
</tbody>
</table>

The results of Duncan’s test regarding the mean tree diameters of all tree species together confirm the previously conducted analysis of variance and point to statistically significant differences between sample plots 1 and 4 and sample plots 2 and 4. The results of Duncan’s test regarding the mean diameters of Hungarian oak and Turkey oak also confirm the previously conducted analysis of variance for these tree species and point to the homogeneity of sample plots. The statistical analysis of the stand state in mixed forests of Hungarian oak and Turkey oak in Romania produced similar results [24].

The estimates of tree crown classes, stem quality and crown quality are given in Table 2. The presented data show that there was a sufficient number of Hungarian oak trees for the selection of final crop trees (future trees) and indicate a favorable relationship between the Turkey oak and Hungarian oak trees and the good quality of the stand, with a small percentage of suppressed Hungarian oak trees.

**Proposed silvicultural operations.** Table 3 shows the average data regarding the distribution of trees per diameter degrees, selected final crop trees and the proposal of tree marking. The data indicate that the future trees are selected from the dominant layer of the stand. Since all these stands have been intensively tended lately, only light thinning is proposed.

Three-dimensional and bird perspectives of the existing (initial) state of the stand and the state of the stand after the selective thinning has been conducted are given in Figure 5.

**DISCUSSION**

The above presented results show that this is a typical even-aged stand, with the regular binomial distribution of the number of trees, a significantly higher share of Hungarian oak compared to Turkey oak, both regarding the number of trees and the wood volume (by 60% regarding the number of trees and by 73% regarding the wood volume). However, despite the share, the mean diameter of Turkey oak trees is by 5.1 cm larger than the mean diameter of Hungarian oak trees, which indicates that it is a biologically stronger species. Based on the share of trees per number of trees and wood volume of the given mixture and their distribution by diameter degrees, it can be clearly concluded that this stand state allows successful natural regeneration of primarily Hungarian oak due to its sufficient participation in the mixture.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Averaged data for the stand state and the marking proposal</strong></td>
</tr>
<tr>
<td><strong>Diameter degree cm</strong></td>
</tr>
<tr>
<td><strong>N trees/ha</strong></td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>Σ</td>
</tr>
</tbody>
</table>

**Legend:** N - number of trees; V - volume; \(d_b\) - mean diameter; \(h_b\) - mean height; \(I_v\) - volume increment; \(p_v\) - percentage of volume increment.
The research on the tree quality shows it is a tended stand. Oaks are prominent heliophytes, which means that the presence of a large number of oak trees in the lower storey would indicate that tending measures have been conducted insufficiently or not conducted at all [25-27]. Hungarian oak trees are predominant in the stand mixture and their quality is not much behind the quality of Turkey oak trees (their stem quality is even better) which means they will take the role of seed bearers and the stand will be restored naturally, with the application of auxiliary measures of soil preparation if necessary. Trees of other species are found in the lower storey, so they do not have any greater influence on the quality of the stand, but certainly have their important function regarding the special purpose and significance of the investigated stands.

The research studies in coppice and degraded forests in a large number of ecological units and in different sites have established parameters that must be considered when determining the degree of stand degradation. They are classified into the following three groups: 1. stand characteristics; 2. site characteristics and management objectives and 3. economic and financial characteristics [21, 28]. Stand characteristics make the group of factors that are most important for the determination of the degree of degradation and the selection of the optimal methods of forest amelioration. The specific parameters are: stand origin, composition and mixture, stand developmental stage, stand quality, stand structure, health state, share of the main tree species.

It is possible to stop the regressive succession and degradation of stands during the regeneration stage provided that we propose an appropriate system of regeneration and constantly apply modern technological solutions in the process of regeneration. The basic criterion of selective thinning in the simulation of thinning operations was the specific silvicultural role of trees. The best trees were selected as final crop trees (future trees) to which the proposed regeneration measures would be applied (silvicultural-ameliorative operations). In doing so,
silvicultural principles were modified and harmonized with the free technique of silviculture, in accordance with the special purpose of the investigated forest complex. The long-term goal of silvicultural treatments is to bring the investigated stands into an optimum state in which the environmental potential of the site will be fully utilized and the forest complex will provide protective, regulatory and social functions. Trees that have a detrimental impact on the development of the stand and make an obstacle to the achievement of silvicultural goals were marked for felling. The intensity of thinning per number of trees was 8.1%, while it was 10.3% per wood volume. Considering the current silvicultural need, selective thinning of very low intensity was performed, removing Turkey oak trees of poor phenotypic characteristics. Due to the length of the production cycle in forestry, it takes a long time to study the effects of the implemented forest management measures, which is often the main problem and a limiting factor in the definition and application of appropriate silvicultural measures. The application of computer modeling and methods of visualization to silvicultural procedures allows fast, precise and timely information about the current stand state, as well as simulation of the development of the stand in the future.

CONCLUSIONS

The investigated stand is a mixed stand of Hungarian oak and Turkey oak (Quercetum fraineteto cerridis Rudski 1945) within the suburban complex of Lipovica Forest in the area of Belgrade - Serbia. Site conditions are suitable for the growth of forest vegetation. According to the analyzed site conditions, stand state and stand quality, it can be concluded that the studied stand is a good-quality coppice stand on a preserved site of Hungarian oak and Turkey oak. Hungarian oak has a greater share in the mixture than Turkey oak, both in the number of trees and in the wood volume, as well as a sufficient number of good-quality future trees. This type of stand state is much rarer in Lipovica forest complex than the type of Hungarian oak and Turkey oak stands in which Turkey oak dominates or has equal participation as Hungarian oak. The proposed tending measures should be applied until new planning documents have been produced. They will define long-term proposals for regeneration measures in order to achieve the desired goal in these stands, given their specific purpose. The investigated stand is suitable for indirect conversion, i.e. natural regeneration using the existing stand, with the aim of creating a good-quality seed forest. A group-selection regeneration system with a general 60-year regeneration period should be applied, with the application of auxiliary measures of natural regeneration. Proper implementation of silvicultural and ameliorative operations, good-quality high forests will be formed with a favorable mixture of Hungarian oak and Turkey oak. In doing so, the stand should be as uneven-aged as possible due to the special purpose of the forest. In that sense, the general regeneration period should be as long as possible.

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YIELD AND NUTRITIVE VALUE OF ITALIAN RYEGRASS (Lolium italicum L.) IS INFLUENCED BY DIFFERENT LEVELS OF NITROGENOUS FERTILIZER

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ABSTRACT

Italian ryegrass is often fertilized with high levels of nitrogen (N) in order to ensure a maximum biomass yield. While overdose of any fertilizer is neither cost-effective nor environmentally sound. In this context, the study was carried out at the agricultural experimental area of İğdır University during 2013, to find out the optimum dose of nitrogenous fertilizers for maximizing the biomass yield as well as nutrients content of Italian ryegrass. Treatments were five levels of nitrogen viz., 0 (N0), 2 (N2), 4 (N4), 6 (N6) or 8 (N8) kg da⁻¹, respectively. Treatments were arranged in a randomized completed block design with three replications. Data on green herbage and dry biomass yield as well as nutrients content such as Calcium (Ca), Magnesium (Mg), potassium (K), phosphorus (P), Crude protein (CP), Crude ash and Tetany ratio (K/(Ca+Mg)) were examined under the different levels of N. Results of the study showed that increasing rate of N fertilizer increased the green herbage as well as dry biomass yield of Italian ryegrass. The highest green herbage and dry biomass yield were recorded for the 8 kg N da⁻¹ (N8) (1142.7 and 473.7 kg da⁻¹) treated grass followed by those treated with a 6 kg N da⁻¹ (N6) (1070.2 and 444.8 kg da⁻¹). Similarly, the maximum seed yield was also recorded in the treatment of 8 kg N da⁻¹, followed by the treatment of 6 kg N da⁻¹. Considering the nutritional values, the levels of N and K in the ryegrass were decreased from the initial growth stages to seed stages. While, CP, Mg, P and Tetany ratios were decreased as growth stages of plant increased. Therefore, the application of 6 to 8 kg N da⁻¹ could be recommended for higher green herbage, dry biomass yield and also improving the nutrients content of Italian ryegrass under the condition of İğdır-Turkey.

KEYWORDS:
Italian ryegrass, harvest time, biomass yield, nutritive value.

INTRODUCTION

Among the fodder or cattle feed crops, ryegrass is characterized by high productivity and an excellent source of various nutrients such as Ca, Mg, K, P, crude protein and crude ash with a favorable tetany ratio, K/(Ca+Mg) [1, 2]. Although Italian ryegrass is native to Europe and neighboring countries, it has the ability to grow over a wider range of environment including Mediterranean to temperate regions, resistant to different diseases, capable of guaranteeing natural resowing and could be cultivated as intercrop with other grasses and legumes [3], as a forage for ruminants [4]. Lolium species is occupied about 23% of the 52 million ha of grassland in Europe. Among the Lolium species, Italian ryegrass is grown for livestock feed and bird seed similar to other millet species. The Italian ryegrass is the most extensively grown grass along with the English ryegrass (Lolium perenne L.). The feed value of the grass is generally considered to equal that of grain sorghum or corn [5]. However, seed production of the grass is highly dependent on sowing rate, inter-row spacing, climate condition, soil fertility and water availability [6, 7]. Pavinato et al. [8], reported that in tropical soils, nitrogen (N) fertilization is one of the most important nutrients that significantly influence the green herbage and dry biomass production of grass through increasing both the tillers plant⁻¹ and the pasture quality [9].

Fertilization is a cultural practice for forage and other crops’ yield as its results are immediate [10]. It is possible to obtain better yield and quality herbage and change greatly depending on cultural practice,
the species, variety and environment [11, 12]. It should be taken into account that the most fertilizer is obtained for maximum yield, quality feed, superior benefit from the applied fertilizer and the beneficial use of fertilizer in the economic direction [10].

Among the plant nutrients, N is an essential plant element that can significantly increase the growth, yield and quality of Italian ryegrass [13]. However, its supply from soil compared to its demand by crops as well as pasture plants is the most limiting amongst soil nutrients. On the other hand, most herbage ryegrasses are grown in soils that supply insufficient N for maximum crop yields. It has been reported that N is considered as one of the most important nutrients limiting to the leaf growth, biomass yield and protein content and quality of ryegrass [9]. In addition, the quantity of N absorbed by the crop is largely dependent on crop cultivars, growth stage as well as soil, and environmental factors such as soil texture, depth, and water holding capacity, amount and distribution of precipitation, length of growing season etc. [14]. Most of the cases, major N absorption occurred immediately before the stem elongation stage for rapid growth [15, 16]. It is reported that the uptake of N is very high from early vegetative stage to maximum vegetative stage crop as compared to reproductive stage in forage plant [17, 18]. However, in an N-rich soil, the absorption by the crops is highly correlated with the crop growth rate and biomass accumulation. Therefore, to obtain maximum yield and to increase N use efficiency, it is very essential to apply the N when crop is physiologically prepared to generate tillers and also environmental condition is favorable to uptake the N towards the root [14]. While, lack of rain or soil water, yield response to N is limited in most of the crops including forage crops [19].

Therefore, to get the maximum green herbage, dry biomass, as well as seed yield of grasses as a fodder, proper soil nutrient balance, should be maintained. The amount of N depends on several factors such as climate, soil productivity, and management [20]. Since research information is lacking for optimum levels of N to maximize the biomass yield as well as seed nutritional quality of Italian ryegrass (Lolium multiflorum westerdwoldicum- Caramba) under the soil and environmental condition of Iğdır, Turkey. The study was, therefore, conducted to evaluate the impacts of various doses of N on green herbage, dry biomass yield and seed nutritional values of ryegrass.

MATERIALS AND METHODS

Experimental sites. The research was carried out at the Faculty of Agriculture, Department of Field Crops in Iğdır University, Turkey during 2013.

Soil properties. Before sowing the crop, the soil sample was taken at 0-30 and 30-60 cm soil depth and was analyzed for properties presented in Table 1. The soil contains 6.53% lime in the clay structure and shows an alkaline (pH 7.98-8.03). The calcareous content is low, the phosphorus content is medium, and the potassium level is very high (Table 1).

Climatic conditions. The temperature has increased by 4.9% during the experimental year compared to long years and seasonal normal temperatures were observed except for the months in which there is an extreme value (Figure 1). The temperature below 0°C was recorded in the months of January and December. Rainfall was above normal and the maximum rainfall occurred from March to July and extreme was recorded during May (Figure 1).

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Soil structure</th>
<th>CaCO₃ (%)</th>
<th>Total salt (mmhos/cm)</th>
<th>pH</th>
<th>P₂O₅ (kg/da)</th>
<th>K₂O (kg/da)</th>
<th>Organic matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>Clay loam</td>
<td>6.53</td>
<td>1.8</td>
<td>7.98</td>
<td>8.0</td>
<td>343</td>
<td>1.6</td>
</tr>
<tr>
<td>30-60</td>
<td>Clay</td>
<td>6.53</td>
<td>1.8</td>
<td>8.03</td>
<td>3.7</td>
<td>248</td>
<td>1.3</td>
</tr>
</tbody>
</table>

TABLE 1

Chemical properties of experimental soils before experimentation

FIGURE 1

Monthly mean temperature (°C) and rainfall (mm) during the growing season in the year 2013

TABLE 2
Green herbage yield (kg da⁻¹) of Italian ryegrass grown under different nitrogen doses

<table>
<thead>
<tr>
<th>Nitrogen Fertilizer</th>
<th>First cutting</th>
<th>Second cutting</th>
<th>Third cutting</th>
<th>Mean of cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0N (kg da⁻¹)</td>
<td>439.3 d</td>
<td>548.7 c</td>
<td>824.0 c</td>
<td>603.9 d</td>
</tr>
<tr>
<td>2N (kg da⁻¹)</td>
<td>502.3 d</td>
<td>670.0 c</td>
<td>1005.0 b</td>
<td>761.1 c</td>
</tr>
<tr>
<td>4N (kg da⁻¹)</td>
<td>727.9 c</td>
<td>814.3 b</td>
<td>1011.0 b</td>
<td>890.4 b</td>
</tr>
<tr>
<td>6N (kg da⁻¹)</td>
<td>913.2 b</td>
<td>1057.7 a</td>
<td>1139.7 a</td>
<td>1070.2 a</td>
</tr>
<tr>
<td>8N (kg da⁻¹)</td>
<td>1005.6 a</td>
<td>1175.9 d</td>
<td>1281.0 a</td>
<td>1142.7 a</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>737.7 c</td>
<td>866.5 b</td>
<td>1076.0a</td>
<td></td>
</tr>
<tr>
<td>F-test</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

dekare or decare (da):10 kg/da is equal to 1 kg/ha; ns: not significant; **: significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05).

**TABLE 3**

<table>
<thead>
<tr>
<th>Nitrogen Fertilizer</th>
<th>First cutting</th>
<th>Second cutting</th>
<th>Third cutting</th>
<th>Mean of cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0N (kg da⁻¹)</td>
<td>161.2 c</td>
<td>203.4 c</td>
<td>370.8 c</td>
<td>254.2 c</td>
</tr>
<tr>
<td>2N (kg da⁻¹)</td>
<td>196.6 c</td>
<td>309.4 b</td>
<td>454.9 b</td>
<td>320.3 d</td>
</tr>
<tr>
<td>4N (kg da⁻¹)</td>
<td>267.2 b</td>
<td>343.0 b</td>
<td>508.1 b</td>
<td>372.4 c</td>
</tr>
<tr>
<td>6N (kg da⁻¹)</td>
<td>347.4 a</td>
<td>444.2 a</td>
<td>542.9 a</td>
<td>444.8 b</td>
</tr>
<tr>
<td>8N (kg da⁻¹)</td>
<td>381.3 a</td>
<td>493.5 a</td>
<td>546.5 a</td>
<td>473.7 a</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>270.7 c</td>
<td>363.9 b</td>
<td>484.6 a</td>
<td></td>
</tr>
<tr>
<td>F-test</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

dekare or decare (da):10 kg/da is equal to 1 kg/ha; ns : not significant; **: significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05).

Experimental treatments and design. Lolium multiflorum cv. Caramba was as plant material in this study and the plant material was collected from Ulusoy Tohumculuk Ltd., Turkey. Year-old grass was used as material. It was carried out on 15 parcels with dimensions of 5.0 x 2.0 = 10 m² and 2.5 kg seeds were used.

Treatments were arranged in a split-plot design as three cutting times (i.e., first cutting was done on 12 June 2015 (at beginning of flowering); second cutting was done on 16 July 2015 (at milk stage) and third cutting was done 30 July 2015 (at seed maturity) were placed in main plot and five levels of nitrogen i.e., 0 (N₀), 2 (N₁), 4 (N₂), 6 (N₃) and 8 (N₄) kg da⁻¹ respectively were arranged in sub-plot. The treatments were replicated thrice.

Data collection. Data on the green herbage, dry matter yield, and nutrients content were recorded according to the following procedure: after weighing the fresh green herbage, the samples were put in an oven for 72 hours at 70°C for drying and calculating dry matter yield. After weighing the green herbage in each cutting, about a 250-300g sample was taken for each plot for nutrients analysis such as Calcium (Ca), Magnesium (Mg), potassium (K), phosphorus (P), Crude protein (CP), crude fat, Crude ash, Neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude fiber according to [21]. Tetany ratio was calculated as (K/(Ca+Mg)) in mEq/Kg DM.

Statistical analysis. The obtained data were evaluated separately and combined using SAS statistical package program. The LSD test was used to compare the difference between the averages [22].

**RESULTS AND DISCUSSION**

Green herbage and dry biomass yields. The results of the study indicated that the green herbage and dry biomass yield of Italian ryegrass (Lolium italicum L.) were dependent on the different levels of N, which are presented in Tables 2 and 3. The results of the study showed that the increased levels of N fertilizer significantly (p<0.05) increased the green herbage and dry biomass yield of Italian ryegrass (Lolium italicum L.). The highest green herbage and dry biomass yield were recorded from 8 kg N da⁻¹ (N6) (1142.7 and 473.7 kg da⁻¹) fertilizer applied treatment, followed by 6 kg N da⁻¹ (N6) (1070.2 and 444.8 kg da⁻¹) fertilizer applied treatment. However, there was not significant statistically between the N6 and N8 fertilizer doses in respect of green herbage yields as a mean of N fertilizers. Whereas, the lowest (737.7 kg da⁻¹) and highest (1076.0 kg da⁻¹) green herbage yields were obtained from the first and latest cutting times, respectively during the first growing season of 2013.

Simić et al. [23], stated that the N application of 50 kg ha⁻¹ was found to be the optimal level for seed production and higher rates of N application (100 to 150 kg ha⁻¹) had either no impact on seed yield or decreased the yield of seed as a result of ryegrass lodging following seed shedding. On the other hand, Kesiktaş [24], concluded that the highest values of green and dried herbage yield, as well as those of ratio and yield of crude protein, were obtained from the application of 15 kg da⁻¹ N. Kusvuran and Tansi [25], the highest green herbage has been obtained from the plots received 20 kg da⁻¹ dose with 2 harvests.

Chemical composition. The chemical composition of seed grain of Italian ryegrass in accordance...
to cutting times is shown in Tables 4-8. It was observed that the CP (crude protein), crude fat and crude ash contents of Italian ryegrass were significantly (p<0.01) decreased in the earlier cutting, while crude fiber content was increased with the earlier cutting rather than the other two cuttings. In case of minerals content, the first cutting showed the maximum N, P, K, and Mg concentration, while Ca concentration was the highest at the time of third cutting (Tables 4-8).

The results of the present study are also similar to the findings of earlier researchers, who noticed that the application of N increased the dry matter yield [26] and crude protein content [27, 28, 29] in the grass of *Panicum* species. Similarly, Gultekin [30] found that green herbage yield, hay yield, the green leaf ratio, dry matter yield, crude protein ratio, and crude protein yield were significantly influenced by the application of different levels of nitrogenous fertilizers. Similarly, [9] reported a positive relationship between N fertilizer levels and CP contents of Italian ryegrass. Staugaard and Rutkauskiené et al. [31], concluded that crude protein content in the dry matter of Italian ryegrass herbage yield in the first cut ranged from 6.19% to 13.88% and in the second cut ranged from 3.19% to 4.75% and these findings are consistent with the results of our study.

Increasing available Mg in crested wheatgrass (*Agropyron spp.*) could reduce the incidence of grass tetany (Hypomagnesemia) in ruminants grazing this forage [32]. Grass tetany (hypomagnesemia) may be an important factor limiting the productivity of annah grazing Russian wild-rye [33]. This nutritional disorder is associated with relatively low concentrations in the forage of Mg and Ca, and high value for K and K/(Ca+Mg).

### TABLE 4

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>K (ppm)</th>
<th>P (%)</th>
<th>N (%)</th>
<th>Crude ash (%)</th>
<th>Tetany ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 N (kg da⁻¹)</td>
<td>46.85</td>
<td>608.0</td>
<td>502.6 b</td>
<td>0.41 b</td>
<td>2.01</td>
<td>10.0 b</td>
<td>0.24</td>
</tr>
<tr>
<td>2 N (kg da⁻¹)</td>
<td>62.55</td>
<td>552.7</td>
<td>614.8 ab</td>
<td>0.74 a</td>
<td>2.04</td>
<td>9.0 b</td>
<td>0.33</td>
</tr>
<tr>
<td>4 N (kg da⁻¹)</td>
<td>80.50</td>
<td>617.6</td>
<td>653.3 a</td>
<td>0.78 a</td>
<td>2.45</td>
<td>8.0 b</td>
<td>0.31</td>
</tr>
<tr>
<td>6 N (kg da⁻¹)</td>
<td>44.50</td>
<td>538.1</td>
<td>635.5 ab</td>
<td>0.68 ab</td>
<td>2.09</td>
<td>8.0 b</td>
<td>0.35</td>
</tr>
<tr>
<td>8 N (kg da⁻¹)</td>
<td>50.35</td>
<td>691.8</td>
<td>615.8 ab</td>
<td>0.78 a</td>
<td>2.40</td>
<td>16.0 a</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>50.95</td>
<td>601.6</td>
<td>604.4</td>
<td>0.68</td>
<td>2.20</td>
<td>10.20</td>
<td>0.30</td>
</tr>
</tbody>
</table>

F-test ns ns ** ns ns ** ns
dekare or decare (da);10 kg/da is equal to 1 kg/ha; ns : not significant, ** , significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05). tetany ratio = K/(Ca+Mg), mEq per kg

### TABLE 5

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>K (ppm)</th>
<th>P (%)</th>
<th>N (%)</th>
<th>Crude ash (%)</th>
<th>Tetany ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 N (kg da⁻¹)</td>
<td>51.10 b</td>
<td>625.4 a</td>
<td>574.7</td>
<td>0.59</td>
<td>1.66</td>
<td>9.0</td>
<td>0.27</td>
</tr>
<tr>
<td>2 N (kg da⁻¹)</td>
<td>101.9</td>
<td>508.5 ab</td>
<td>612.3</td>
<td>0.47</td>
<td>1.09</td>
<td>9.0</td>
<td>0.33</td>
</tr>
<tr>
<td>4 N (kg da⁻¹)</td>
<td>106.9</td>
<td>390.3 b</td>
<td>490.7</td>
<td>0.42</td>
<td>1.09</td>
<td>5.0</td>
<td>0.34</td>
</tr>
<tr>
<td>6 N (kg da⁻¹)</td>
<td>121.7</td>
<td>385.9 b</td>
<td>525.1</td>
<td>0.52</td>
<td>0.54</td>
<td>6.0</td>
<td>0.36</td>
</tr>
<tr>
<td>8 N (kg da⁻¹)</td>
<td>120.5</td>
<td>461.8 b</td>
<td>517.6</td>
<td>0.48</td>
<td>0.71</td>
<td>7.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>100.4</td>
<td>474.38</td>
<td>544.08</td>
<td>0.496</td>
<td>1.018</td>
<td>7.2</td>
<td>0.32</td>
</tr>
</tbody>
</table>

F-test ** ns ns ns ns ns ns
dekare or decare (da);10 kg/da is equal to 1 kg/ha; ns : not significant, ** , significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05). tetany ratio = K/(Ca+Mg), mEq per kg

### TABLE 6

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>K (ppm)</th>
<th>P (%)</th>
<th>N (%)</th>
<th>Crude ash (%)</th>
<th>Tetany ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 N (kg da⁻¹)</td>
<td>105.9</td>
<td>470.3 ab</td>
<td>386.1</td>
<td>0.28</td>
<td>0.48 b</td>
<td>13.0</td>
<td>0.23</td>
</tr>
<tr>
<td>2 N (kg da⁻¹)</td>
<td>108.3</td>
<td>434.7 b</td>
<td>427.8</td>
<td>0.35</td>
<td>0.47 ab</td>
<td>11.0</td>
<td>0.27</td>
</tr>
<tr>
<td>4 N (kg da⁻¹)</td>
<td>137.2</td>
<td>492.9 ab</td>
<td>231.7</td>
<td>0.41</td>
<td>0.53 a</td>
<td>12.0</td>
<td>0.12</td>
</tr>
<tr>
<td>6 N (kg da⁻¹)</td>
<td>143.4</td>
<td>632.6 a</td>
<td>297.5</td>
<td>0.44</td>
<td>0.34 ab</td>
<td>11.0</td>
<td>0.13</td>
</tr>
<tr>
<td>8 N (kg da⁻¹)</td>
<td>137.2</td>
<td>489.4 ab</td>
<td>544.3</td>
<td>0.34</td>
<td>0.36 ab</td>
<td>11.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>126.4</td>
<td>503.98</td>
<td>377.48</td>
<td>0.364</td>
<td>0.436</td>
<td>11.6</td>
<td>0.21</td>
</tr>
</tbody>
</table>

F-test ns ** ns ns * ns ns

dekare or decare (da);10 kg/da is equal to 1 kg/ha; ns : not significant, ** , significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05). tetany ratio = K/(Ca+Mg), mEq per kg

### TABLE 7
Chemical contents of Italian ryegrass grown under different nitrogen doses in the average of time

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>K (ppm)</th>
<th>P (%)</th>
<th>N (%)</th>
<th>Crude ash (%)</th>
<th>Tetany ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 N (kg da⁻¹)</td>
<td>76.3</td>
<td>567.9</td>
<td>487.8</td>
<td>0.43</td>
<td>1.25</td>
<td>10.67</td>
<td>0.25</td>
</tr>
<tr>
<td>2 N (kg da⁻¹)</td>
<td>90.9</td>
<td>498.6</td>
<td>551.6</td>
<td>0.52</td>
<td>1.20</td>
<td>9.67</td>
<td>0.31</td>
</tr>
<tr>
<td>4 N (kg da⁻¹)</td>
<td>124.9</td>
<td>500.3</td>
<td>458.6</td>
<td>0.53</td>
<td>1.36</td>
<td>8.33</td>
<td>0.26</td>
</tr>
<tr>
<td>6 N (kg da⁻¹)</td>
<td>68.2</td>
<td>535.4</td>
<td>486.0</td>
<td>0.54</td>
<td>0.99</td>
<td>8.33</td>
<td>0.28</td>
</tr>
<tr>
<td>8 N (kg da⁻¹)</td>
<td>101.0</td>
<td>530.9</td>
<td>559.2</td>
<td>0.53</td>
<td>1.15</td>
<td>11.33</td>
<td>0.29</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>92.26</td>
<td>526.62</td>
<td>508.64</td>
<td>0.51</td>
<td>1.19</td>
<td>9.666</td>
<td>0.28</td>
</tr>
</tbody>
</table>

F-test ns ns ns ns ns ns ns

dekare or decare (da); 10 kg da is equal to 1 kg/ha; ns: not significant; **, significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05). tetany ratio = K/(Ca+Mg), mEq per kg

### TABLE 8
The nutrient content at different cutting times of Italian ryegrass grown in Iğdır conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>K (ppm)</th>
<th>P (%)</th>
<th>N (%)</th>
<th>Crude ash (%)</th>
<th>Tetany ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>56.95 b</td>
<td>601.6 a</td>
<td>604.4 a</td>
<td>0.68 a</td>
<td>2.20 a</td>
<td>10.2 a</td>
<td>0.30 a</td>
</tr>
<tr>
<td>Second</td>
<td>119.4 a</td>
<td>447.4 b</td>
<td>544.1 a</td>
<td>0.49 b</td>
<td>1.02 b</td>
<td>7.2 b</td>
<td>0.32 a</td>
</tr>
<tr>
<td>Mean of N levels</td>
<td>92.25</td>
<td>517.63</td>
<td>508.67</td>
<td>0.51</td>
<td>1.19</td>
<td>9.67</td>
<td>0.28</td>
</tr>
</tbody>
</table>

F-test ** * * * * ***

ns : not significant, ***, significant at 1% level; a,b,c,d Means in a row with different superscripts are significantly different (p<0.05). tetany ratio = K/(Ca+Mg), mEq per kg

### TABLE 9
A relationship between nutrient contents of Italian ryegrass through the application of different levels of nitrogen

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>K (ppm)</th>
<th>P (%)</th>
<th>N (%)</th>
<th>Crude ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mg</td>
<td>-0.624**</td>
<td>0.219</td>
<td>0.476**</td>
<td>0.589**</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>-0.133</td>
<td>0.549**</td>
<td>0.636**</td>
<td>0.770**</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P</td>
<td>-0.496**</td>
<td>-0.118</td>
<td>-0.099</td>
<td>0.381</td>
<td>-0.049</td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>-0.550**</td>
<td>0.367</td>
<td>0.351</td>
<td>-0.102</td>
<td>0.007</td>
<td>1</td>
</tr>
<tr>
<td>Crude ash</td>
<td>-0.999</td>
<td>-0.431</td>
<td>-0.381</td>
<td>-0.118</td>
<td>-0.049</td>
<td>1</td>
</tr>
</tbody>
</table>

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

*. Correlation is significant at the 0.05 level (2-tailed).

### CORRELATIONS

The relationship between N and soil moisture is extremely important for biomass and yield of the plant. The results of the present study reported that an increased level of N not only increased the seed yield, seed quality, and nitrogen use efficiency but also increased the water use efficiency and that an improved understanding of the balance between N and water availability is needed. The relationships of various chemical components of biomass and seed yield under different cutting times were evaluated, which are shown in Table 9. The results of the present study showed that except Ca application of different levels of N has positive relation with other nutrients content.

### CONCLUSIONS

From the results and discussion of the present study, it can be concluded that increasing levels of nitrogen up to 8 kg N da⁻¹ increased the green herbage as well as dry biomass yield of Italian ryegrass. It was also found that 6 and 8 kg N da⁻¹ N produced a statistically similar yield as well as improved the quality of the biomass. Considering the nutritional values, the levels of N, P, K and Mg were decreased from the initial growth stages to seed stages of ryegrass with the application of different levels of N. While, crude protein, Ca and Tetany ratio were decreased as stages of plant increased. Therefore, 6 and 8 kg N da⁻¹ may be recommended for higher green herbage, dry biomass yield and also for improving the quality content of Italian ryegrass under the condition of Iğdır-Turkey.

### REFERENCES


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INVESTIGATION OF POTENTIAL ANTI-CANCER AND ANTI-INFLAMMATORY EFFECTS OF Datura stramonium ETHANOLIC EXTRACTS AGAINST SELECTED HUMAN CANCER CELL LINES

Mehluka Alper*
Department of Molecular Biology and Genetics, Faculty of Science, Mucela Sitki Kocman University, Mugla, Turkey

ABSTRACT

In this study, the different biological properties particularly associated with anti-cancer and anti-inflammatory effects of the D. stramonium extracts from Edirne (Turkey) were examined for the first time.

The ethanolic extracts of leaves (DSL) and seeds together with flowers (DSSF) from D. stramonium L. were used. The cytotoxic activity was determined using MTT assay. Analysis of the cell cycle distribution and the cell death were performed using flow cytometry. The levels of the VEGF and the pro-inflammatory cytokines (IL-1α, IL-6 and TNF-α) were determined by ELISA.

DSL extract was more cytotoxic than DSSF extract. The DSL extract at 72 h exhibited selective cytotoxicity against HeLa cells with IC50 value of 265.7 μg/mL and MCF-7 cells with IC50 value of 272.5 μg/mL compared to healthy BEAS-2B cells with IC50 value of 622 µg/mL. The effect of the DSL extract on the cell cycle progression changed according to the type of cell lines. The DSL extract at 800 µg/mL was found to induce necrotic cell death in all the cancer cell lines tested. The VEGF secretion was determined to reduce by 36% in A549 cells and by 12% in PC-3 cells after treatment with 300 µg/mL DSL extract. The levels of pro-inflammatory cytokines (IL-6, TNF-α) secretion varied according to cell lines.

The findings of the present study suggest that D. stramonium may be an important source for novel anti-cancer agents.

KEYWORDS:
Datura stramonium, anti-cancer, anti-inflammatory, cancer cell lines

INTRODUCTION

Cancer is one of the most common causes of death in the World and continues to be a growing major health problem [1]. In cancer treatment, there are various procedures like immunotherapy, stem cell transplantation, surgery, radiotherapy, chemotherapy and they can cause serious side effects such as limited bioavailability, toxicity and nonspecificity [2]. Moreover, chemotherapy which is one of the major strategies of cancer treatment is usually associated with the development of multidrug resistance [3]. Therefore, recent researches focus on overcoming the side effects of chemotherapy and developing new anticancer agents with no toxic effects [4].

More than 60% of the currently used anticancer agents were originated from natural resources. Plants have historically been the main sources of natural products. Also, the currently used effective cancer chemotherapeutics such as vinristine, etoposide, paclitaxel (Taxol®), docetaxel, topotecan, and irinotecan are plant-derived agents [5].

There are more than 3000 plants with anticancer properties in the world [6]. The secondary metabolites of the plants can play the significant role as both potential anticancer agents and cancer chemoprevention [7,8]. Environmental factors were reported to be one of the important determinants of the difference in secondary metabolites of plant species [9]. Therefore, the screening of therapeutic potential of plants from different environmental and ecological conditions may contribute to developing pharmacologically active compounds for cancer treatment.

Datura stramonium L. (DS) belonging to Solanaceae family is a widespread annual plant. This plant is commonly known as Angel’s trumpet, Loco-weed, Jimson weed, Datura or thorn apple [10, 11]. It is also named in our country such as “seytan elması, boru çıçeği, abu zambak, cin ou, tatula” [12]. All parts of the plant are poisonous. On the other hand, the parts of the plant are practically used in indigenous and traditional folk medicine [13]. In different studies, the plant has been stated to possess medicinal values such as antiasthmatic, antimicrobial, acaricidal, anti-cancer, anti-inflammatory, antifungal, nematocidal activities and has phytochemical contents including different compounds like alkaloids, saponins, tannins, glycosides with various biological activities [10, 13, 14, 15]. However, the anti-cancer and anti-inflammatory properties of D. stramonium has been poorly evaluated.
Therefore, the present study for the first time aimed to investigate the various biological properties particularly associated with anti-cancer and anti-inflammatory effects of ethanolic extracts from *D. stramonium* collected from Edirne province (Turkey) on different cell lines.

**MATERIALS AND METHODS**

*Collection of plant species.* *D. stramonium* (DS) specimens were collected from Edirne province in Turkey in September 2015, and authenticated by Dr. Fatma Güneş at the Department of Pharmaceutical Botany, Trakya University, Edirne. The voucher specimen was deposited at Trakya University Herbarium.

*Preparation of plant extracts.* Aerial parts of the plant were washed, air-dried under shade at room temperature and separated into two parts: leaves (DSL) and seeds together with flowers (DSSF). Each 10 gr of pulverized parts of DS were extracted with absolute ethanol (Merck, USA) in a shaking water bath (Stuart, SBS40, UK) for 6 at 53°C at least twice. The extracts were filtered using Whatman filter paper no. 1 and concentrated in a vacuum rotary evaporator (IKA, RV 10, USA) at 42–49°C. The crude extracts were stored at -20°C and protected from light until use. The stock solutions of the extracts were prepared in 10% DMSO (Applichem, USA) and serial dilutions were freshly performed with the growth medium. The final concentration of DMSO in cells tested did not exceed 0.1%.

*Cell culture conditions.* MCF-7 (breast cancer cell line), A549 (non-small-cell lung cancer cell line), HeLa (cervical cancer cell line), PC-3 (prostate cancer cell line), Daudi (Burkitt’s lymphoma, CCL-213) and BEAS-2B (bronchial epithelial cell line) human cell lines used in this study were obtained from ATCC. The cell lines were cultured in RPMI-1640 medium (Biochrom, Germany) with stable L-glutamine (Biochrom, Germany) containing 10% heat-inactivated fetal bovine serum (FBS) (Biochrom, Germany), 100 units/mL penicillin and 100 mg/mL streptomycin (Biochrom, Germany) at 37°C in a humidified incubator with 5% CO₂ and 95% air. When the cells reached 80% confluence, they were passaged and used for the assay.

*Cell cytotoxicity assay.* The MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide) (Applichem, USA) assay [16] was used to evaluate the cytotoxic effects of the extracts against different cell lines. In brief, the cell lines at a density of 2x10⁴ cells/well were added into 96-well microplates (Greiner, Germany) in triplicate and incubated for 24 h. To screen the cytotoxic effects of the extracts, the cell lines were exposed to the extracts at 800 µg/mL for 72 h. For the dose and time course, experiments were performed for six different concentrations (800-25 µg/mL) of the extract for 24, 48 and 72 h under the same conditions. The untreated cells served as a control. At the end of incubation time, the medium in each well was discarded and replaced with 100 µL of fresh growth medium. Then, 10 µL of MTT (5 mg/mL) in phosphate-buffered saline (PBS) was added into each well and the microplate was incubated for a further 4 h at 37°C. After the medium with MTT was removed, the blue formazan crystals were dissolved in 100 mL of DMSO (Applichem, USA). The optical density of each well was read with a microplate reader (Thermo Scientific, Multiscan FC, USA) at 540 nm. The percentage of the viable cells was calculated using the following equation:

\[
\text{Cell viability \%} = \frac{\text{Mean Abs of treated cells}}{\text{Mean Abs of untreated cells}} \times 100
\]

*Analysis of cell cycle distribution.* The percentages of MCF-7, A549, HeLa and PC-3 cells in each cell cycle phase were determined by propidium iodide (PI) (Sigma-Aldrich, USA) staining. Briefly, the cells were plated at 5x10⁵ cells/well in 6 well-plates. After 24 h incubation, the cells were treated with 800 µg/mL DSSL extract for 24 h. The control cells were exposed to DMSO at 1% final concentration. Then, the cells were collected by trypsinization, washed with cold PBS and fixed in absolute ethanol. After 48 h of storage at -20°C, the cells were centrifuged at 1200 rpm for 10 min at 4°C and cell pellets were washed with cold PBS. Later, the cells were resuspended in 1 mL PBS containing 0.1% (v/v) Triton X-100 (Amresco, USA) and incubated with 100 µL of RNase A (200 µg/mL) (Applichem, USA) at 37°C for 30 min. Subsequently, each cell suspension was treated with 100 µL PI (1mg/mL) and incubated at room temperature for 15 min in the dark. These stained cells were analyzed by BD FACSCanto flow cytometry (BD Biosciences, San Jose, CA) using Modfit LT 3.0 software to detect the cell percentage in each phase of the cell cycle.

*Analysis of cell death.* An annexin V-FITC apoptosis detection kit (eBioscience, BMSF500FI/100) was used for detection the extent of the cell death in treated and control cells, according to the manufacturer’s directions. In brief, MCF-7, A549, HeLa and PC-3 cells were cultured at 5x10⁴ cells/well in 6-well plates and treated with the extract according to the method of cell cycle analyses described above. The cells treated with DMSO at 1% final concentration served as the control. After treatment, the cells were collected from each well by trypsinization and washed with cold PBS twice. Cell suspensions were centrifuged at 800 rpm for 5 min at 4°C. After careful removing supernatants, cells were resuspended in 190 µL binding buffer and 5 µL
Annexin V-FITC was added to each cell suspension. Cells were incubated at room temperature for 10 min in the dark and then centrifuged. Cells were again dissolved in 190 μL binding buffer. Then, each cell suspension was stained with 10 μL PI (20 μg/mL) at room temperature in the dark and analyzed with BD FACSDiva software v6.13 using BD FACSCanto flow cytometry (BD Biosciences, San Jose, CA).

Enzyme-linked immune sorbent assay (ELISA). The supernatants of the cell cultures were used for quantification of VEGF (vascular endothelial growth factor) in A549 and PC-3 cells, and for quantification of the pro-inflammatory cytokines (IL-1α, IL-6, and TNF-α) in A549 and Daudi cells by using human ELISA kits (Boster Biological Technology, USA). Briefly, cells were seeded at 2x10^5 cells/well in a 6-well plate and incubated for 1 h. After treatment with 300 μg/mL (approximately IC_{50} value for cancer cells tested except for Daudi) DSL extract for 6 h, the supernatants of treated and untreated (control) cell cultures were collected and centrifuged at 14000 rpm for 30 second. These supernatants were aliquoted and stored at -20°C until the time of testing. A 100 μL of supernatant was then assayed for detection of the level of VEGF or inflammatory each cytokine produced through ELISA kits following the manufacturer’s protocol. The absorbance of each well was measured at 450 nm by the usage of a microplate reader (Thermo Scientific, Multiscan FC, USA).

Statistical analysis. The data were presented as the mean ± standard error (SE). GraphPad Prism software version 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used to perform statistical analyses and to calculate IC_{50} values.

RESULTS

The cytotoxic effects of the plant extracts in several cell lines. The cytotoxic effects of the crude extracts of the DS against MCF-7, A549, HeLa, PC-3, and BEAS-2B cells were determined by the MTT assay. Firstly, the ethanolic DSL and DSSF extracts at 800 μg/mL were separately tested for 72 h on the cancer cell lines used for MTT assay to detect the effective extract. According to the results, DSL extract exhibited more than 65% cytotoxicity on all cancer cell lines whereas DSSF extract cause less cytotoxicity than DSL extract (Figure 1). So, dose and time course experiments for 24, 48 and 72 h were performed for DSL extract in concentration range between 800 μg/mL to 25 μg/mL. In addition, BEAS-2B healthy cells were treated with same DSL extract concentration only for 72 h.

As shown in Figure 2, the DSL extract generally decreased the cell viability in a time- and concentration dependent manner. This extract was also found to be effective to different extent according to the cell lines tested. The most statistically significant cytotoxic effects in BEAS-2B were seen at concentrations of only 800 and 400 μg/mL for 72 h in contrast to the cancer cells. The approximate IC_{50} values (50% inhibitory concentrations) of the DSL extract against the cell lines tested were summarized in Table 1. The DSL extract at 24 h did not demonstrate more than 50% cytotoxicity on cancer cells except for PC-3 cells. At 72 h, DSL extract showed the highest cytotoxicity against HeLa cells with IC_{50} value of 265.7 μg/mL and MCF-7 cells with IC_{50} value of 272.5 μg/mL compared to normal BEAS-2B cells with IC_{50} value of 622 μg/mL. The IC_{50} value of this extract at 72 h were found to be 361 μg/mL and 369 μg/mL for A549 and PC-3 cells, respectively. These findings revealed that the most sensitive cells to the DSL extract at 72 h were HeLa and MCF-7.

Flow cytometric analysis of the cell cycle distribution. The cell cycle distribution in cells treated with 800 μg/mL DSL extract or with only DMSO at 1% final concentration (control) for 24 h were investigated using flow cytometry (Figure 3). There were increases in the percentage of the MCF-7 cells in the S phase from 41.61% to 45.08% and G2 phase from 17.34% to 25.06% after treatment, accompanied by a decrease in the percentage of MCF-7 cells in G1 phase. Treatment with this extract caused 6.31% and 15.50% increases of A549 cells in the S and G2 phase in comparison with control cells, respectively.
FIGURE 2

The cytotoxic effects of ethanolic extract of leaves of *D. stramonium* against MCF-7 (A), A549 (B), HeLa (C), PC-3 (D) cancer cell lines and BEAS-2B (E) normal cell line in different concentrations.

Cell viabilities of cancer cells were determined using MTT assay for 24, 48 and 72 h. Data was presented as mean± standard error (SE) from three independent experiments. Asterisks indicate statistical significance in cell viability of treated cells in comparison with untreated cells (control). ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 and ns: non significant (P> 0.05).

**TABLE 1**

Approximate IC₅₀ values of ethanolic extract of leaves of *D. stramonium* against different cell lines based on time course

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td></td>
<td>436.3±0.24</td>
<td>272.5±0.16</td>
</tr>
<tr>
<td>A549</td>
<td></td>
<td>410±0.35</td>
<td>361±0.13</td>
</tr>
<tr>
<td>HeLa</td>
<td></td>
<td>664±0.29</td>
<td>265.7±1.05</td>
</tr>
<tr>
<td>PC-3</td>
<td>629±0.12</td>
<td>528±1.03</td>
<td>369±1.05</td>
</tr>
<tr>
<td>Beas-2B</td>
<td></td>
<td></td>
<td>622±1.06</td>
</tr>
</tbody>
</table>

Note: a: Value was not calculated because there was no more than 50% cytotoxic effect. b: Value was not calculated for BEAS-2B cells.
FIGURE 3
Analysis of cell cycle distributions by flow cytometry.
MCF-7 (A), A549 (B), HeLa (C), PC-3 (D) cells were either treated with 1% DMSO (control) (a) or treated with DSL extract at 800 µg/mL (b) for 24 h. The percentages of distribution of cells in the different phases of the cell cycle were shown as a bar graph (E).
and concomitant decrease in the percentage of the cells in G1 phase. In addition, treated PC-3 cells were arrested especially in S phase (from 17.70% to 41.76%) and G2 phase (from 11.10% to 17.66%) compared to control cells with a parallel decrease of the cells in G1 phase. However, an increase in the percentage of the HeLa cells was detected in G1 phase (from 49.09% to 56.87%). The data obtained from this assay clearly indicated that the DSL extract was likely changing the cell phase distribution according to the cell lines used.

**Flow cytometric analysis of the cell death.**
The percentage of apoptosis and necrosis in the cells were analyzed using an annexin V-FITC apoptosis detection kit by flow cytometry for 24 h. As shown in Figure 4, DSL extract at 800 µg/mL was found to induce the apoptosis and necrosis in MCF-7 compared with the control cells. After treatment, the percentages of the apoptotic (quadrant 2 and 4) and necrotic (quadrant 1) MCF-7 cells increased from 3.3% to 17.1% and from 0.7% to 43%, respectively. In addition, the percentage of necrosis in other treated
cells increased from 1.7% to 11.2% for A549 cells, 4.2% to 16.1% for HeLa cells and 1.1% to 17.0% for PC-3 cells compared to control cells. Therefore, these results show that the DSL extract induces necrotic cell death in almost all cell lines tested, indicating that the extract at 800 μg/mL is toxic to the cells. These findings were consistent with cell cycle analysis, because disruption of cell cycle progression may ultimately lead to apoptotic/necrotic death [17].

These results might indicate that the DSL extract has antiangiogenic properties, because the VEGF is known as an angiogenic factor [20].

**FIGURE 5**

**Effects of DSL extract on VEGF secretion of A549 and PC-3 cells.**

The VEGF concentration in supernatants of the cells treated with the extract at 300 μg/mL for 6 h was determined by ELISA. The values represent the mean of three independent experiments ± SE. The concentration of VEGF in control (untreated) cells was taken as 1-fold. ****p<0.0001, versus control.

**Detection of the VEGF level.** It was reported that VEGF expression was determined in A549 [18] and PC-3 [19] cell lines. In this study, the VEGF levels in supernatants of the A549 and PC-3 cell cultures also investigated using human VEGF ELISA assay. These findings showed that VEGF secretion was reduced by 36% in A549 cells treated with 300 μg/mL DSL extract compared to untreated cells. However, the level of secreted VEGF in PC-3 cells was decreased by 12% after treatment (Figure 5).

**DISCUSSION**

The investigation of new agents with selective and low side-effects continues to be an important case in cancer research. Natural products are one of the main sources of the potential pharmaceutical agents used for the prevention and treatment of cancer [5]. Plants have a strong potential for development of new anticancer agents to be used clinically due to their phytochemical properties [21]. It is known that the various bioactive phytochemicals exhibit anti-cancer activity by serving as anti-inflammatory agents, the regulator of cell cycle and apoptosis and the like [2]. So, the evaluation of the potential anti-cancer properties of the plants can provide a significant contribution to studies about cancer therapy. Although previous studies showed the various pharmacological activities of *D.*

**FIGURE 6**

**Effects of DSL extract on IL-1α, IL-6, and TNF-α secretion of A549 (A) and Daudi (B) cells.**

The pro-inflammatory cytokines concentration in supernatants of the cells treated with the extract at 300 μg/mL for 6 h were determined by ELISA. The values represent the mean of three independent experiments ± SE. The concentrations of each cytokine in control (untreated) cells was taken as 1-fold. ****p<0.0001, versus control.
stramonium [reviewed by 11, 13, 14], there is not much research on the anti-cancer and anti-inflammatory effects of this plant in the literature.

In the present study, the ethanolic extracts of leaves (DSL) or seeds together with flowers (DSSF) of D. stramonium collected from Turkey were separately tested on different cell lines for cytotoxic effects using MTT assay for the first time according to the available literature. When the cytotoxic effect of the extracts at 800 μg/mL was screened for 72 h, it was observed that DSL extract showed more than 65% cytotoxicity whereas DSSF extract caused less than 25% cytotoxicity. Based on these results, the DSL extract was selected for further dose and time course experiments, the analyses of the cell cycle distribution, apoptosis, as well as ELISA.

The DSL extract generally increased cytotoxicity in a dose and time-dependent manner. It was observed that DSL extract at 72 h exhibited the high cytotoxic effects against HeLa cells with an IC₅₀ value of 265.7 μg/mL and MCF-7 cells with an IC₅₀ value of 272.5 μg/mL compared to healthy BEAS-2B cells with an IC₅₀ value of 622 μg/mL. Also, this extract was detected to be relatively cytotoxic on A549 (IC₅₀ value of 361 μg/mL) and PC-3 cells (IC₅₀ value of 369 μg/mL). Based on the results, the DSL extract appears to exhibit the selective cytotoxicity to HeLa and MCF-7 cancer cells relative to BEAS-2B normal cells. Unlike the present study, Sasaki et al. expressed that D. stramonium agglutinin inhibited the proliferation of four human glial tumour cells, U251 (glioblastoma), SF-539 (gliosarcoma), SNB-75 (astrocytoma), and SNB-78 (astrocytoma) [22]. The therapeutic dose of D. stramonium as an Ayurvedic anti-cancer herb was reported to be 0.05–0.1 g along with some side effects such as vomiting, hypertension, loss of consciousness [23]. D. stramonium aqueous leaf extract was demonstrated to induce oxidative stress in different human cancer cell lines [24]. Merza et al. investigated the activity of the acetic extract of D. stramonium seeds on cancer cell lines. These researchers reported that the extract exhibited cytotoxic effect by decreasing the viability of AMN3 (mammary adenocarcinoma) (42.91%) and brain cell lines (32.79%) and caused little effect on the viability of normal cell line Rf3 (rat embryonic fibroblast) [25]. Khalighi-Sigaroodi et al. stated the first report on cytotoxicity and antioxidant activity of five plant species collected from different regions of Iran. When these species were tested by brine shrimp lethality assay, methanol extracts and chloroform fractions of D. stramonium leaves were reported to show the highest cytotoxic activities with LC₅₀ values of 21.66 μg/mL and 4.29 μg/mL, respectively [26]. In addition, methanolic seeds extract of D. stramonium collected from Konya caused a reduction in the cell proliferation of human lymphocytes [27]. In another study, it was declared that the EC₅₀ value of fraction-10 from D. stramonium leaves for the human peripheral blood mononuclear cells (PBMC) was 19.1 μg/mL and fraction-10 pretreated PBMC exhibited enhanced cytotoxicity against A549 and MCF-7 cell lines [28]. The CTC₅₀ value (inhibit cell growth by 50%) of the compound isolated from the ethyl acetate fraction of D. stramonium flowers for liver cancer HePG2 cell lines was found to be 131.53 μg/mL [29]. Iqbal et al. explained that methanol extract of seeds of D. stramonium on MCF-7 cells showed increasing cytotoxicity with increasing concentrations of the extract and its IC₅₀ value was 113.05 μg/mL [30]. All these findings support that the level of cytotoxicity may alter depending on the location and the part of the plant harvested as well as the selected solvent for extraction and the cell lines used. These results also confirm that there may be present potential anti-cancer substances in D. stramonium.

One of the common features of cancer is the alteration of cell cycle progression [31]. It has been emphasized that the targeted regulation of the cell cycle is important for the treatment of cancer [32]. Also, various natural compounds can inhibit cancer cell growth as inhibitors of cell cycle progression [33]. Although it was declared that the other extract of D. stramonium seeds has the ability to inhibit mitosis in cancer cells [34], the effect of the DSL extract from Edirne (Turkey) on cell cycle progression was investigated for the first time in the present study. The DSL extract treatment at 800 μg/mL for 24 h caused a significant cell cycle arrest at the S phase in PC-3 cells (about 24%) and the G2 phase in A549 cells (about 15.50%). The percentage of MCF-7 and HeLa cells after the treatment was increased by about 8% in the G2 and G1 phases of the cell cycle, respectively, compared to normal cells. Based on these results, it may be suggested that the DSL extract induced the cell cycle arrest and the cytotoxicity observed against cancer cell lines may be originating from this property of the extract.

The flow cytometry analyses showed that the percentage of necrotic cells was higher than that of apoptotic cells among all cells treated with DSL extract at 800 μg/mL for 24 h. So, the research result suggests that the main cell death pathway in treated cancer cells is necrosis. Unlike this study, Akal et al. displayed that methanolic seeds extract of D. stramonium exhibited apoptotic effect according to TunEL assay results [27]. Karmakar et al. stated that alkaloids—scopolamine, trigonelline and tyramine compounds obtained from activity-guided fractionation of the MeOH extract of D. stramonium leaves exhibited TRAIL (a potent inducer of apoptosis in most cancer cells)-resistance overcoming activity at 50, 150, and 100 μM, respectively in TRAIL-resistant AGS cells [35]. Although it is expressed that plant-derived anti-cancer drugs can induce non-apoptotic cell deaths [36], it is still unclear whether necrosis plays an important role in the formation of the tumor. In addition, it has been stated that the necrosis of tumor cell can create an
inflammatory response, and an immune response to potentially malignant cells, so it may prohibit development of the tumor [37]. Necrosis has long been regarded as an uncontrolled, non-programmed form of cell death. However, the necrotic cell death was interestingly noted to be controllable [38]. Thus, the findings obtained in the present study may provide a significant contribution to the literature.

The development of new blood vessels from preexisting ones is called angiogenesis and this process is important in the growth and metastasis of cancer tissue [39]. VEGF is known as a key regulator of angiogenesis in cancer [40]. So, VEGF is considered as a rational target for anti-cancer therapy. The extract of seeds of *D. innoxia* was reported to inhibit angiogenesis by suppressing VEGF in cervical cancer HeLa cells by Pandey et al. [41]. Also, Khoshkoo et al. presented the anti-angiogenesis properties of *D. innoxia* [42]. In the present study, the effect of the DSL extract on the secretion of VEGF in A549 and PC-3 cells for 6 h were investigated. Based on the literature, this study revealed for the first time that the treatment with the DSL extract at 300 μg/mL reduced the level of VEGF in A549 and PC-3 cells by 36% and 12%, respectively, compared to untreated cells. So, these findings suggest that DSL extract has a potential antiangiogenic effect.

The pro-inflammatory cytokines like IL-1, IL-6, and TNF may enhance tumor cell survival [43]. Therefore, the DSL extract at 300 μg/mL was tested for its anti-inflammatory activity using A549 and Daudi cells for 6 h in this present study. For the purpose, relative levels of the secreted pro-inflammatory cytokines (IL-1α, IL-6, and TNF-α) in treated cells were determined by comparing those with the untreated cells. The data herein showed for the first time that the DSL extract at 300 μg/mL caused an increase not exceeding 20% in the level of secreted TNF-α and IL-6 in A549 and Daudi cells, respectively. Also it was observed that the level of IL-6 and TNF-α did not significantly alter in A549 and Daudi cells, respectively compared to untreated cells. In addition, the level of IL-1α in treated cells was found to slightly decrease in comparison to untreated cells. In a previous study, the leaf or whole plant of *D. stramonium* has been stated to use in Madhya Pradesh as anti inflammatuar and antispasmodic [44]. Unlike present study, Khabali et al. investigated the anti-inflammatory effect the alcoholic extract of seed of *D. stramonium* on inflammation and reported that the extract has the competence to markedly decrease the amount of inflammation in particular the acute form [45]. Also, Sonika et al. stated that the ethanolic extracts of fruits of *Coriandrum sativum*, leaves of *D. stramonium* and *Azadirachta indica* exhibited significant anti-inflammatory activity comparable to the standard drug Diclofenac sodium against carrageenan induced rat paw edema method [46]. In addition, the chemical composition and anti-inflammatory property of *D. stramonium* oil growing in Nigeria was firstly reported by Aboluwodi et al. [47]. These findings may suggest that the anti-inflammatory effect of *D. stramonium* may change based on the organisms, the cell type and the testing method chosen.

As a result, the ethanolic extracts of leaves of *D. stramonium* caused cytotoxic effects against different cancer cell lines, and exhibited the selective cytotoxicity to HeLa and MCF-7 cancer cells relative to healthy BEAS-2B, and arrested the cell cycle progression, and induced the necrosis, and reduced the level of VEGF secretion, and affected the level of pro-inflammatory cytokines (IL-6, TNF-α) secretion.

**CONCLUSION**

In conclusion, the leaves of *D. stramonium* may be an effective source for potential novel agents in the treatment of cancer. Further studies are necessary for isolation and identification of biologically active substances from the leaves of the plant.

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PURIFICATION, CHARACTERIZATION OF GLUTATHIONE S-TRANSFERASE FROM THE GILL TISSUE OF LAKE VAN FISH AND INHIBITION EFFECTS OF SOME METAL IONS AND PESTICIDES

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ABSTRACT

Glutathione-S-Transferases (GSTs) are xenobiotic metabolizing enzymes that protect cells from toxic drugs and environmental electrophiles. Furthermore, these enzymes have affected the regulators of oxidative stress and toxicity [1]. In earlier studies; GST enzymes have been purified and characterized from many living things such as sheep, bird, fish, bacterium, bovine and human. This study proves the purification and characterization of GST enzyme (E.C. 2.5.1.18) from the Gill Tissue of Lake Van Fish by affinity chromatography. Inhibitory effects of some metal ions and pesticides on GST activity were determined with using the CDNB method under in vitro conditions. Purification degree for the purified enzyme was controlled by SDS-PAGE. Optimal pH, optimal ionic strength, optimal temperature and stable pH were determined as 7.3, 120 mM, 35°C, 8.0, respectively. Inhibitory effects of Al³⁺, Ba²⁺, B³⁺ and Se²⁻ metal ions and Oxamyl, Diniconazole, Carbofuran, Tebuconazole and Atrazine pesticides have been examined on the purified GST enzyme. Inhibition graphics have been drawn in order to find the IC50 values of metals and pesticides show inhibition from Activity % -[Inhibitor] graphs. Consequently, in vitro inhibition rank order was determined as Al³⁺ > Ba²⁺ > Se²⁻ > B³⁺ for metal ions, Carbofuran > Tebuconazole > Oxamyl > Atrazine > Diniconazole for pesticides.

KEYWORDS:
Affinity, Metal Ion, Pesticide, Inhibition, Gill

INTRODUCTION

Air, water, soil and other natural sources can be contaminated with different chemicals because of technological reasons. At the head of these chemicals; there are heavy metals and pesticides. These chemicals entering the metabolism by the way of respiration, water and foods lead to toxic effects in the body [2].

All living organisms have developed protective mechanisms that including detoxification and an antioxidant protective system that covers glutathione reductase, glutathione peroxidase and glutathione S-transferases against xenobiotics and oxidative stress [3]. Glutathione S-transferases, which is one of the natural protective systems have a significant role in the detoxification of electrophilic xenobiotics such as anticancer drugs, herbicides, pesticides, chemical carcinogens and environmental pollutants [4]. It is anticipated that the enzyme protects the cells against pesticides, drugs and foreign substances such as carcinogenic [5]. Glutathione S-transferases catalyse reactions between glutathione and lipophilic compounds with electrophilic centres, leading to neutralisation of toxic compounds, xenobiotics and products of oxidative stress [6].

The inhibition effects of numerous substances like medical drugs, various metals, anions and pesticides have been reported in literature. Normal enzyme activity is changed with high levels of chemicals and this affects the metabolism, especially inhibition of specific enzymes and the effects can be both dramatic and systemic [7, 8].

Lake Van is the largest alkaline lake in the worldwide with an area of 3.713 km² and a 430-km shore. The lake surface lies 1.646 m above the sea level and the lake average depth is 171 m. The lake water is brackish with a salinity between 21.5 and 22.5%, highly alkaline with a pH between 9.7 and 9.9 and contains sulfates with concentrations comparable to seawater. Besides, its brackish water (0.22%) and high pH (9.8) greatly limits the type of living organisms [9]. Lake Van Fish is an anadromous species that migrates to the streams (salinity 0.02%, pH 8.42) flowing into Lake Van (salinity 0.22%, pH 9.8) during the spawning period (April–July) [10]. Lake Van Fish (Alburnus tarichi Güldenstädt, 1814) is the only vertebrate species adapted to the extreme conditions of Lake Van. Even though some other fish species were introduced into
the lake, they died in a short time [11]. About 10,000 t of Lake Van Fish are fished per year. Thus, this species is a substantial protein source for the people inhabiting the region.

Fish are used as practical experimental organisms for the valuation of the aquatic ecosystems subjected to environmental pollution and are related to biochemical changes [12]. It is important to realize that the detoxification mechanism of aquatic species is contaminated with pesticides, metals, persistent, and herbicides pollutants [13]. The gill is the first tissue contacting with the contaminants in the water. Owing to its small diffusion distance and large surface area between the blood and water, the gills are principally affected by pollutants such as metals [14].

The aim of our study is to purify the GST enzyme from the Gill Tissue of Lake Van Fish, characterize it and examine the inhibitory effects of Al³⁺, Ba²⁺, B³⁺ and Se²⁻ metal ions and Oxamyl, Dimiconazole, Carbofuran, Tebuconazole and Atrazine pesticides.

**MATERIALS AND METHODS**

**Chemicals.** N,N,N’,N’- Tetramethylethylene-diamine (TEMED), Glutathione–Agarose, 1-Chloro-2,4-dinitrobenzene, L-Glutathione reduced (GSH), Coommassie Brilliant Blue R-250, Bromine Thymol Blue, protein assay reagents were obtained from Sigma-Aldrich Co. All other chemicals were of analytical grade and obtained from Merck.

**Glutathione-Agarose Affinity Chromatography.** 1 g lyophilized powder of glutathione-agarose was weighed for 10 ml of bed volume and washed several times with 200 ml distilled water to remove solid particles. Washing swelled the gel, which was deaerated by using a water trompe and suspended by adding stabilization buffer (0.05 M K-phosphate (pH= 7.5), 1 mM EDTA, and 1 mM DTT). The suspended gel was packed in a cooled column with a 1x10 cm closed system. After precipitation, the gel was washed by using a peristaltic pump with stabilization buffer. Stabilization of the column was clear from the fact that the absorbance and pH of the eluate and buffer were identical. The affinity column was thus prepared.

**Purification of GST enzyme from the Gill Tissue of Lake Van Fish by affinity chromatography.** Fish from Van Lake were brought to our research laboratory according to the cold chain rules. Once the gills of the fish had separated, the gills separated into small pieces. The fish gills were homogenized with liquid nitrogen. The homogenate was taken to 2 volumes of buffer solution (10 mM KH₂PO₄/150 mM NaCl, pH = 7.4) The tissue samples were centrifuged at 12.500 rpm for 60 min and the plasma and precipitate were removed. The homogenate was applied to the prepared Glutathione–Agarose affinity column equilibrated with 10 mM KH₂PO₄/150 mM NaCl, (pH = 7.4). The affinity gel was washed with 10 mM KH₂PO₄/0.1 M KCl, (pH = 8.0). The Lake Van Fish Gill Tissue GST enzyme were eluted with 50 mM KH₂PO₄/2.5–10 mM GSH (pH = 7.5). All of the purification steps were performed at 4°C.

**Determination of GST activity.** Glutathione S-transferase activity was performed by using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to Habig et al. Among its many other substrates, the aromatic electrophile 1-chloro-2,4-dinitrobenzene is one that is the most often used substrate in GST enzyme activity determination. The reaction product, dinitrobenzene S-glutathione (DNB-SG), displays maximum absorbance at 340 nm. Activity measurements were thus carried out by measuring the absorbance increase at this wavelength [15].

**Protein determination.** During each purification step, protein determination was performed spectrophotometrically at 595 nm according to the Bradford method, using bovine serum albumin as the standard [16].

**SDS polyacrylamide gel electrophoresis.** After the purification steps, SDS polyacrylamide gel electrophoresis was performed to verify enzyme purity. It was carried out in 15% and 3% acrylamide for the running and the stacking gel, respectively, containing 0.1% SDS according to Laemmli procedure. A 20 μg sample was applied to the electrophoresis medium. Gels were stained for 2 h in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, then stain removed with several changes of the same solvent without dye [17] (Figure 1).

**Optimum pH Determination.** In order to determine the optimum pH, K-phosphate buffers were used in the pH range of 5.0 to 8.0.

**Optimum Temperature Determination.** For determination of the optimum temperature, enzyme activity was assayed at different temperatures in the range from 5°C to 70°C. The desired temperature was provided by using a Grant bath.

**Optimum Ionic Strength Determination.** For the determination of optimum ionic strength, enzyme activity was determined by using different concentrations of K-phosphate buffer, pH: 7.3, in the range from 10mM to 1000mM.
Lane (1) 170 kDa, 130 kDa, 100 kDa, 70 kDa, 55 kDa, 40 kDa, 35 kDa, 25 kDa standard proteins.
Lane (2) GST enzyme purified from the Gill Tissue of Lake Van Fish.

For this aim, equal volumes of the buffers K-phosphate at pH of 7.0, 7.3 and 8.0, and purified enzyme were mixed and kept in a refrigerator (+4°C). The enzyme activity was assayed during a week at twenty-four hours intervals.

**Stable pH Determination.** For this aim, equal volumes of the buffers K-phosphate at pH of 7.0, 7.3 and 8.0, and purified enzyme were mixed and kept in a refrigerator (+4°C). The enzyme activity was assayed during a week at twenty-four hours intervals.

**In vitro effects of compounds.** The inhibitory effects of some metals and pesticides were analysed at different inhibitor concentrations. Compounds showing inhibitory effects were tested in triplicate at each concentration usage. We measured GST activ-
ity in the presence of inhibitor concentrations. Control activity in the absence of inhibitor was taken as 100%. For each metal and pesticides, activity (%) vs. inhibitor concentration graphs were drawn. Ki constants were calculated by using the Cheng-Prusoff equation to determine the constants Ki of each metals and pesticides causing the inhibition [18].

RESULTS AND DISCUSSION

In this experimental study, Lake Van Fish Gill Tissue GST enzyme was characterized for the first time. The GST enzyme was purified at about 1543.51-fold with a yield of 82.25%, and a specific activity of 11344.83 EU/mg proteins. Parallel results have been observed for the enzyme from different sources. For instance, rainbow trout erythrocytes GST was purified 11026-fold with a yield of 59%, and a specific activity of 16.54 EU/mg proteins [19] (monopterus albus) fishy gill GST was purified 300-fold with a yield of 14%, and a specific activity of 13.07 EU/mg proteins [20], human erythrocytes GST was purified 1143-fold with a yield of 80%, and a specific activity of 16.00 EU/mg proteins [21]. When other studies in the literature are analyzed, it can be seen that the enzyme has been purified at low yields in 2-3 steps. However, we purified the enzyme with higher efficiency in a single step by using affinity chromatography.

For the aim of finding $K_M$ and $V_{\text{max}}$ values for GSH and CDNB substrates of GST enzyme purified from the Gill Tissue of Lake Van Fish, activity measurement was performed with five different CDNB concentration at the stable GST concentrations. $1/[\text{CDNB}]-1/V$ Lineweaver-Burk plots were drawn by using the obtained data [22]. With the aid of this graph, $K_M$ and $V_{\text{max}}$ values of the purified enzyme were determined to be 1.0574 mM and 0.373 EU/mL, respectively (pH: 6.0 and 20°C) for CDNB. Similarly by performing the activity measurements with GSH’s five different concentrations at the stable CDNB concentration $1/[\text{GSH}]-1/V$ Lineweaver-Burk plots were drawn. With the aid of this graph, $K_M$ and $V_{\text{max}}$ values of the purified enzyme were determined to be 0.1590 mM and 0.0854 EU/mL, respectively (pH: 6.0 and 20°C) for GSH.

To determine molecular weight of the enzyme, SDS-PAGE was done. The molecular weight was determined to be 32.218 kDa. Similar results have been observed for the enzyme from different sources. For example, rainbow trout liver GST is 23 kDa (homodimer), Atlantic salmon GST and brown trout GST are approximate 23.7 kDa [23], Lettuce (Lactuca sativa) GST is 23 kDa [24], Tilapia (Oreochromis niloticus) fishy gill GST is 25.46 kDa and (monopterus albus) fishy gill GST is 26 kDa [20].

The enzyme was seen to exhibit the highest activity at 35°C in a study of temperatures between 5°C-70°C. Huang et al. found that the optimum temperature of (monopterus albus) fishy gill GST enzyme as 45°C [20]. Comakli et al. found that the optimum temperature of rainbow trout erythrocytes GST enzyme as 30°C [19].

As a result of the characterization of the Lake Van Fish Gill Tissue GST enzyme, optimal pH, optimal ionic strength, optimal temperature and stable pH were determined as 7.3, 120 mM, 35°C, 8.0, respectively.

Today, all living things can unfortunately be exposed to the effects of different kinds of chemical substances. This situation can be the result of people’s unconsciously use of environment. Although there is no connection with the atmosphere of such a work environment, people and other organisms are primarily affected by the waste created by people. Particularly, the elimination of waste materials from factories in various countries has been made by burying into the ground or releasing to the air. As a result, many toxic substances such as heavy metals and pesticides, have been passing into the soil, water, plants, and then from there to animals and people. So today, especially enzyme activity studies made by using these substances are very popular [25]. Especially, scientists have reported that it causes the toxic effects such as the inhibition effect of metal ions on different enzymes in protein structure, which play an important role in the execution of metabolic activities in living organisms [26].

Metals are components found in trace quantities in the aquatic environment. But, development in industry, agriculture, and mining has led to an increase in their levels [27]. This increase has become one of the most important problems of environmental toxicology. This has become very threatening for living organisms, especially for living organisms in aquatic environments containing specific enzymes. It is known that enzymes catalyze nearly all of the reactions that occur in living systems. Contaminants such as metal ions take effect by increasing or decreasing enzyme activity even at very low concentrations. This can be achieved by binding to non-metals containing an unbound electron pair such as N, O, and S present in the structure of the active enzymes [25, 26]. Some heavy metals such as Ni, Ag, and Cd, which fish are exposed to, accumulate in their tissues. The human, at the top of the food chain, is at the greatest risk of exposure to health problems as a result of consuming these living things [28]. In a study by Ozaslan and colleagues [29], it was reported that Cd$^{2+}$, Ag$^+$, Zn$^{2+}$ and Cu$^{2+}$ metals inhibited Lake Van Fish Gill Tissue GST enzyme. In another study by Kuzu and colleagues [30], it was reported that Cd$^{2+}$, Ag$^+$, Ni$^{2+}$ and Cu$^{2+}$ metals inhibited Lake Van Fish Gill Tissue CA I and CA II isoenzymes.
and pesticides were added to the reaction medium. GST enzyme, different concentrations of metal ions (Al³⁺, Ba²⁺, B³⁺ and Se²⁻) (Table 2) and pesticides (Oxamyl, Diniconazole, Carbofuran, Tebuconazole and Atrazine) (Table 3) on the Lake Van Fish Gill Tissue GST enzyme in a single step by affinity chromatography and analyzed characteristic features. In order to analyze the effects of the metal ions, the determination of different kinds of enzymes, the determination of structural and kinetic features is going on today.

The enzyme activity was measured and an experiment was done in the absence of inhibitor was used as control (100% activity). The IC₅₀ values were obtained from activity (%) vs. inhibitor concentration plots. Kᵢ constants were calculated using the Cheng Prusoff equation to determine Kᵢ constants for the metal ions and pesticides causing the inhibition. IC₅₀ values of Al³⁺, Ba²⁺, B³⁺ and Se²⁻ metals were calculated as 0.072, 0.082, 0.100, and 0.083 mM, respectively. IC₅₀ values of Oxamyl, Diniconazole, Carbofuran, Tebuconazole and Atrazine pesticides were calculated as 0.062, 1.604, 0.0415, 0.056, and 0.750 mM, respectively. Kᵢ values of Al³⁺, Ba²⁺, B³⁺ and Se²⁻ metals were calculated as 0.0028, 0.0032, 0.0040, and 0.0033 mM, respectively. Kᵢ values of Oxamyl, Diniconazole, Carbofuran, Tebuconazole and Atrazine pesticides were calculated as 0.0028, 0.0032, 0.0040, and 0.0033 mM, respectively. Consequently, in vitro inhibition rank order was determined as Al³⁺ > Ba²⁺ > Se²⁻ > B³⁺ for metal ions, Carbofuran > Tebuconazole > Oxamyl > Atrazine > Diniconazole for pesticides.

According to this study Al³⁺, Ba²⁺, B³⁺ and Se²⁻ metals (Table 2) and Oxamyl, Diniconazole, Carbofuran, Tebuconazole and Atrazine pesticides (Table 3) were found to inhibit the enzyme at much lower concentrations. In literature, it has also been reported that metals have negative effects on metabolism and embryonic development and genotoxic effect in fish [33]. In recent years, industrial development, rapid population growth, agricultural activities, and mining have also caused the aquatic environment to be exposed to these negative effects [28]. Consequently, in fish that are at the top of the aquatic food chain, chemicals such as heavy metals accumulate in body tissues and reach high levels and show toxic effects [34]. In the present study, we can say that metals and pesticides have negative effects on health. Because of this, attention should be paid to industrial and metal residues.

**ACKNOWLEDGEMENTS**

This study is supported by Agri Ibrahim Cecen University Scientific Research Projects Coordination (SYO.15.002).

The authors report no conflicts of interest.

### TABLE 1
Summary of purification procedure for Lake Van Fish Gill Tissue GST enzyme.

<table>
<thead>
<tr>
<th>Purification Steps</th>
<th>Activity (EU/ml)</th>
<th>Total volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (EU/mg)</th>
<th>Specific activity (EU/mg)</th>
<th>Yield %</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>240</td>
<td>20</td>
<td>32.65</td>
<td>653</td>
<td>4800</td>
<td>7.35</td>
<td>100.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Glutathione-agarose affinity chromatography</td>
<td>658</td>
<td>6</td>
<td>0.058</td>
<td>0.348</td>
<td>3948</td>
<td>11344.83</td>
<td>82.25</td>
<td>1543.51</td>
</tr>
</tbody>
</table>

### TABLE 2
Kᵢ values and IC₅₀ values for some metal ions of Lake Van Fish Gill Tissue GST enzyme.

<table>
<thead>
<tr>
<th>Type of metal</th>
<th>Average values of IC₅₀ (mM)</th>
<th>Average values of Kᵢ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B⁺⁺</td>
<td>0.100</td>
<td>0.0040</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>0.082</td>
<td>0.0032</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>0.072</td>
<td>0.0028</td>
</tr>
<tr>
<td>Se²⁻</td>
<td>0.083</td>
<td>0.0033</td>
</tr>
</tbody>
</table>

### TABLE 3
Kᵢ values and IC₅₀ values for pesticides of Lake Van Fish Gill Tissue GST enzyme.

<table>
<thead>
<tr>
<th>Type of pesticide</th>
<th>Average values of Kᵢ (mM)</th>
<th>Average values of IC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxamyl</td>
<td>0.062</td>
<td>0.0024</td>
</tr>
<tr>
<td>Diniconazole</td>
<td>1.604</td>
<td>0.0644</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.0415</td>
<td>0.0016</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>0.056</td>
<td>0.0022</td>
</tr>
<tr>
<td>Atrazine</td>
<td>0.750</td>
<td>0.0301</td>
</tr>
</tbody>
</table>

With a specific role in the metabolism of a wide group of electrophilic substrate that include antibiotic, analgesic, and anticancer medications, glutathione S- transferases [31] constitute a group of multifunctional enzymes which are involved in conjugating both endogenous and exogenous electrophilic compounds with reduced glutathione (GSH) and thus catalyzing their conversion into less toxic metabolites [32]. The properties of Glutathione S-transferases which have been purified from mammals such as human, mouse, bovine and rabbit have been characterized in a detailed way. But, the GST enzyme in fish is not as well-characterized as the GST enzymes in living organisms. Besides, the purification of different kinds of enzymes, the determination of structural and kinetic features is going on today. These studies are very crucial in terms of drug design and eliciting of mechanisms of the enzymes. For this reason, the research that is made by us is very important.

Consequently, we purified Lake Van Fish Gill Tissue GST enzyme in a single step by affinity chromatography and analyzed characteristic features. In order to analyze the effects of the metal ions (Al³⁺, Ba²⁺, B³⁺ and Se²⁻) (Table 2) and pesticides (Oxamyl, Diniconazole, Carbofuran, Tebuconazole and Atrazine) (Table 3) on the Lake Van Fish Gill Tissue GST enzyme, different concentrations of metal ions and pesticides were added to the reaction medium.
REFERENCES


[18] Cheng, Y.C., Prussoff, W.H. (1973) Relationship between the inhibition constant (Kᵢ) and the concentration of inhibitor which causes 50 percent inhibition (I₅₀) of an enzymatic reaction. Biochemical Pharmacology. 22, 3099-3108.


EVALUATION OF CARROT (DAUCUS CAROTA L.)
GERMLAMPS UNDER DROUGHT STRESS

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Saba Manzoor1, Nasim A Yasin2, Asghri Bano1, Philipp W Simon4

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ABSTRACT

A field experiment was designed in which 19 genotypes were evaluated in RCBD, with three
recursions, inseeded under drought conditions. After 30 days of stress all the genotypes were
morphologically, biochemically and physiologically evaluated. The obtained results suggest that whole
studied genotypes respond to stress and all the studied
attributes were affected significantly at
\(P<0.005\). Three genotypes i.e. Ames31193, Ames26383 and Ames29111 have minimum
drought susceptibility index as well as highest
drought tolerance efficiency and therefore these are
considered as drought tolerant and can be used for
further breeding programmes.

KEYWORDS:
Drought stress, Drought susceptibility Index, Genotypes, Biochemical, Morphological, Carrot.

INTRODUCTION

Plants face different biotic and abiotic stresses that
lead to reduce their yield [1]. In the category of
abiotic stress, drought stress has a major contribu-
tion i.e. 16% of all other stresses [2, 3]. Major reason
behind this continuous increment of drought stress is the increasing pressure on water resources
that results in the reduction of total crop yield up to
82% [4, 5, 6]. In Pakistan same scenario exists due
to limited and irregular pattern of rain fall [7, 8].

Vegetables play a significant role in human diet and limited supply of water drastically effects
their yield [9]. Carrot is a root vegetable that consists of about 80-90% water and slightly reduction
in water supply drastically affects its yield as well as nutritional components [10]. Carrot is a very
important vegetable crop due its nutritional (Vita-
min B) and medicinal (Anti-oxidant) constituents
[11, 12].

Under stress conditions plants exhibit different morphological, physiological and biochemical adapt-
tations that protects them from the harmful ef-
fects of stress [13]. Morphological adaptations in-
clude decrease in leaf area\(^1\), root diameter, and root
length while biochemical attributes that play signif-
icient role under stress conditions are total soluble
proteins, total soluble sugars and reducing sugars
[14, 15, 16].

The aim of current research work was to eval-
uate drought resistant genotypes that can be used
for further breeding programs by using above men-
tioned standards.

MATERIALS AND METHODS

In present research work 19 wild genotypes of
carrot were evaluated against drought stress in Ja-
hurabad, Pakistan. RCBD design was applied by
using two treatments T\(_0\) (normal) and T\(_1\) (Stress),
with three replicates. Until germination in both
conditions proper irrigation was carried out. After 15
days of complete germination In T\(_0\) 100% field
capacity while in T\(_1\) 50% field capacity was man-
aged.

After 90 days of sowing, five plants from each
replicate were selected to study morphological and
biochemical parameters.

Biochemical attributes were studied in molecular
lab of department of Botany, university of Sar-
godha by using UV-1100 Absorption spectropho-
tometer. Activity of total soluble proteins was
measured by the method of Roensen and Johnson
[18]. Concentration of total soluble sugars and re-
ducing sugars were measured by the methods of
Yemm and Willis [18] and Muhammad and Tabas-
sum [19] respectively.

Results of current research work was statisti-
cally analysed by using descriptive stat, analysis of
covariance and principal component analysis for all
the operations XLSTAT and Statistix 8.1.
TABLE 1
Descriptive stats of the effect of drought for different traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>Treatment</th>
<th>Mean</th>
<th>Reduction %</th>
<th>Minimum</th>
<th>Maximum</th>
<th>S.D.</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL</td>
<td>N</td>
<td>16.04</td>
<td>17.337</td>
<td>9.53</td>
<td>33.7</td>
<td>5.22</td>
<td>1.199</td>
</tr>
<tr>
<td>(cm)</td>
<td>S</td>
<td>13.259</td>
<td>7.45</td>
<td>30.233</td>
<td>4.97</td>
<td>1.141</td>
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</tr>
<tr>
<td>RW</td>
<td>N</td>
<td>2.217</td>
<td>10.17</td>
<td>0.21</td>
<td>11.33</td>
<td>2.42</td>
<td>0.556</td>
</tr>
<tr>
<td>(gm)</td>
<td>S</td>
<td>1.992</td>
<td>0.14</td>
<td>0.14</td>
<td>11</td>
<td>2.38</td>
<td>0.547</td>
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<tr>
<td>RD</td>
<td>N</td>
<td>0.421</td>
<td>25.106</td>
<td>0.015</td>
<td>1.7833</td>
<td>0.37</td>
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<tr>
<td>(cm)</td>
<td>S</td>
<td>0.315</td>
<td>0.0073</td>
<td>1.6033</td>
<td>0.34</td>
<td>0.078</td>
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<tr>
<td>LA</td>
<td>N</td>
<td>444.230</td>
<td>273.00</td>
<td>1828.386</td>
<td>340.88</td>
<td>78.20</td>
<td></td>
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<tr>
<td>(cm²)</td>
<td>S</td>
<td>417.175</td>
<td>6.090</td>
<td>248</td>
<td>1719</td>
<td>321.26</td>
<td>73.70</td>
</tr>
<tr>
<td>TP</td>
<td>N</td>
<td>9.962</td>
<td>3.129</td>
<td>43.05</td>
<td>11.91</td>
<td>2.732</td>
<td></td>
</tr>
<tr>
<td>(mg gm⁻³ of tissue)</td>
<td>S</td>
<td>9.545</td>
<td>4.1924</td>
<td>2.94</td>
<td>42.34</td>
<td>11.54</td>
<td>2.648</td>
</tr>
<tr>
<td>TS</td>
<td>N</td>
<td>3.694</td>
<td>3.049</td>
<td>4.396</td>
<td>0.483</td>
<td>0.110</td>
<td></td>
</tr>
<tr>
<td>(mg gm⁻³ of tissue)</td>
<td>S</td>
<td>3.98</td>
<td>-7.74</td>
<td>3.289</td>
<td>4.631</td>
<td>0.462</td>
<td>0.106</td>
</tr>
<tr>
<td>RS</td>
<td>N</td>
<td>3.239</td>
<td>2.793</td>
<td>3.95</td>
<td>0.379</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>(mg gm⁻³ of tissue)</td>
<td>S</td>
<td>3.479</td>
<td>6.892</td>
<td>3.12</td>
<td>4.540</td>
<td>0.300</td>
<td>0.068</td>
</tr>
</tbody>
</table>

N= Normal, S= Stress, RL= Root length, RW= Root Weight, RD= Root diameter, LA= Leaf Area, TP= total soluble protein contents and TS= total soluble Sugars, RS= Reducing Sugars

TABLE 2
Response of selected genotypes against stress

<table>
<thead>
<tr>
<th>SR</th>
<th>Genotype</th>
<th>DSI %</th>
<th>DTE %</th>
<th>SR</th>
<th>Genotype</th>
<th>DSI %</th>
<th>DTE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1</td>
<td>0.765014</td>
<td>72.09302</td>
<td>11</td>
<td>Ames29084</td>
<td>0.841494</td>
<td>79.30029</td>
</tr>
<tr>
<td>2</td>
<td>Ames27397</td>
<td>0.980041</td>
<td>92.35669</td>
<td>12</td>
<td>Ames27396</td>
<td>0.96468</td>
<td>90.90090</td>
</tr>
<tr>
<td>3</td>
<td>Ames25740</td>
<td>0.936583</td>
<td>88.26125</td>
<td>13</td>
<td>P1 279764</td>
<td>0.846174</td>
<td>79.74138</td>
</tr>
<tr>
<td>4</td>
<td>P1</td>
<td>0.809994</td>
<td>92.44645</td>
<td>14</td>
<td>Ames30198</td>
<td>0.978837</td>
<td>92.24319</td>
</tr>
<tr>
<td>5</td>
<td>P1 252370</td>
<td>0.834013</td>
<td>78.59532</td>
<td>15</td>
<td>P1 478883</td>
<td>0.871744</td>
<td>82.15103</td>
</tr>
<tr>
<td>6</td>
<td>P1 252348</td>
<td>0.944987</td>
<td>89.05325</td>
<td>16</td>
<td>Ames26381</td>
<td>0.869239</td>
<td>81.91489</td>
</tr>
<tr>
<td>7</td>
<td>P1 252338</td>
<td>0.921593</td>
<td>86.84864</td>
<td>17</td>
<td>Ames31193</td>
<td>0.620672</td>
<td>58.49057</td>
</tr>
<tr>
<td>8</td>
<td>P1 250706</td>
<td>0.860063</td>
<td>81.05023</td>
<td>18</td>
<td>Ames26383</td>
<td>0.685573</td>
<td>64.60674</td>
</tr>
<tr>
<td>9</td>
<td>P1 250487</td>
<td>0.974238</td>
<td>91.80978</td>
<td>19</td>
<td>Ames29111</td>
<td>0.696379</td>
<td>65.625</td>
</tr>
<tr>
<td>10</td>
<td>P1 250235</td>
<td>0.977804</td>
<td>92.14586</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DSI %= drought susceptibility Index, DTE %= drought tolerance efficiency

RESULTS AND DISCUSSION

The data of descriptive statistics is presented in Table 1, which reflects the response of different growth parameters (root length, root weight, root diameter, leaf area, total soluble protein contents, total soluble Sugars and reducing Sugars) under normal and stress conditions. The reduction % for studied attributes is 17.33, 10.17, 25.10, 6.09, 4.19, 5.97 and 6.89 of tissue respectively.

Response of studied genotypes, under drought stress, is presented in Table 2. It is obvious from the results that three genotypes (i.e. Ames31193, Ames26383 and Ames29111) have lowest drought susceptibility index that means these genotypes are highly tolerant to drought stress, while the genotype P1 652351 has DSI 0.765, and presents tolerate behaviour against stress. Response of six genotypes (P1 652370, 0.860063, Ames29084, P1 279764, 0.478883 and Ames26381) depicts that these are susceptible to stress. Under stress conditions nine genotypes (i.e. Ames27397, Ames25740, P1 652391, P1 652348, P1 652388, P1 269487and P1 163235) have highest DSI and they are highly susceptible to stress.

Figure 1 depicts the distribution of 19 studied genotypes into four classes on the basis drought susceptibility index (DSI). According to data presented in Figure 1 the highest frequency was presented by 1st class (HS) which contain 9 genotypes and minimum were in 4th class (T) that contain only 1 genotype i.e. 2nd class presents highly tolerant genotypes i.e. P1 652351, Ames27397 and Ames25740.

Scarcity of water is one of major growth limiting factors of plants [21]. Results of Table 3 depict a highly significant relation to applied drought treatments and studied parameters. On the basis of LSD values it is obvious in the table that highly significant difference exists between two treatments as well as their relation to traits (i.e. RW, RL, RD, LA, TP, TS and RS).

According to Table 3 as a result of drought stress root length, root weight, root diameter and leaf area plant¹ are significantly (P ≤ 0.01) reduced. This decrease in morphological parameters is an adoptive measurement of plants to survive through harsh environments. The major reason behind this reduction in root is its location as interfaces between plant and soil. Our results are in accordance with the findings of Franco et al. [22] and Nahar et al. [23], who reported that root system is highly affected by drought stress due to its direct interaction with soil.
**FIGURE 1**
Frequency distribution of 19 studied genotypes on the basis of drought susceptibility index
HS = Highly susceptible, HT = Highly tolerant, S = Susceptible, T = Tolerant

**TABLE 3**
Randomized Complete Block ANOVA for Root length

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENOTYPE</td>
<td>18</td>
<td>156.020</td>
<td>34.7324</td>
<td>162.96***</td>
<td></td>
</tr>
<tr>
<td>TREATMENT</td>
<td>1</td>
<td>220.435</td>
<td>1.4507</td>
<td>19.68***</td>
<td></td>
</tr>
<tr>
<td>MEAN OF NORMAL</td>
<td>16.041</td>
<td>2.2179*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN OF STRESS</td>
<td>13.260</td>
<td>1.9923*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.4326</td>
<td>0.1010</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| GENOTYPE     | 18 | 0.755     | 23428.7 | 17.33*** |
| TREATMENT    | 1  | 3.019     | 34429.1 | 264.25*** |
| MEAN OF NORMAL | 0.4217 | 372.97* |
| MEAN OF STRESS | 0.3158 | 338.21* |
| LSD          | 0.0505 | 4.2453 |

| GENOTYPE     | 18 | 825.118 | 1.33643 |
| TREATMENT    | 1  | 4.972   | 1.56949 |
| MEAN OF NORMAL | 9.9628 | 3.9290 |
| MEAN OF STRESS | 9.5451 | 3.6943 |
| LSD          | 0.2913 | 0.0743 |

| GENOTYPE     | 18 | 0.65896 | 41.20*** |
| TREATMENT    | 1  | 1.63893 |          |
| MEAN OF NORMAL | 3.4792  |          |
| MEAN OF STRESS | 3.2394  |          |
| LSD          | 0.0742 |          |

RL= Root length, RW= Root Weight, RD= Root diameter, LA= Leaf Area, TP= total soluble protein contents and TS= total soluble Sugars, RS= Reducing Sugars.

Shortage of water effects the biomass production in root as our results in Table 3 depicts, as a result of drought stress root weight of plant is significantly (P ≤ 0.01) reduced. During drought conditions plants close their stomata to prevent water loss which correspondingly permits less CO₂ intake into the plants. This is associated with reduction in photosynthetic activity leading to a reduced biomass production [24]. Limited supplies of water have deep impact on the morphology of the roots. Under stress environment thin roots are produced to enhance surface to volume ratio. Table 2 shows the same response of root diameter that is significantly (P ≤ 0.01) reduced under drought stress. Leaves are the primary sites for photosynthesis through which transpiration occur. In short time limiting supply of water, plant close their stomata to control transpiration rate but prolonged duration of stress results in reduction of leaf size as shown in Table 3. Our results were supported by the findings of Ramanjulu and Sudhakar, [25], Yordanov et al., [26], Mwanamwengu et al., [27] who reported that as a result of drought stress leaf area is decreased.

Plants respond to environmental stress by adopting different biochemical measures [28, 29]. As presented in Table 3 highly significant (P ≤ 0.01) reduction occur in the accumulation of total soluble proteins, total soluble sugars and reducing sugars. Decrease in the activity level of total soluble proteins under stress seems due to multiple reasons e.g. During water stress rate of photosynthesis decreases followed by the shortage of material for protein synthesis that decrease protein level [30]. During stress period, a continuous reduction of total soluble proteins is also induced by proteolysis [31]. Findings of Iqbal and Banó [32], Bayramov et al. [33] also supports our results.

Data presented in Table 3 depicts a strong relation of stress with concentration of total soluble sugars and reducing sugars. Soluble sugars acts as
an osmylates that play a vital role in defensive system of plants against stress and maintain turgidity in its tissues [34]. During stress conditions starch degradation increases and as a result of which soluble sugar level increases that protects cell membranes [35]. Our results also collaborate with the findings of Hassini et al. [36], Luo et al. [37] and Parida et al. [38].

PCA was performed on studied attributes with 19 genotypes by taking parameters as vectors. Observations (genotypes) were spread into two dimensional spaces accordingly. Another supplementary variable was added into the analysis to split the treatment (Normal and Stress). Expression of genotypes was bisected with respect to the treatment applied. Transformed variable PC1 and PC2 were appeared to express 58.68% variation collectively, PC1 (eigenvalue 2.34) explained 33.43% variability and PC2 (eigenvalue 1.76) depicts 25.23% variation (Table 3).

Root diameter and root weight were found highly correlated with PC1 while root length and leaf area were extended to PC2. According to figure 2 genotype i.e. Ames 27397 attained the highest values of RW, RL and LA while four genotypes P1 652351, P1 652370, P1 280706 and Ames26381 have highest root lengths. Genotypes Ames27396, P1 478883 and Ames31193 have lowest root weight, root diameter and root length.

REFERENCES


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BIOLOGICAL SYNTHESIS OF SILVER NANO PARTICLES FROM THE FISH PATHOGEN ESCHERICHIA HERMANNII AND ITS ANTIMICROBIAL AFFECTS AGAINST HUMAN MICROBIOLOGICAL PATHOGENS

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2Ahi Evran University, Department of Medical Services and Techniques, Health Services Vocational College, Kirşehir, Turkey
3Ondokuz Mayis University, Medical Faculty, Department of Family Medicine, Samsun, Turkey

ABSTRACT

The purpose of this study was to investigate the antimicrobial activity of silver nanoparticles (AgNPs) biosynthesized by isolated Escherichia hermannii obtained from aquacultured fish on several human pathogens. AgNPs were isolated once E. hermannii had been isolated from the organs of Cyprinus carpio L. and Tinca tinca. The biosynthesis of AgNPs was measured using a UV-visible and FTIR analysis. The antimicrobial activity of AgNPs was evaluated using the well diffusion method. MIC values were determined and compared with the results obtained using commercial antibiotics for positive control. The antimicrobial activities of AgNPs at two different concentrations (0.5μM, 1μM) were measured using the diameter of inhibition zone (DIZ). Although AgNPs exhibited several degrees of antimicrobial effect on the microorganisms (DIZ values between 8 and 25 mm) included in the study, the highest effects were observed against Listeria monocytogenes, Salmonella typhimurium, Candida albicans (DIZ=25mm), and Shigella dysenteriae (DIZ=20mm) at a concentration of 1 mM AgNPs. No antimicrobial effect was observed against Proteus mirabilis. The results obtained are thought to be an effective antibacterial agent against human pathogens by the biosynthesis AgNPs of E. hermannii and based on the data of the obtained indices, these AgNPs can be considered as an alternative to today's antibiotics.

KEYWORDS:
Escherichia hermannii, Antimicrobial activity, Silver nanoparticles, Pathogenic bacteria.

INTRODUCTION

Nanotechnology can be defined as the study, design, creation, synthesis, manipulation and application of functional materials, devices, and structures through the control of matter at the nanometer level (1-100 nm). In recent years, metal nanoparticles have received considerable attention in terms of new applications in biotechnology, catalysis, electronics, and optics [1, 2]. One of the most interesting effects of metal nanoparticles is their antimicrobial activity [3]. Silver nanoparticles (AgNPs) have attracted particular interest in this context [4]. The antibacterial effect of AgNPs is mostly related to their accumulation on the pathogen microorganism cell wall, which causes "pits" and eventually leads to cell death [5]. These features helped them exhibit high antimicrobial (antifungal and antiviral) and bactericidal activity against Gram-positive and Gram-negative bacteria, including highly multi-resistant strains [6].

Eco-friendly green synthesis has recently become important due to the use of biological organisms such as bacteria, molds and microalgae as an alternative method for the synthesis of AgNPs [7, 8]. Even more limited data are therefore available concerning the use of biologically synthesized AgNPs in fish flora against other pathogens. This study was therefore planned to investigate the antibacterial effects of biologically synthesized AgNPs in Escherichia hermannii obtained from fish cultures against several human pathogens. Whereby E. hermannii is able to tolerate toxic hydrocarbons and metals contaminated environments, it has been potentially used in areas such as bioremediation, biodegradation, industrial waste treatment, municipal wastewater treatment, organic waste disposal and composting [9, 10]. In the scientific literature, there is no evidence that E. hermannii may have adverse effects on animal or plant populations in the environment. The aim of this study was to investigate the antibacterial effect of biologically synthesized AgNPs from E. hermannii obtained from fish against several human pathogen organisms.

MATERIALS AND METHODS

Isolation and identification of bacterial strains. The bacteria in this study were isolated from the organs of Cyprinus carpio L. and Tinca
tinca collected from Hırfanlı Dam Lake (Turkey). Nutrient agar was planted from fish organs and incubated at 37°C for 72 hours. Individual colonies of bacteria which varied in shape and color were selected and purified by streaking on nutrient agar. Bacteria were identified using the VITEK 2 system, and 37 isolated bacteria were collected, purified and identified. E. hermannii was selected from these 37 isolates for this study.

**Synthesis of AgNPs.** The reactions were carried out under an atmosphere of air. In a typical synthesis procedure, AgNO₃ was dissolved in deionized water. For AgNP synthesis, E. hermannii nutrient broth was inoculated and then centrifuged, and the resulting supernatant was used. The supernatants were added to 100 ml of 0.5 mM and 1 mM silver nitrate solution in 250 ml aliquots and incubated at 30°C for 18, 24 and 72 hours. The blank test was also performed in the absence of silver nitrate [11]. All studies were conducted in triplicate.

**UV-visible spectroscopic analysis.** Preliminary identification of AgNPs was carried out using UV-visible spectroscopy. UV-vis spectrum analysis was performed in the range of 420-480 nm (Thermo Scientific spectrophotometer Genesys 10S). The reduction of Ag⁺ ions was measured at time intervals of 18, 24 and 72 hours. AgNPs were visually examined for light brown to brown changes in the color of the culture medium, and nanoparticles were confirmed by measuring AgNPs with the UV-vis spectrum.

**Fourier Transform Infrared (FTIR) spectroscopy analysis.** FTIR spectroscopy was used for determination of the bond stretching vibration present in the AgNPs. Infrared analysis was carried out at the Ahi Evran University Central Laboratory, Kırşehir, Turkey, using a Thermo Scientific (Nicolet 6700) FTIR device. The FTIR spectrum of the dried samples was recorded at a range of 4000 to 450 cm⁻¹ at a resolution of 4 cm⁻¹ [12].

**Determination of antibacterial activity and minimum inhibitory concentration (MIC) determination.** The AgNPs synthesized from E. hermannii were tested for antimicrobial activity by the well-diffusion method against human pathogenic bacteria (Table 1). Bacterial cultures were also produced in Trypticase Soy Broth. Six-millimeter diameter wells were then made on Triptcase Soy Agar plates using gel puncture.

For the biosynthesized AgNPs, sterile distilled water was used as a control, and 11 other wells were loaded with 75 μL of AgNPs at 5 μg/ml, 2.5 μg/ml, 1.25 μg/ml, 0.75 μg/ml, 0.37 μg/ml, 0.187 μg/ml, 0.09 μg/ml, 0.046 μg/ml, 0.023 μg/ml and 0.01 μg/ml concentrations of nanoparticle solution. After incubation at 37°C for 48 hours, the diameters (in mm) of the different levels of zone of inhibition were measured. A broth microdilution method was used to determine the toxicity of AgNPs to pathogenic bacteria. Similar concentrations of AgNPs exhibited antimicrobial activity using the agar well-diffusion method. Bacterial growth was assessed in the presence and absence of AgNPs. Commercial antibiotic discs (Amoxicillin 10 mcg, Gentamicin 10 mcg and Nystatin 50 mcg) applied as positive control.

**Antimicrobial activity index.** Antimicrobial index of individual algal extracts were calculated as formula below [13].

\[
\text{Antimicrobial Index} = \frac{\text{Extract inhibition zone/ Antibiotic inhibition zone}}{100}
\]

**Statistical analysis.** Agar well diffusion and MIC were performed in triplicate, and the results were expressed as mean (±) standard deviation.

**Ethical Approval.** Ethical approval for this study was granted by the Ahi Evran University Ethical Committee.

<table>
<thead>
<tr>
<th>Human Pathogen Microorganisms</th>
<th>Code</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>ATCC 29906</td>
<td>gm negative</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>709 Roma</td>
<td>gm positive</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ATCC 35152</td>
<td>gm positive</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 29213</td>
<td>gm positive</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 27853</td>
<td>gm negative</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>ATCC 51342</td>
<td>gm negative</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>ATCC 14028</td>
<td>gm negative</td>
</tr>
<tr>
<td>Shigella dysenteria</td>
<td>ATCC 11835</td>
<td>gm negative</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC 25924</td>
<td>gm negative</td>
</tr>
<tr>
<td>Enterococcus fecalis</td>
<td>ATCC 29212</td>
<td>gm positive</td>
</tr>
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<td>Aeromonas hydrophila</td>
<td>ATCC7966</td>
<td>gm negative</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC 10774</td>
<td>gm positive</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>ATCC 10231</td>
<td>gm positive</td>
</tr>
</tbody>
</table>
RESULTS

Synthesis of AgNPs. In the current study, E. hermannii showed biosynthesis of AgNPs, this was observed by the visual observations of the changes in color of silver nitrate solution from light brown to brown at room temperature. The appearance of a yellow-brown color in AgNO₃-treated culture supernatant suggested the formation of AgNPs. A change in color indicates that aqueous silver ions have been reduced to AgNPs due to stimulation of surface plasmon vibrations in nanoparticles.

UV-visible spectroscopic analysis. AgNPs and their stability were characterized using UV-vis spectrophotometry. E. hermannii reactions were determined at different times (18, 24 and 72 hours). The UV-vis spectrum graphic is shown in Figure-1. Optically measured by UV-vis spectrophotometer showed absorbance peaks shifted from 420 to 480 nm.

FTIR. FTIR measurement was carried out to identify possible interaction between silver salts and protein molecules, which might account for the reduction of silver ions and stabilization of AgNPs (Figure 2). The assignments of the individual bands are listed in Table 2. The amide linkages between amino acid residues in proteins give rise to well-known signatures in the infrared region of the spectrum in E. hermannii. A dominant absorption that is often attributed to O-H stretching of hydroxyl groups was recorded at 3200 cm⁻¹. The strong and broad absorption band (3200 cm⁻¹) should be attributed mainly to N-H stretching vibrations of nucleic acids components. The IR absorption of cell proteins delivered several amide-related bands. These dominated at 1626 cm⁻¹ (amide I), 1537 cm⁻¹ (amide II) [14]. The peaks appearing in the region 1626 cm⁻¹ are attributed to the stretching vibration of the (C=O) group that is characteristic of proteins shifted after synthesis of AgNPs. This result shows that the protein molecules may be involved in AgNPs formation.

FIGURE 1
UV-visible absorption spectra of AgNPs synthesized by the culture supernatant of E. hermannii

FIGURE 2
FTIR spectral analysis of silver nanoparticles synthesized from E. hermannii
Antibacterial assay. The effect of AgNPs on bacteria was examined using the disk diffusion method. Biologically synthesized AgNPs exhibited considerable antibacterial activity. The biosynthesized nanoparticles showed antimicrobial activity against most bacteria at concentration of 0.37 μg/mL, 0.187 μg/mL and 0.023 μg/mL. The synthesized AgNPs exhibited antibacterial activity on the basis of both the well-diffusion method and growth curve analysis.

The antimicrobial activity of AgNPs was investigated against various bacteria, such as P. mirabilis, B. cereus, L. monocytogenes, S. aureus, P. aeruginosa, E. aerogenes, S. typhimurium, S. dysenteria, E. coli, E. feacalis, A. hydrophila, B. subtilis and C. albicans, using the well-diffusion method. The mean values of three replicates of the diameter of inhibition zones (in millimeters) around each well with AgNP solution are shown in Table 3. The highest level of antimicrobial activity was determined against L. monocytogenes, S. typhimurium and C. albicans with a DIZ measurement of 25 mm each for 1 mM AgNPs. The lowest level of activity was observed against S. aureus (DIZ= 8 mm).

The Gram-negative bacterium P. mirabilis exhibited no zone of inhibition. All test bacteria (approximately 10^8 cells/mL) were produced for 24 hours at different AgNP concentrations (0.01-5 μg/mL). Following this incubation, the viability of bacterial cells was determined by CFU counting. The MIC values of both AgNPs are presented in Table 3. The results show the effectiveness of AgNPs against S. aureus, E. coli, E. feacalis, B. subtilis and C. albicans with low MIC (0.01 μg/mL). The inhibition of AgNPs synthesized by E. hermannii against some pathogenic microorganisms in their agar cultures are represented in Figure 3.

### TABLE 2

<table>
<thead>
<tr>
<th>Main Peak (cm⁻¹) AgNO₃+ E. hermannii</th>
<th>Typical Band Assignment from the Literature</th>
<th>Wavenumber Range (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3200 Water -O(H) stretching</td>
<td>3646-3026</td>
<td></td>
</tr>
<tr>
<td>1626 Protein -N(H) stretching (amide A)</td>
<td>1709-1587</td>
<td></td>
</tr>
<tr>
<td>1537 Amide II band</td>
<td>1577-1481</td>
<td></td>
</tr>
<tr>
<td>1375 Amide I band</td>
<td>1408-1358</td>
<td></td>
</tr>
<tr>
<td>1290 Lipid δ(N(CH₃)₃) bending of methyl</td>
<td>1294-1194</td>
<td></td>
</tr>
<tr>
<td>1030 Nucleic Acid δ(C-P-O) stretching of phosphodiester</td>
<td>1136-980</td>
<td></td>
</tr>
<tr>
<td>816 Fingerprint region</td>
<td>900-600</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>Human Pathogen Microorganisms</th>
<th>0.5 mM AgNPs (DIZ mm)</th>
<th>1 mM AgNPs (DIZ mm)</th>
<th>Antibiotic Positive Control</th>
<th>AgNPs: AgNO₃+ E. hermannii (MIC (μg/mL))</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. mirabilis</td>
<td>NCD*</td>
<td>NCD*</td>
<td>AMP¹ 17 20 0</td>
<td>0.046 (±0.001)MIC</td>
</tr>
<tr>
<td>B. cereus</td>
<td>10 (±0.3) 15 (±0.2)</td>
<td>21 22 0</td>
<td>0.046 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>20 (±0.5) 25 (±0.4)</td>
<td>19 21 0</td>
<td>0.01 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>5 (±0.2) 8 (±0.2)</td>
<td>23 24 0</td>
<td>0.01 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>8 (±0.2) 10 (±0.2)</td>
<td>26 25 0</td>
<td>0.09 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>13 (±0.3) 16 (±0.1)</td>
<td>16 15 0</td>
<td>0.023 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>22 (±0.2) 25 (±0.2)</td>
<td>14 17 0</td>
<td>0.046 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>S. dysenteria</td>
<td>20 (±0.2) 22 (±0.2)</td>
<td>12 13 0</td>
<td>0.09 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>8 (±0.2) 10 (±0.2)</td>
<td>11 21 0</td>
<td>0.01 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>E. feacalis</td>
<td>18 (±0.2) 20 (±0.2)</td>
<td>22 13 0</td>
<td>0.01 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>12 (±0.2) 15 (±0.1)</td>
<td>20 21 0</td>
<td>0.023 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>7 (±0.2) 10 (±0.1)</td>
<td>25 25 0</td>
<td>0.01 (±0.001)MIC</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>24 (±0.2) 25 (±0.4)</td>
<td>0 0 24</td>
<td>0.01 (±0.001)MIC</td>
<td></td>
</tr>
</tbody>
</table>

*MIC=Minimum Inhibitory Concentration; **Negative Control: flask containing inoculum and peptone water medium, devoid of nanoparticles; **NCD=No cultivable cells detected

1: Ampicillin (AMP) 10 mcg; 2: Gentamicin (GEN) 10 mcg; 3: Nystatin (NS) 50 mcg
The comparison of the antibacterial activity of (AgNO\textsubscript{3} + E. hermannii) AgNPs with commercial antibiotics was evaluated as the antimicrobial index. These data are shown in Table 4. Antimicrobial indexes were found to be noteworthy compared to those of commercial antibiotics.

**DISCUSSION**

The most important outcome of this study is that biologically synthesized AgNPs from E. hermannii exhibited excellent antimicrobial effects on several human pathogens in different concentrations. Although several studies have examined the antimicrobial effects of AgNPs, the methodology of our study enabled us to investigate these effects on several human microbiological pathogens using biologically synthesized AgNPs obtained from aquaculture fishes [15]. Since conservative chemical AgNP production involves biological and environmental hazards, the eco-friendly biosynthesis is a more favorable option. Our results confirm that AgNPs have antimicrobial effects even in low concentrations (0.5 mM) since they are known to be non-toxic to hosts at low concentrations [16]. These properties (high efficacy, and low toxicity) make them ideal candidates for research into aquacultural infections. Although the exact mechanism of biosynthesis of AgNPs is not yet fully understood, nitrate reductase enzyme is believed to play a major role [17]. It has been hypothesized that silver ions require the NADPH-dependent nitrate reductase enzyme for their reduction, which is secreted by microorganisms in the extracellular environment [18]. Nitrate reductase is known to shuttle electrons from nitrate to the metal group, and its role in the in vitro synthesis of AgNPs under anaerobic conditions has previously been documented [18, 19].

**TABLE 4**

**(AgNPs: AgNO\textsubscript{3} + E. hermannii) antimicrobial index**

<table>
<thead>
<tr>
<th>Human Pathogen Microorganisms</th>
<th>Antibiotics</th>
<th>0.5 mM AgNP</th>
<th>1 mM AgNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. mirabilis (ATCC 29906)</td>
<td>AMP</td>
<td>NCD\textsuperscript{*}</td>
<td>NCD\textsuperscript{*}</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>48</td>
<td>71</td>
</tr>
<tr>
<td>B. cereus (Roma 709)</td>
<td>AMP</td>
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<td>68</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>105</td>
<td>131</td>
</tr>
<tr>
<td>L. monocyctogens (ATCC 35152)</td>
<td>AMP</td>
<td>95</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
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<td>35</td>
</tr>
<tr>
<td>S. aureus (ATCC 29213)</td>
<td>AMP</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>31</td>
<td>38</td>
</tr>
<tr>
<td>P. aeruginosa (ATCC 27853)</td>
<td>AMP</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>87</td>
<td>106</td>
</tr>
<tr>
<td>S. typhimurium (ATCC 14028)</td>
<td>AMP</td>
<td>157</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>129</td>
<td>147</td>
</tr>
<tr>
<td>S. dysenteria (ATCC 11835)</td>
<td>AMP</td>
<td>166</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>153</td>
<td>169</td>
</tr>
<tr>
<td>E. coli (ATCC 25924)</td>
<td>AMP</td>
<td>72</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>38</td>
<td>48</td>
</tr>
<tr>
<td>E. faecalis (ATCC 29212)</td>
<td>AMP</td>
<td>81</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>138</td>
<td>153</td>
</tr>
<tr>
<td>A. hydrophila (ATCC 7966)</td>
<td>AMP</td>
<td>NCD\textsuperscript{*}</td>
<td>NCD\textsuperscript{*}</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>57</td>
<td>71</td>
</tr>
<tr>
<td>B. subtilis (ATCC 6633)</td>
<td>AMP</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>C. albicans (ATCC 90029)</td>
<td>NS</td>
<td>100</td>
<td>104</td>
</tr>
</tbody>
</table>

\textsuperscript{*}NCD=No Culturable Cells Detected

1: Ampicillin (AMP) 10 mcg; 2: Gentamicin (GEN) 10 mcg; 3: Nystatin (NS) 50 mcg

**FIGURE 3**

The inhibition of pathogen microorganism by AgNPs synthesized by E. hermannii.

Characterization of synthesized silver nanoparticles. The principal strength of this study lies in its well-planned methodology. First of all, we set out to confirm the biosynthesis of AgNPs using AgNO₃ from E. harnani isolated from the organs of Cyprinus carpio L. and Tinca tinca, two species of fish collected from the Hirfanli dam reservoir. During the reaction, the color changed to a typical brown and surface plasmon formed, which indicates the aqueous reduction of aqueous silver ions to AgNPs due to excitation of surface plasmon vibrations in nanoparticles [6]. Similar observations have previously been reported when global AgNPs with an average size of 4-12 nm were biosynthesized from supernatant from E. harnani [20]. The biosynthesis of AgNPs from E. harnani was also confirmed by UV-vis spectroscopy and FTIR measurements. A wide variety of analytical techniques are used for the characterization of metal NPs (including particle size, shape, crystallinity, fractal dimensions, pore size and surface area). UV-vis spectroscopy measurements used to characterize nanoparticle morphology in our study, revealed peaks at around 420 and 480 nm in the spectra. In one recent study, a strong, broad peak was observed between 430-450 nm and 400-430 nm measured in the UV-vis absorption spectrum in AgNPs biosynthesized from K. marxianus and C. utilis [12]. Measurements within this range are therefore regarded as a quite specific finding for AgNPs [21]. FTIR measurements also revealed that vibrations at primary and secondary amines, indicating that the biologically synthesized AgNPs contained E. harnani supernants. It has also been observed that intracellular protein can bind to nanoparticles through their free amine groups or cysteine residues, or through amide groups. This process therefore probably forms a coating around AgNPs and stabilizes the aqueous synthetic medium [18]. This suggests that the biomolecules may possibly produce stable AgNPs in an aqueous medium [12, 22].

Analysis of the antimicrobial activity of AgNPs. Several Gram-positive and negative human pathogens were selected for this study in order to investigate the antimicrobial affect of AgNPs (Table 1). According to the DI& measurements, with the exception of P. mirabilis, all the pathogens in this study were affected by the antimicrobial effect of AgNPs and their reproduction was inhibited at various levels (Table 3). The highest antibacterial activity (25 mm) was exhibited against L. monocytogenes, S. typhimurium and C. albicans, followed by S. dysenteria at 22 mm, and E. feacalis at 20 mm. The lowest antibacterial activity (8 mm) was exhibited against S. aureus. Our results are compatible with previous findings in the literature. Previous studies have described many bacterial species, such as E. harnani, Enterobacter aeruginosa, Bacillus brevis, Citrobacter sedlakii, Pseu-

donas putidae, Escherichia coli [6], Lactobacillus spp., Pedicoccus pentosaceus, Enterococcus faecium and Lactococcus garvieae [23], Sphingo- monas paucimolils, Serratia spp. and Pseudomonas aeruginosa [24], as particularly efficient at synthesizing AgNPs. Anandalakshmi et al. (2016) reported AgNPs exhibited the highest antibacterial activity against E. coli and B. subtilis [25]. The antibacterial efficacy of AgNPs against the fish pathogen Aeromonas hydrophila may be useful as a disinfectant or antimicrobial agent for better fish health management [26]. Along with the MIC, the prepared AgNPs exhibited strong antibacterial activity against Gram-positive, Gram-negative and different Candida species at concentrations ranging between 4 and 32 μg/ml [11]. Another study analyzed the synthesis of silver and gold nanoparticles using cashew nut shell liquid and their antibacterial activity against fish pathogens. Silver and gold nanoparticles exhibited significant antibacterial activity, minimum inhibitory concentrations and minimum bactericidal concentrations of bacteria related to fish diseases [27].

AgNPs smaller than 20 nm in diameter bind to the sulfur-containing proteins of the bacterial cell membrane. This increases the permeability of the cell membrane, leading to the death of bacterial cells [5]. Morones et al. (2005) reported the lethal effects of AgNPs (at a range of 10-15 nm) on both Gram-negative and Gram-positive bacteria [28]. In our study, we also determined the antimicrobial effects of AgNPs synthesized from E. harnani against Gram-positive and Gram-negative bacteria were also determined. DeVault Green et al. (2011) reported that silver ions exhibited greater bactericidal activity against Gram-negative bacteria [29]. The thickness of the peptidoglycan layer on the wall cell of Gram-positive bacteria is thought to prevent the effect of silver ions to some extent. Jones et al. (2004) study of AgNP toxicity in human pathogens opened a new door for antibacterial and antifungal agents [30]. Kim et al. (2007) reported that AgNPs exhibited antimicrobial activity and can be used as a medical application to treat infections in burns [31]. AgNPs have also been shown to prevent bacterial colonization on various surfaces, such as catheters and human skin.

CONCLUSION

The contamination of water resources has led to increases in bacterial fish diseases. This is an important cause of economic losses in the aquaculture industry. Treatment options for bacterial fish diseases with agents such as immunostimulants, probiotics or vaccines are currently used for the control of bacterial infections. However one of the main concerns in this context is the increase in antibiotic resistance in microorganisms due to inap-
propriate use of antibiotics. The growth of antibacterial resistance in aquatic bacteria in aquacultures has also led to the need to develop new antibiotics to treat infections. Recent trends have shifted to the use of AgNPs with low environmental and cytotoxic impacts, and high antimicrobial activities and biological synthesis as antibacterial agents. This study investigated the antimicrobial properties of AgNPs synthesized from *E. hermannii*, a pathogenic bacterium in humans and fish, and determined their effects on human pathogens. The results obtained represent preliminary data for the evaluation of AgNPs in disease management in aquaculture. Additional in vivo studies are now needed to investigate the safety and efficacy of AgNPs in aquaculture fish.

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RESEARCH ON ENVIRONMENTAL EFFICIENCY OF CHINA'S STRATEGIC EMERGING INDUSTRIES BASED ON DEA MODEL

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ABSTRACT

At present, China is in the period of accelerating industrialization and urbanization, and the economy maintains rapid growth. However, the proportion of resource-processing and high-energy-consuming industries is too large, causing the sustainable development of the economy to face the dual pressures of scale expansion and resources and environment. The traditional industrial development model urgently needs to change. To this end, the paper will study the environmental efficiency problems faced by China's strategic emerging industries, use data envelopment analysis methods to evaluate the environmental efficiency of China's strategic emerging industries, and analyze the factors affecting environmental efficiency, in order to solve China's strategic emerging industries. The environmental efficiency issue proposes corresponding policy recommendations, and it is expected to provide theoretical support and policy recommendations for China to develop strategic emerging industries and seize the commanding heights of economic science and technology.

KEYWORDS:
Strategic Emerging industry, Environment efficiency, DEA

INTRODUCTION

At present, China is in the period of accelerating industrialization and urbanization, and the economy maintains rapid growth. However, the proportion of resource-processing and high-energy-consuming industries is too large, causing the sustainable development of the economy to face the dual pressures of scale expansion and resources and environment. The traditional industrial development model urgently needs to change. To this end, we will accelerate the cultivation and development of China's strategic emerging industries, adjust and optimize the industrial structure, and shift from a quantitative and consumption-oriented economic growth mode to a quality and efficient economic growth mode, focusing on the balance of quality and efficiency in economic growth. And the coordination of industrial structure is an important way to achieve sustainable economic development in China. In the coming period, the development process and prospects of strategic emerging industries will be highly concerned both in theory and in practice. In 2010, the State Council clearly proposed to focus on the development of strategic emerging industries, and determined the key directions, main tasks and supporting policies for the development of strategic emerging industries. The government has introduced a series of supporting policies and special plans to promote the development of strategic emerging industries. However, in recent years, while the rapid development of China's strategic emerging industries; blind investment in strategic emerging industries has emerged, with emphasis on speed rather than quality, which has had a negative impact on the ecological environment. In this context, this paper discusses the environmental efficiency level of China's strategic emerging industries and its influencing factors, and then proposes policy recommendations for the development of strategic emerging industries. It has important practical significance for government's developing strategic emerging industries, optimizing resource allocation and realizing green economy development.

LITERATURE REVIEW

With the rise of strategic emerging industries, domestic and foreign scholars have obtained rich research results in this field, which provides important reference for this study. Domestic scholar Zhang Guoqiang et al. (2010) analyzed the impact of enterprise technology innovation on strategic emerging industries, and studied the relationship between the two through empirical analysis [1]; Li Guangkai and Shen Kunrong (2011) researched the technological innovation ability of strategic emerging industries, and evaluated the technological progress and the dynamic mechanism of technological innovation in China's economic growth [2]. Liu Meiping (2011) proposed a symbiotic model of
technological innovation path by analyzing the constraints of technological innovation in strategic emerging industries based on the perspective of strategic emerging industry technology innovation [3]. Yu Xindong and Niu Shaofeng (2011) analyzed the relationship between industrial restructuring and energy consumption structure, and then conducted a detailed analysis of the role of strategic emerging industries in optimizing energy consumption structure [4]. Zhu Ruibo and Liu Wei (2011) proposed that the government fosters a strategic emerging industry development mechanism consisting of four parts: the innovation chain integration mechanism, the industrial chain integration mechanism, the innovation chain and the industrial chain integration mechanism, and the social system supporting mechanism [5]. Li Jinhua (2011) analyzed the developmental plans of strategic emerging industries in China and developed countries, and analyzed the driving effects and industry agglomeration of China’s strategic emerging industries [6]. Lu Xiaojun (2012) proposed the necessity of developing China’s strategic emerging industries by analyzing the background of strategic emerging industries and the main content and characteristics of strategic emerging industries [7]. Lin Xuejun (2012) proposed that strategic emerging industries are characterized by orientation, externality, innovation, risk and regionality [8]. Huang Qicai (2013) used the industrial agglomeration index to analyze the evolution of the strategic emerging industries relying on the sector from the perspective of time, industry and geography [9]. Gao Baoshong (2013) uses factor analysis method to select economic indicators from the aspects of industrial development potential, location advantage, technological innovation ability and related driving ability, and objectively evaluate the development level of strategic emerging industries in Hebei Province [10]. Yu Dengke et al. (2013) based on the enterprise survey of strategic emerging industries in Nanchang High-tech Zone, analyzed the status quo of science and technology resource input and output of strategic emerging industries in Jiangxi Province, and constructed an evaluation model for the input and output efficiency of strategic emerging industries. The relative efficiency of the input and output of science and technology resources in the ten strategic emerging industries in Jiangxi Province was measured [11]. Li Hongjin and Li Shenghui (2013) used the panel data of listed companies in our country as the research sample, and used the stochastic frontier model to measure the innovation efficiency of strategic emerging industries, and then compare and analyze them [12]. Liu Hongyu et al. (2012) proposed that in the burgeoning stage, strategic emerging industries depend on the macro guidance of the government; in the formation stage, strategic emerging industries rely on the joint action of policy guidance and market competition; at the maturity stage, strategic emerging industries pass Endogenous development forms a cluster effect and gives full play to the leading role of the market [13]. Shen Junxi (2012) believes that it is necessary to build an industry-university-research cooperation in the development of strategic emerging industries based on the characteristics of different stages of development of strategic emerging industries [14]. Gao Changshui (2012) believes that we should learn from the successful experiences of Western countries in the selection of strategic emerging industries [15]. Zhong Qingliu (2010) proposed a strategic emerging industry promotion strategy that should adhere to R&D priorities and innovation-driven rather than investment-driven [16]. Xiong Yongqing and Li Shicai (2010) analyzed the feasibility of cultivating and promoting strategic emerging industries, and established a three-dimensional comprehensive evaluation system, hoping to better promote the development of strategic emerging industries [17].

In summary, domestic and foreign scholars have achieved rich research results. However, since “strategic emerging industries” are the policy concepts proposed by China, foreign countries have not clearly defined “strategic emerging industries”. The research on the environmental efficiency of domestic strategic emerging industries is also due to the late start and the development is not mature enough. At present, most of the research is based on foreign classical theories and methods, using domestic strategic emerging industry data for empirical testing, and the results of domestic research vary widely. At the same time, there is a lack of systematic research on the environmental impact of strategic emerging industries, especially the lack of empirical analysis on the environmental impact of strategic emerging industries. Based on this, this paper focuses on the environmental efficiency of China’s strategic emerging industries, and analyzes the main factors affecting environmental efficiency, providing a theoretical reference for improving the development of China’s strategic emerging industries and exploring their sustainable development.

**METHODS**

**Measurement model selection.** Data envelopment analysis (DEA) was proposed by the famous operations researcher Charnes, Cooper and Rhodes in 1981[18]. It is based on the concept of relative efficiency, with convex analysis and linear programming as tools, and calculations have the same type. The relative efficiency between the decision making units (DMUs) is evaluated by the evaluation objects and has been applied to performance evaluation in various fields.

The basic principle of the DEA method is: there are n decision units $DMU_j$ $(j = 1, 2, \cdots, n)$,
and their inputs and output vectors are:
\[ X_j = (x_{1j}, x_{2j}, \ldots, x_{nj})^T > 0, \]
\[ Y_j = (y_{1j}, y_{2j}, \ldots, y_{nj})^T > 0, \quad j = 1, \ldots, n. \]

Since the status and role of various inputs and outputs vary during the production process, the DMU must be "integrated" in terms of its inputs and outputs, i.e., they are considered to have only one input and one output process that produces an overall quantity, so that each input and output need to be given the right weight. Assume that the weight vectors of inputs and outputs are \( v = (v_1, v_2, \ldots, v_m)^T \) and \( u = (u_1, u_2, \ldots, u_n)^T \), respectively, so that the following definition can be obtained.

\[
\theta_j = \frac{u^T y_j}{v^T X_j} = \sum_{i=1}^n u_i y_{ij} \sum_{i=1}^n v_i x_{ij}, \quad (j = 1, 2, \ldots, n)
\]

Let \( \theta_j \) be the efficiency evaluation index of the \( j \)-th decision unit \( DMU_j \).

According to the definition, we can always choose the appropriate weight vector to make \( \theta_j \) as large as possible. If you want to know a decision unit, assuming that \( DMU_o (o \in \{1, 2, \ldots, n\}) \) is relatively "optimal" in the \( n \) decision units, what is the maximum value of \( \theta_o \) when \( u \) and \( v \) change as much as possible? The value of \( \theta_o \), Charnes et al., in 1981 [18] proposed the following CCR (three authors acronym) model:

\[
\text{Maximize} \quad \frac{\sum_{i=1}^n u_i y_{io}}{\sum_{i=1}^n v_i x_{io}} = \theta_o
\]

\[
\text{subject to} \quad \sum_{i=1}^n u_i y_{ij} \leq \theta v_i x_{ij}, \quad j = 1, 2, \ldots, n,
\]

\[
\sum_{i=1}^n v_i x_{ij} \leq 1, \quad j = 1, 2, \ldots, n,
\]

\[
u_i, v_i \geq 0, \quad \forall r, i.
\]

When calculating the efficiency value, the CCR model often has multiple effective decision-making units (efficiency value of 1), which makes the comparison analysis between the effective decision-making units. Andersen and Petersen (1993) [7] in order to achieve complete ordering of decision-making units, the evaluated decision-making units are removed from the efficiency boundary, based on the remaining decision-making units, a new efficiency boundary is formed, and the decision-making unit of the rejection is calculated. Since the culled decision unit is not surrounded by the efficiency boundary, the calculated new efficiency value will be greater than 1 for the effective decision unit, and the invalid efficiency value will remain unchanged for the invalid decision unit.

Less than 1, so that the entire decision unit can achieve full ordering. Since the efficiency of an effective decision unit is greater than 1, there is a concept of super-efficiency. The super-efficient DEA model based on the CCR model is:

\[
\text{Minimize} \quad \theta
\]

\[
\text{subject to} \quad \sum_{j=1}^m x_{ij} \lambda_j \leq \theta x_{io}, \quad i = 1, 2, \ldots, m,
\]
\[
\sum_{j=1}^n y_{ij} \lambda_j \geq y_{io}, \quad r = 1, 2, \ldots, s,
\]
\[
\lambda_j \geq 0, \quad j \neq o.
\]

Rajiv et al. (2006) [19] confirmed that super-efficiency is highly susceptible to outliers, so this method can be used to detect the presence of outliers in the data set.

**Index system construction.** The selection of environmental efficiency measurement indicators for strategic emerging industries is as follows. It is assumed that there are three input variables in the production process: capital input, labor input, and technology input; output variables are divided into expected output and undesired output, among which expected output is: industrial growth, technological growth, and undesired output. Wastewater discharge, chemical oxygen demand, sulfur dioxide emissions, soot emissions, carbon dioxide emissions, dust emissions and solid waste production. Following the principles of science, availability, and operability, and drawing on previous research, the index system for evaluating the environmental efficiency of strategic emerging industries in China is shown in Table 1.

**Selection of samples.** This paper selects 200 strategic emerging industry listed companies from 2010 to 2017. In order to ensure the accuracy of financial data, some ST listed companies are excluded. The data comes from a series of research databases such as Guotai An, Ruisi and Juchao. The sample distribution is as follows: Table 2.

**Untargeted data of original indicator data.** Although there is no requirement for the unit of input and output indicators when using the DEA model to measure efficiency, there are certain limits on the value of input and output. Usually, both input and output indicators need to be greater than or equal to zero. If the indicator has a negative value, the DEA model will not be able to effectively measure the efficiency. This paper adopts the data processing method commonly used by scholars as follows:

\[
y_i = \frac{x_i}{\max_{1 \leq i \leq n} x_i}
\]
TABLE 1
Indicator System

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Index classification</th>
<th>Index composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input indicator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>capital</td>
<td>fixed assets investment (ten thousand yuan)</td>
<td></td>
</tr>
<tr>
<td>labor</td>
<td>Average number of employees in labor (person)</td>
<td></td>
</tr>
<tr>
<td>Technology</td>
<td>Technology R&amp;D expenditure (ten thousand yuan)</td>
<td></td>
</tr>
<tr>
<td>wastewater pollution</td>
<td>wastewater discharge (10,000 tons)</td>
<td></td>
</tr>
<tr>
<td>Chemical oxygen demand (tons)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sulfur dioxide emissions (tonnes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undesired output</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exhaust gas pollution</td>
<td>Carbon dioxide emissions (tons)</td>
<td></td>
</tr>
<tr>
<td>Dust emissions (tons)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Output indicator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid waste pollution</td>
<td>solid waste production (10,000 tons)</td>
<td></td>
</tr>
<tr>
<td>Expected output</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDP</td>
<td>GDP (ten thousand yuan)</td>
<td>Technology growth (number of patents)</td>
</tr>
</tbody>
</table>

TABLE 2
Distribution of the sample industry

<table>
<thead>
<tr>
<th>Sub-sectors</th>
<th>Number of companies</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-end equipment manufacturing</td>
<td>30</td>
<td>15%</td>
</tr>
<tr>
<td>Energy saving and environmental protection</td>
<td>34</td>
<td>17%</td>
</tr>
<tr>
<td>Biological and Pharmaceutical</td>
<td>21</td>
<td>10.50%</td>
</tr>
<tr>
<td>New material</td>
<td>28</td>
<td>14%</td>
</tr>
<tr>
<td>New energy vehicles</td>
<td>15</td>
<td>7.50%</td>
</tr>
<tr>
<td>New energy</td>
<td>42</td>
<td>21%</td>
</tr>
<tr>
<td>Information Technology</td>
<td>30</td>
<td>15%</td>
</tr>
</tbody>
</table>

TABLE 3
Strategic Emerging Industry Environmental Efficiency Measurement Results

<table>
<thead>
<tr>
<th>Year</th>
<th>High-end equipment manufacturing</th>
<th>Energy saving and environmental protection</th>
<th>Biological and Pharmaceutical</th>
<th>New material</th>
<th>New energy vehicles</th>
<th>New energy</th>
<th>Information Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>0.6721</td>
<td>0.7012</td>
<td>0.7532</td>
<td>0.7331</td>
<td>0.6509</td>
<td>0.7489</td>
<td>0.7621</td>
</tr>
<tr>
<td>2011</td>
<td>0.6876</td>
<td>0.7232</td>
<td>0.7777</td>
<td>0.7678</td>
<td>0.6687</td>
<td>0.7588</td>
<td>0.7789</td>
</tr>
<tr>
<td>2012</td>
<td>0.6891</td>
<td>0.7454</td>
<td>0.7912</td>
<td>0.7783</td>
<td>0.6908</td>
<td>0.7698</td>
<td>0.7823</td>
</tr>
<tr>
<td>2013</td>
<td>0.7121</td>
<td>0.8518</td>
<td>0.7991</td>
<td>0.7909</td>
<td>0.7012</td>
<td>0.7812</td>
<td>0.7829</td>
</tr>
<tr>
<td>2014</td>
<td>0.7321</td>
<td>0.8681</td>
<td>0.8423</td>
<td>0.8412</td>
<td>0.7112</td>
<td>0.7942</td>
<td>0.8213</td>
</tr>
<tr>
<td>2015</td>
<td>0.7678</td>
<td>0.8721</td>
<td>0.8556</td>
<td>0.8512</td>
<td>0.7329</td>
<td>0.8067</td>
<td>0.8462</td>
</tr>
<tr>
<td>2016</td>
<td>0.7988</td>
<td>0.8733</td>
<td>0.8813</td>
<td>0.8656</td>
<td>0.7562</td>
<td>0.8819</td>
<td>0.8629</td>
</tr>
<tr>
<td>2017</td>
<td>0.8213</td>
<td>0.8921</td>
<td>0.8909</td>
<td>0.8903</td>
<td>0.7882</td>
<td>0.8902</td>
<td>0.8692</td>
</tr>
<tr>
<td>Mean</td>
<td>0.7351</td>
<td>0.8159</td>
<td>0.8239</td>
<td>0.8148</td>
<td>0.7125</td>
<td>0.8039</td>
<td>0.8132</td>
</tr>
</tbody>
</table>

\[
y_j = \frac{\max x_j + \min x_j - x_i}{\max x_j}
\]

\[
y_j = \frac{\max x_j - \min x_j}{\max x_j - \min x_j}
\]

\[
y_j = \frac{x_i - \max x_j}{\max x_j - \min x_j}
\]

\[
y_j = \frac{x_j - \max x_j}{\max x_j - \min x_j}k + q
\]

RESULTS

Strategic emerging industry environmental efficiency measurement results. The DEAP2.1 software was used to substitute the input and output indicators into the model. The results are shown in Table 3.

(1) From the overall level. The environmental efficiency of listed companies in strategic emerging
industries between 2010 and 2017 was not optimal overall, and the average environmental efficiency was only 0.789. Among them, only 14 companies have an environmental efficiency of 1, as shown in Table 4, that is, 14 listed companies have reached the DEA effective status. The rest of the companies are in the DEA inactive state, and the company with the lowest environmental efficiency has an efficiency value of only 0.213, reflecting a very low level of environmental efficiency.

<table>
<thead>
<tr>
<th>Strategic emerging industry</th>
<th>FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of valid companies</td>
<td>proportion</td>
</tr>
<tr>
<td>Not effective</td>
<td>186</td>
</tr>
<tr>
<td>effective</td>
<td>14</td>
</tr>
</tbody>
</table>

(2) From the industry point of view, the industries with higher environmental efficiency are the bio-pharmaceutical industry (0.8239) and the energy-saving and environmental protection industry (0.8159), followed by the new materials industry (0.8148), the new energy industry (0.8040) and the information technology industry (0.8132), the worst environmental performance is the high-end equipment manufacturing industry (0.7351) and the new energy automotive industry (0.7125). Compared with the other five strategic emerging industries, the environmental efficiency of the new energy automobile industry and the high-end equipment industry is relatively low, and the environmental efficiency of these two industries urgently needs to be improved.

(3) From the perspective of composition, as shown in Table 4, the environmental efficiency of China's strategic emerging industries in the industrial level between 2010 and 2017 did not reach 1. According to the definition of DEA measurement efficiency, there is no strategic emerging industry. The production frontier is achieved between 2010 and 2017, and it is relatively effective. It shows that the environmental effects of the seven specific strategic emerging industries in China are not optimal, and there is room for improvement and optimization. Therefore, it is necessary to further analyze the listed companies that influence main factors of strategic emerging industries environmental efficiency, so as to propose solutions.

Result of the affecting factors on environmental efficiency of China's strategic emerging industries. This paper establishes a panel data model to verify the factors affecting the environmental efficiency of China's strategic emerging industries, explores the reasons for the low environmental efficiency of China's strategic emerging industries, and provides a theoretical basis for solving environmental efficiency problems.

This paper selects environmental efficiency (ETE) as an indicator of the environmental efficiency of China's new energy industry enterprises. The factors that influence the factors are as follows: technical innovation (TECHA): expressed by the number of invention patents; R&D investment (RD): expressed by R&D expenditure; market concentration (MC): the proportion of the total output value of large and medium-sized enterprises to the total output value of the industry said. Ownership structure (OS): expressed as the proportion of the total output value of state-owned enterprises to the total output value of the industry. Environmental Regulation (ER): Expressed by the collected sewage charges. The construct the following multiple linear regression model based on the determined variables:

\[ \text{ETE}_{it} = \alpha_0 + \alpha_1 \text{TECHA}_{it} + \alpha_2 \text{RD}_{it} + \alpha_3 \text{MC}_{it} + \alpha_4 \text{OS}_{it} + \alpha_5 \text{ER}_{it} + \mu_{it} \]

Among them, \( i \) represent the i-th strategic emerging industry listed company, and \( t \) represents the year. ETE stands for environmental efficiency, and the value of this variable comes from the above calculation results.

Multiple regression analysis of the model was performed by using software. The results are as follows:

(1) The impact of technological innovation on environmental efficiency. The estimation coefficient of the number of patented inventions is positive, indicating that the increase in the number of patented inventions has contributed to the improvement of the environmental efficiency of China's strategic emerging industries. With the increase in the number of patented inventions, the technological innovation has been expanded, which has enhanced the ability of technological innovation, strengthened the internal promotion of technological innovation to environmental efficiency, and further improved the environmental efficiency of China's strategic emerging industries.

(2) The impact of R&D expenditure on environmental efficiency. The estimated coefficient of expenditure is positive, indicating that China's strategic emerging industries will further promote the technological innovation level of China's strategic emerging industries with the increase of R&D expenditures, and then increase technological innova-
Suggestion

New innovation strategy to “open innovation and leading innovation”. Strategic emerging industries are knowledge- and technology-intensive industries, and technological innovation is particularly important for the development of strategic emerging industries. In the new era, with the continuous expansion of the scope, scale and speed of technological innovation, and the increasing uncertainty and complexity of technological innovation, the technological innovation environment under the background of globalization has undergone fundamental changes, and China’s strategic emerging industries are gradually shifting to a new innovative strategy of “open innovation and leading innovation”. On the basis of rational allocation of internal and external resources, explore more effective innovation methods, shorten the cycle of technological innovation, and achieve breakthroughs in technological innovation to improve the efficiency of technological innovation and further promote the rapid development of China’s strategic emerging industries.

Increase investment in research funding and enhance technological innovation capability. Strategic emerging industries belong to knowledge-industry-intensive industries, and their development momentum is mainly based on technological innovation, while the investment in research funding affects the output of technological innovation results to a large extent. In the above, there is a positive correlation between environmental efficiency and research funding, and the increase in research funding contributes to the improvement of environmental efficiency. Therefore, while China's strategic emerging industries are developing, they must increase investment in research funding, increase the output of technological innovations, and achieve major innovations and breakthroughs in technology to cope with the continuous upgrading of technology. While advancing the process of technological innovation, we will enhance the technological innovation capability of China's strategic emerging industries. Then, while developing China's strategic emerging industries, with the intrinsic promotion of environmental efficiency through technological innovation, we will further promote the environmental efficiency of China's strategic emerging industries.

Improve market competition mechanism and increase market competition. In today's era, after-sales service and technology competition have become the main form of competition, especially in the technology-intensive strategic emerging industry market. Therefore, in the process of developing strategic emerging industries, the competition form of strategic emerging industry markets is changed to increase the degree of market competition. While improving the competitive mechanism of strategic emerging industry market, a competition model that emphasizes the coexistence of social and economic benefits will be formed to enhance the efficiency of competition in strategic emerging industry markets. By improving the market competition mechanism, increasing the degree of market competition, and acting on technological innovation, we will further promote the environmental efficiency of China's strategic emerging industries.

Increase government control. Government regulation plays a very important role in improving the environmental efficiency of strategic emerging industries. Through regulation, the government can strengthen environmental constraints and realize the
transformation of enterprises from traditional development mode to new industrialization road. China is still in the stage of industrialization development. At present, an important task is to gradually abandon the traditional industrialization road and realize the path of new industrialization. Taking a new road to industrialization is an inevitable requirement for adapting to sustainable economic development and scientific and technological progress in a global context. The characteristics of new industrialization are that it requires economic benefits, high technology content, low environmental pollution, low resource consumption, and full utilization of human resources. Therefore, the government must increase its control and achieve supervision. In addition to some nationally-used management systems, for example, each region should improve the distribution and trading system of emission rights, so that enterprises should bear the social costs of environmental pollution. The government should adapt to local conditions and adopt different environmental regulation systems for different regions. For example, for some regions where the economy is not very developed, the level of economic development can be considered first, and environmental issues should be properly regulated. Only when the economic level is improved can we obtain better environmental protection support. Conversely, for some economically developed but heavily polluted areas, environmental issues should be considered firstly and economic development is second, so that the transformation of the economic development mode can achieve the ultimate sustainable development.

Promote local government cooperation and achieve win-win development. Strategic emerging industries are likely to cause inconsistencies in the initial stage of development due to different resource endowments, and regional differences occur. China's economic policy is to lead the rich after the first rich, and ultimately achieve common prosperity, which should also be reflected in the development of the industry. Once the technology achieves breakthroughs, its learning is very fast. For local governments, they should achieve joint research and knowledge sharing in the breakthrough of some basic technologies, and greatly reduce the research cost to achieve win-win development.

ACKNOWLEDGEMENTS

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OPTIMIZATION OF EXISTING SOLID WASTE LANDFILL SITES USING GIS AND MCDA: THE CASE OF GIRESUN, TURKEY

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ABSTRACT

Rapid population growth, industrial development and urbanization in developing countries such as Turkey have brought with them the problem of urban solid waste. In a world where natural resources are being depleted rapidly and environmental destruction is increasing along with the daily rise in global warming and pollution of water resources, solid waste must be stored or disposed of more effectively. Appropriate site selection for solid waste disposal involves many environmental, economic and socio-political factors and is one of the biggest problems in waste management. In the process of collection, transportation and storage of urban solid wastes, and especially in the selection of landfill sites, spatial/map-based models are being used effectively. This study was carried out using Geographic Information Systems (GIS) and Analytic Hierarchy Process (AHP) in the selection of a solid waste landfill site. The study evaluated the suitability of the solid waste storage site in Çavuşlu/Giresun. The negative environmental effects were widely discussed in addition to the topic having raised concern among the local population. Consequently, nine criteria related to the selection of landfill sites were examined: geology, distance from rivers, lakes and dams (m), slope (°), land use/landcover, soil quality, distance from roads (m), sensitive and protected areas, distance from a fault line and population (km²). Each criterion was weighted using AHP and mapped via GIS and conformity analyses were performed. As a result, it was determined that the 90,000 m² Çavuşlu/Giresun solid waste landfill site, still in active use, was at an average level of suitability.

KEYWORDS: Solid waste management, Landfill, GIS analysis, AHP, MCDA

INTRODUCTION

Today, the world population continues to grow rapidly, with the majority of the population living in urban areas. Unplanned construction, damage to the water basins, air and water pollution and generation of solid waste are the source of significant environmental problems [1]. Solid wastes from industrial enterprises and urban housing create serious environmental problems [2]. Wastes vary according to socio-economic status, household size and the season. Due to the dynamic nature of the composition and amount of solid waste, it is difficult to manage its disposal economically and environmentally [5].

Due to the increasing risk factors and inadequate financing of solid waste management, its importance is heightened with every day that passes [3, 4]. Poorly managed waste leads to significant detrimental effects on health, the local and global environment and the economy [5]. Therefore, an integrated solid waste management plan covering all stages from waste generation to final disposal methods is essential for both the environment and public health.

Today, various techniques such as landfills, heat treatment, biological treatment and recycling are used for solid waste management [6, 7]. The landfill is the most common solid waste disposal method in many countries [2, 8]. The first and most important step in landfill solid waste storage is site selection [2, 9]. In the literature, many studies on solid waste site selection have been performed using multi-criteria decision analysis (MCDA) integrated with geographic information systems (GIS) [5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31].

In this study, GIS techniques were combined with analytic hierarchy process (AHP) methods for the selection of a solid waste disposal site. The method was applied around the area still being used for landfill/waste storage in Çavuşlu/Giresun in northern Turkey, and the results were evaluated.

MATERIALS AND METHODS

The study area of the Çavuşlu environs is located in the district of Göreme in the eastern part of Giresun Province. According to the data of 31 December 2017, the population of Göreme District is 17,803 and that of the Çavuşlu municipality is 2,063. The solid waste storage area in the study is located in the Çavuşlu stone quarry which makes up a large
part of the stone reserve and is within the boundaries of the Çavuşlu Municipality in Görele District of Giresun Province and within 1 km of the Giresun - Trabzon coastal road. The allocated area is 17.93 hectare in size and belongs to the General Director of Real Estate. It is 69 km from the Giresun provincial capital, 6 km from the central capital of Görele district and 1 km from the Çavuşlu town center. The site is 70 m to the east of the Çavuşlu Stream and the Obakiran Stream passes through the middle of the site (Fig. 1).

**Identification of criteria and data collection.**
In this part of the study, two stages are discussed for the formation of the model at the site selection point. In the first stage, the scope of the study was determined by taking into account the following factors: the Restrictions for the Regulation of Landfill Waste Storage (Article. 15, Resmi Gazette [Official Gazette]: 26.03.2010, Resmi Gazette No: 27533) [53], the European Union (EU) criteria for solid waste disposal, the criteria set by the World Health Organization (WHO), the criteria considered by the World Bank and Turkey's laws-regulations and 10th Development Plan on solid waste. In the second stage, the selection criteria were determined by using the values obtained by examining the reports published by national and international academic studies and reports by agencies, institutions and non-governmental organizations on the determination of landfill sites.

**Restrictions for landfill waste storage regulation.** The legal regulations for landfill waste storage were examined and the spatial data layers were defined separately for the limitations mentioned in the legislation. The related spatial and verbal data corresponding to these limitations were obtained via data collection methods.

The limitations can be summarized as follows:
- Distance to residential units,
- Air transportation safety,
- Distance to forest areas and afforestation areas,
- Distance to flora - fauna areas,
- Distance to underground and surface waters,
- Geological/geotechnical status
- Topographical structure,
- Hydrogeological status,
- Flood, landslide, avalanche, erosion and high earthquake risk,
- Rainfall,
- Natural or cultural heritage status,
- Distance to linear engineering structures (pipelines, power transmission lines, etc.),
- Soil quality,
- Existing mines and quarries,
- Drinking water basins,
- Recreation and resort areas [28].

**FIGURE 1**
Map of study area
### TABLE 1

<table>
<thead>
<tr>
<th>References</th>
<th>Aim</th>
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### Literature review to determine site selection factors.

The criteria to be used and at the same time the factors in the selection and also the factor weights of these criteria were determined from the literature review.

As a result of the detailed literature review and assessments (Table 1), the suitability of the factors determined according to the literature, the degree of impact of these factors on the site selection process in addition to the degree of conformity of the sub-criteria were evaluated. It was seen that these factors included the factors within the regulation of solid waste storage. As a result of the studies conducted, in order to increase the efficiency of the model to be formed, the factors affecting the site selection and the sub-factors expressing the transition difficulties of these factors were developed (Table 2). As previously stated, many studies were examined from the point of determining factors, including law-related regulations, academic studies, reports, etc. After determination of the factors, methods to determine the weight values of these factors were established.

### Relation of criteria to weight.

The selection of a landfill waste storage site is a complex problem that involves the evaluation of multiple factors (political, social, environmental and economic) from among different criteria. In order to solve such complex problems, MCDA integrated with GIS can explore many different disciplines at the same time and make it easier for the user to evaluate the alternatives.

Among the general methods found in the literature, the AHP, the Simple Weighted Total Method and the Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) are among the MCDA methods frequently used in solid waste site studies. Within the scope of this study, AHP was used for solid waste disposal site selection.

### Analytical Hierarchy Process.

The AHP is a structured technique for analyzing complex problems involving multiple interrelated criteria [48]. It is very important to determine the weight of the criteria in MCDA. Evaluation criteria are defined according to their importance. Thus, after all criteria are ranked in a hierarchical manner, a pair comparison matrix is created so that a comparison can be made for each criterion (Table 3). Weighting with AHP has been widely used in many applications [7, 9, 10, 17, 22, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49].
| TABLE 2 |
|-----------------|-----------------|-----------------|
| **Factors**     | **Sub-factors** | **Rating Score** | **Weight (%)** |
| Population (km²) | >3000           | 10              |                |
|                 | 2001–3000       | 8               |                |
|                 | 1001–2000       | 6               | 0.268          |
|                 | 501–1000        | 4               |                |
|                 | 0–500           | 1               |                |
| Distance from rivers, lakes, and dams (m) | 0–500       | 10              |                |
|                 | 501–2000        | 3               | 0.231          |
|                 | >2000           | 1               |                |
| Slope (°)       | 0               | 5               | 0.144          |
|                 | 1               | 6               |                |
|                 | 11–15           | 3               |                |
|                 | 16–20           | 4               |                |
|                 | 21–25           | 5               |                |
|                 | 26–30           | 6               |                |
|                 | 31–35           | 7               |                |
|                 | 36–40           | 8               |                |
|                 | 41–45           | 9               |                |
|                 | >45             | ∞               |                |

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\[ \lambda_{max} = 9.731; \text{Consistency Ratio (CR)} = 0.063 \leq 0.1 \]
The general definition of the method consists of objectives, criteria, possible sub-criterion levels and options. In this study, the suitability classification was made from 0 to 10, where 0 represents the least appropriate and 10 represents the most appropriate areas. In this context, the factors and sub-factors affecting landfill site selection are shown in Table 2.

**Consistency check.** Following the creation of the eigenvector matrix of the AHP (Table 3), it was necessary to evaluate its consistency. The required level of consistency was evaluated using the following index:

\[ CR = \frac{CI}{RI} \]

where CR is the consistency ratio, CI is the consistency index and RI is the random index.

According to the AHP theory, the consistency ratio (CR) ≤ 0.1 should be (Consistency Ratio (CR) = 0.063 ≤ 0.1). Since the CR value was lower than (0.1), the consistency of the weights was confirmed.

**RESULTS**

The thematic maps in Figure 2 show the spatial distribution of the values of the analyzed study area parameters.

**Population (km²).** Point location datasets were created for provincial, district, municipality, neighborhood and village central administrative units. The data were completed by integrating the data obtained from local administrations, TURKSTAT and the National Address Database on existing administrative units, population, etc.

Storage sites in these areas may be detrimental to the inhabitants and therefore areas with less population density were selected. The data for population density of at least 500 km² and not more than 3000 km² were categorized and scored. The weight and score values for each sub-criterion are shown in the comparison matrix and the results are given in Figure 2.

**Distance from rivers, lakes and dams (m).** According to the research of Kazakis et al. (2015), the risk of flooding is high at a distance of less than 100 m from a river, whereas the risk decreases at distances greater than 3000 m. In this context, considering the topographic structure of the study area, buffer zones in the range of 0-500 m were determined for this criterion. A 500-m buffer zone was scored as 10, a 501-2000-m buffer zone as 3, and a buffer zone of >2000 m as 1. The results are shown in Figure 2.

**Slope (°).** The slope is an important criterion for the mountainous terrain of the study area, as natural disasters like landslides and rock falls are frequently seen in the steep hillside. The slope is the main parameter for construction work in the area and for the operation of a facility. Steep slopes are generally technically unsuitable for land construction. Very steep areas (>45°), steep areas (25-45°), moderately steep areas (20-25°), sloping areas (10-20%) and slightly sloping areas (<10%), respectively, were scored between 10 and 1. Areas with <5% sloping were considered the most suitable areas, with a value of 1, and minor sloping areas of 6-10% were given a score of 2. The results are shown in Figure 2.

**Land use/Landcover.** Land use is an important environmental factor in landfill construction. There is no classification of land use types in the region. Therefore, the sub-criteria were defined and rated, based on general land use, as dense forest, seasonal agriculture, farmland, wetlands, rocky areas, open areas, residential areas, industrial areas and other areas. The results are shown in Figure 2.

**Soil.** According to soil damage and limitations, there are eight land use capability classes for soil (Class I –VIII). In Classes I – IV, under good land administration, crops adapted to the region and the forest, pasture and meadow plants can be cultivated in a good manner. Classes V, VI and VII are suitable for growing native plants. In Class VIII, although a product can be obtained with very efficient and expensive improvement efforts, under current market conditions the investment expenditures cannot be met. In the study, soil classifications were scored and after being weighted with AHP, the results are shown in Figure 2.

**Distance from roads (m).** The distance of the landfill site to a road must not be less than 500 m. To this purpose, 500 m-buffer zones were created around the roads. A 500-m buffer zone was scored as 10, a 501-1000 m-buffer zone as 6 and a buffer zone of >1000 m as 0. The results are shown in Figure 2.

**Geology.** The general the geological structure of the region is associated with the Pontide tectonic zone and is part of the Pontid-Elbrus island arc series developed during the Jurassic and through the Pliocene Period. The study area was under the influence of the Caledonian, Hercynian and Alpine orogenesis. The Alpine volcanism that began in the region in the Jurassic Period became very severe and widespread in Upper Cretaceous-Eocene times. At the beginning of the Jurassic Period, the North Anatolian Tethys began to open in the south of the Eastern Pontides. There is clear evidence that the North Anatolian
FIGURE 2
Thematic maps generated from the study area parameters: (a) population (km²); (b) distance from rivers, lakes and dams (m); (c) slope (°); (d) land use/landcover; (e) soil quality; (f) distance from roads (m); (g) geology; (h) sensitive and protected areas; (i) distance from a fault line.

FIGURE 3
Cost surface map

Tethys crust began to fall under the Pontides at the beginning of the Lower Cretaceous Period. Alluvion, basalt-andesite and extrusive rock were determined as sub-criteria for the selection of appropriate storage sites for the study area, and buffer zones were created in the selection region. The results are shown in Figure 2.

**Sensitive and protected areas.** These areas are not suitable for landfills. In this study, a 0-3000-m buffer zone was scored as 10 and a >3000 m-buffer zone as 1. The results are shown in Figure 2.

**Distance from a fault line.** These areas are not suitable for landfills. In the study, a 0-5000 m-buffer zone was scored as 10 and a >5000 m-buffer zone as 1. The results are shown in Figure 2.

The cost surface map created by using the criteria and sub-criteria defined for the study area is given in Figure 3.

Using the cost surface map, a conformity assessment was made of the 90,000 m² existing storage site in the study area (Fig. 4).

In the analyses, the conformity values of the cost surface map of the study area were evaluated as 1.303–9.906 and the average pixel value of 4.790 indicated that the 90,000 m² solid waste storage site is at an average level of conformity. This average pixel value was found by dividing the total number of pixel values in the current area of 90,000 m² by the total number of pixels.
DISCUSSION

Selecting a landfill site for solid waste disposal in developing countries is a difficult process [7]. In recent years, many articles on solid waste management have been published. These are mostly focused on MCDA techniques. The AHP is the most widely used MCDA method used for solid waste site selection found in the literature (Coban et al. 2018) in addition to the PROMETHEE, ELECTRE, TOPSIS and VIKOR methods [5]. In this context, Perkoulidis et al. (2010) used another MCDA method (ELECTRE) for a different kind of regional waste management [50]. Khalili and Duecker (2013) conducted a MCDA which required the participation of various stakeholders from different environmental, industrial, social and economic sectors at every stage of the design of sustainable environmental management systems [51]. Aghaji et al. (2016) stood by TOPSIS as the best MCDA method [52].

In Turkey, a majority of the generated solid waste is deposited in open dumps and there is no systematic solid waste management strategy at a national level [7]. Solid waste site selection is a complex problem that involves the evaluation of multiple factors (technical, political, social, environmental and economic) from amongst different criteria. When using MCDA to solve such complex problems, the most suitable tool that can examine many different disciplines at the same time is GIS. Integrated with GIS and remote sensing, MCDA is a powerful tool that can be effective in site selection.

It is cost effective, fast and capable of managing large amounts of complex data from different sources.

CONCLUSION

In the study, GIS and remote sensing techniques were combined with AHP methods and a suitability assessment was performed for the landfill site. For this purpose, nine criteria were determined including population (km²), distance from rivers, lakes and dams (m), slope (°), land use/landcover, soil quality, distance from roads (m), geology, sensitive and protected areas and distance from a fault line, along with related sub-criteria.

Evaluation factors in the first stage included the Restrictions for the Regulation of Landfill Waste Storage (Article. 15, Resmi Gazete [Official Gazette]: 26.03.2010, Resmi Gazete No: 27533), the European Union (EU) criteria for solid waste disposal, the criteria set by the World Health Organization (WHO), the criteria considered by the World Bank and Turkey's laws-regulations and 10th Development Plan on solid waste. In the second stage, the selection criteria were determined by using the values obtained by examining the reports published by national and international academic studies and reports by agencies, institutions and non-governmental organizations on the determination of landfill sites. The criteria were weighted using AHP and mapped using GIS techniques.
As a result, using the nine criteria and sub-criteria in a GIS environment, a cost surface map was created and a conformity analyses was performed. In addition, sites in the study area that may be suitable for solid waste storage were identified and mapped. Moreover, despite the fact that local inhabitants were opposed to it and although it could lead to environmental problems, the evaluation of the Çavuşla/Giresun solid waste landfill site, still in active use, has determined that the 90,000 m² area is at an average level of suitability for solid waste storage.

ABBREVIATIONS


REFERENCES


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ARE RESULTS OF ENVIRONMENTAL MONITORING OF THE MIDDLE SPOTTED WOODPECKER (LEIOPICUS MEDIUS) INFLUENCED BY THE METHOD USED?

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ABSTRACT

The Middle Spotted Woodpecker is listed among bird species of European importance (Annex I of the Birds Directive) for which Special Protection Areas (SPA) are designated in forest habitats of Europe as a part of the Natura 2000 network. In one of these areas, in SPA Litovelské Pomoravi, a study of abundance of the Middle Spotted Woodpecker in a floodplain forest during the breeding season was investigated. The number of individuals counted at the point transect, using the standard method of monitoring of this species in SPAs in the Czech Republic, was assessed. The results of the standard method were compared with those obtained by the special playback technique. The latter method has increased the number of recorded individuals of the Middle Spotted Woodpecker considerably: twice in 2007 and 2008 and even three times in 2010. Authors discuss the reactions of the Middle Spotted Woodpecker to the reproduced recordings of its calls and differences in vocalisation in the Great and Middle Spotted Woodpeckers. Differences in vocalisation of juveniles of the Great Spotted and Syrian Woodpeckers are also shortly discussed. The importance of application of a proper method for population monitoring of the Middle Spotted Woodpecker in SPAs is suggested in the context of management of its breeding habitats.

KEYWORDS:
Point count method, playback technique, monitoring of Special Protection Areas, Natura 2000, Litovelské Pomoravi.

INTRODUCTION

The Middle Spotted Woodpecker (Leiopicus medius), distinctive in the forest bird community of forest ecosystems due to the signalling character of its colourful plumage [1], is considered to be a biological indicator of sustainability of management of lowland deciduous forests in the European temperate zone [2]. Presence and abundance of the Middle Spotted Woodpecker can be regarded an indicator of the “wildlife-friendly” forest management [3]. The distribution of the Middle Spotted Woodpecker in forest habitats is markedly aggregated [4]. An extensive analysis of factors affecting the occurrence of the Middle Spotted Woodpecker based on the use of forest inventory data has shown that the proportion of old oaks in the stand and the uneven-aged structure of the forest are very important predictors of the species occurrence [5]. Despite the Middle Spotted Woodpecker prefers forests dominated by old oaks, the predictive model of habitat suitability in three forest types in Poland suggested that the species shows a certain level of flexibility in habitat use [6]. Due to its habitat preferences, the Middle Spotted Woodpecker is a good model species for predictions of shifts in the species distribution ranges in forest landscapes as a result of climate changes [7]. The Middle Spotted Woodpecker is listed in Annex I of the Birds Directive [8] as one of the species for which Special Protection Areas (SPAs) are designated in the European Union member states as a part of the Natura 2000 network. All SPAs established for the conservation of the Middle Spotted Woodpecker include deciduous lowland forests as a dominant habitat type, and the forest management is significantly influenced by the protection regime of the SPA [9]. Exact data on the presence and abundance of the Middle Spotted Woodpecker in old forest stands [10] are important not only for the establishment of SPAs but also for regular monitoring and planning of forest management focused on protected phenomena in the SPAs [11].

In the Czech Republic, altogether 7 SPAs were designated to protect the populations of the Middle Spotted Woodpecker in lowland forests [12]. For monitoring of the Middle Spotted Woodpecker in these SPAs, the standard point count method is used [13]. In one of these areas, in Litovelské Pomoravi [14], we compared the results of this standard method with the results of monitoring of the species based on the playback technique, using the reproduced recordings of its calls [15]. The aim of the study was to identify an optimal method of monitoring of the Middle Spotted Woodpecker in floodplain forests, which will provide more precise information on abundance of the
target species in the forest ecosystem. The reactions of the Middle Spotted Woodpecker to the reproduced recordings of its calls and differences in vocalisation of the Middle Spotted Woodpecker and other forest woodpeckers in the floodplain forest environment are also discussed in the paper. The importance of monitoring data on the Middle Spotted Woodpecker populations for the management of lowland deciduous forests in the temperate zone of Europe is stressed in the end.

**MATERIAL AND METHODS**

The Litovelské Pomoravi was designated as SPA in 2005 in order to protect the breeding populations of the Common Kingfisher (*Alcedo atthis*), Collared Flycatcher (*Ficedula albicollis*) and Middle Spotted Woodpecker. Totally, 239 bird species have been recorded in this SPA. Of them, 117 species breed in the area at present [16]. Hardwood floodplain forests are the prevailing type of forest ecosystem in the study area [17]. They reach 54 km² in size and are surrounded by intensively managed agricultural landscape of the Morava river alluvium. From the phytosociological point of view, the studied forest area belongs to the hardwood elm-oak floodplain forests of the *Querco-Ulmetum* association, with local representation of willow shrubs of soil and sand deposits of the *Salicetum triandrae* association, softwood floodplain forests of lowland rivers of the *Salicetum albae* association and alder carrs of the *Calamagrostio canescensis-Alnetum* association [18].

Monitoring of birds using the point count method with a transect of twenty census points, delimited by the coordinates 49°42′19.38″N, 17° 6′35″E and 49°41′22.40″N, 17° 8′19.64″E, was carried out in the years 2005–2012. At each census point, acoustically and visually detected birds were recorded for the time interval of five minutes. Immediately after the five-minute observation was finished, a recording of the contact and territorial call of the Middle Spotted Woodpecker 47 seconds in length [19] was reproduced and the number of individuals of the Middle Spotted Woodpecker (as well as other woodpecker species) which reacted to the playback and the type of their reaction were registered. The research focused on Middle Spotted Woodpecker was carried out in the years 2007–2008 and 2010.

The statistical analysis of data was based on the assumption that for each monitoring period (2007, 2008 and 2010), the mean number of recorded individuals of the Middle Spotted Woodpecker per one census point would be higher using the playback technique (mean μ₁) than using the standard point count method (mean μ₂). To verify this assumption, the null hypothesis „Mean number of individuals of the Middle Spotted Woodpecker per one census point is the same using both methods (i.e. H₀: μ₁ = μ₂)” was tested against the alternative one-tailed hypothesis (H₁: μ₁ > μ₂), which is based on the above assumption. Since this is a test of equality of means of two dependent samples, the pair t-test was used for each monitoring period separately. The analysis was carried out using the Stata 12 program [20].

**RESULTS**

The playback technique has increased the number of recorded individuals (registrations) of the Middle Spotted Woodpecker (MSW). Only in the first monitoring period (2007), the mean number of recorded individuals was not significantly higher using the playback technique than using the standard point count method. In the other three monitoring periods, the difference in favour of the playback technique was statistically significant, at 90% (second measurement in 2007), 95% (2008) and 99% (2010) levels of confidence, respectively, see Table 1.

In the year 2008, the MSW reacted to the recording at 12 census points (60 %), at 9 of them by approaching and emitting an alarm call and at 3 points by approaching without vocalisation. In the year 2010 at the census point no. 9, three individuals of the MSW reacted to the recording: one only approached without vocalisation, another one with a contact call and the other with a territorial call. Besides approaching, the reactions of the MSW to playback often included the contact call „kik kik kik“, which was emitted sharply and in short intervals. Quite exceptionally, the MSW reacted using the croaky territorial voice. Only rarely we recorded single cases when the MSW was registered during the standard point count but did not react to the subsequent playback. The Great Spotted Woodpecker (*Dendrocopos major*) and Black Woodpecker (*Dryocopus martius*) reacted to the recording of the MSW only exceptionally.

**DISCUSSION AND CONCLUSION**

Breeding sites and fidelity of MSW to European temperate oak-dominated forests is in the focus of ornithological research [21]. In the Czech Republic, the courtship in the MSW occurs intensively during March and April. At that time, the species is conspicuous due to its territorial call [22]. There is recommended the turn of March/April to be suitable as the first period and the turn of April/May as the second period for the standard species monitoring. However, in forest habitats, the MSW is rather inconspicuous and quiet, it rarely drums and its determination is complicated by the similarity of its contact call with that of the Great
TABLE 1

Comparison of recorded individuals of the Middle Spotted Woodpecker using two different counting methods

<table>
<thead>
<tr>
<th>Date of the counting</th>
<th>16. 4. 2007</th>
<th>14. 5. 2007</th>
<th>15. 4. 2008</th>
<th>26. 4. 2010</th>
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<tbody>
<tr>
<td>Point number</td>
<td>N1</td>
<td>N2</td>
<td>N1</td>
<td>N2</td>
</tr>
<tr>
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<tr>
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<td>19</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>20</td>
<td>Total number</td>
<td>7</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Mean number (µ1; µ2)</td>
<td>0.35</td>
<td>0.45</td>
<td>0.2</td>
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<tr>
<td></td>
<td>pair-t-test</td>
<td>0.623</td>
<td>1.422</td>
<td>1.831</td>
</tr>
<tr>
<td></td>
<td>(H0: µ1 = µ2) vs. (H1: µ1 &gt; µ2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value for t-test</td>
<td>0.27</td>
<td>0.086</td>
<td>0.041</td>
</tr>
</tbody>
</table>

(N1 = number of individuals recorded using the standard point count method, N2 = number of individuals recorded using the playback technique)

Spotted Woodpecker. Based on our field experience, during counts carried out in mid-April, the MSW uses the „croaky” territorial call only rarely. E.g. in the Vrapac floodplain forest on 14 April 2007, we observed mating of the MSWs which did not show any vocal activity. At the turn of April/May, the species produces practically no territorial calls and its detectability decreases significantly. Therefore, the playback technique seems to be a suitable method for an objective assessment of its numbers.

When using the playback of MSW calls, we had the opportunity to compare the contact and alarms calls of the MSW and the Great Spotted Woodpeckers. While the typical croaky territorial call of the MSW is unambiguous for species identification, determination of contact or alarms calls of the two species can be problematic. Glutz and Bauer [23] mentioned the one-syllable call „gug“ to be rather infrequent in the MSW, in comparison with the Great Spotted and Syrian Woodpeckers (Dendrocopos syriacus). These authors also mention that when disturbed (e.g. at a nest), the MSW produces a repeated row of syllables „geeg-gegegeg…“ which are higher pitched, shorter and louder. Based on our field experience, it seems that the MSW’s call is a rather slow „kitikkitikit“ or „kikkikkikiki“ and it sounds softer in comparison with other woodpeckers. The Great Spotted Woodpecker produces sharp and rather fast „kik“, or only one-syllable „kik“ sometimes changing to rattling „trrrrr“. In the Great Spotted Woodpecker, the syllables are repeated in longer intervals and sound rather one-syllable. In the MSW, the syllables merge into a group and are connected freely. Both species can change the pitch and intensity of the call (in a syllable as well as in a series) according to the current situation. Therefore, species identification based on vocalisation is quite difficult in the forest habitat. The distance from which the call is coming is also important for the determination. Based on our field experience, the Great Spotted Woodpecker (unlike the MSW) reacts to human presence much more often and more intensively and its abundance can be thus overestimated during the standard monitoring. Determination of the two woodpecker species is further complicated by the fact that the calls of fledged juveniles of the Great Spotted Woodpecker are obviously softer than those of adult individuals, which was repeatedly verified in the field during this study. The call of a juvenile Great Spotted Woodpecker rather resembles the call of the Syrian Woodpecker. If the call sounds like a typical call of the Syrian Woodpecker or a typical call of the Great Spotted Woodpecker, the identification is unambiguous. If the call sounds softer and is between the call of the Syrian Woodpecker and that of the Great Spotted Woodpecker in pitch, the bird is a juvenile Great Spotted Woodpecker.
The assessment of abundance of the MSW using the playback technique and effectiveness of this method compared to the territory-mapping method was carried out by the study [24]. According to these authors, for the assessment of distribution, abundance as well as for monitoring of the MSW, three visits of the breeding site should be sufficient, provided that they include the use of playback. In south-eastern Poland, authors of the study [25] found out that the application of the playback method during March and April significantly increased the number of localised breeding territories of the MSW.

If the methods of field monitoring of the MSW are aimed to provide precise data on its population numbers in forest habitats and thus be an objective basis for preparation of forest management strategies (especially in SPAs of the Natura 2000 network), they should, in our opinion, include the playback technique based on reproduction of call recordings. Forest management taking account of conservation of the MSW populations should be focused on preservation of old oaks and their maintenance in forest stands [26]. Local breeding populations of the MSW can be strongly influenced by fragmentation of forest habitats and by introduction of coniferous tree species into oak-dominated forests. Dead very large trees are a limited resource for nesting of the MSW in lowland managed forests, because MSW (as weak excavator) may benefit from an increase in dead wood availability leading to nest niche shifts into more favourable substrates for cavity excavation [27]. An optimal approach to support of nesting population of MSW is probably to conserve selected large oak-dominated lowland forests in the form of strictly protected areas [28] from which no dead wood is removed [29].

ACKNOWLEDGEMENTS

Field monitoring of the Middle Spotted Woodpecker in floodplain forests of SPA Litovalské Pomoravi was supported by the Czech Society for Ornithology as a part of the Czech national monitoring programme for Special Protection Areas in the Natura 2000 network. The authors thank Eva Cepakova for translation of the text to English. The authors are grateful to anonymous referees for their comments on the manuscript.

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TREATMENT OF WASTEWATER BY USING ALUMINUM AND STAINLESS STEEL ELECTRODES - ASSESSMENT WITH RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, the treatment of wastewater from metal plating process by electrocoagulation (EC) process using aluminum and steel electrode was examined. In order to determine the optimum operating conditions, the Response-Surface Method was used and the experimental sets were formed by the central composite design. As a result of optimization studies, optimum conditions for copper removal with aluminum electrode were that current density was 48 mA/cm², reaction time was 26 min, initial pH value was 9. Optimum conditions for chromium (VI) removal were that current density was 28 mA/cm², reaction time was 30 min, and initial pH value was 5, respectively. As a result of optimization with steel electrode, optimum conditions for current removal of copper and chromium (VI) were that current density was 48 mA/cm², reaction time was 23 min, initial pH value was 7, respectively and optimum conditions for chromium (VI) removal were that current density was 48 mA/cm², reaction time was 10 min, the initial pH value was 9. As a result of the experiments, copper and chromium removal efficiencies were obtained as 94.27%, 82.49% in aluminum electrode and 91.06%, 95.91% in steel electrode, respectively. In addition, during the treatment of the metal plating industry wastewater under the optimum operating conditions, the total consumption of the aluminum electrode for copper and chromium was found to be 7.79 and 5.25 €/m³, respectively. For the removal of copper and chromium during the use of steel electrodes, electricity consumption cost was calculated as 3.44 and 1.44 €/m² respectively.

KEYWORDS:
Electrocoagulation, RSM, Metal Working Wastewater, Aluminum, Steel

INTRODUCTION

Metal plating wastewater includes various surfactants, degreasing agents, oil, grease, phosphate, ammonia, suspended solids, cyanide compounds and heavy metals. In such wastewater, heavy metals such as copper, nickel, cadmium, chromium, zinc and silver can be found in high concentrations [1]. Depending on the materials and chemicals used in the metal plating process, the character of the wastewater changes. When the metal plating wastes are discharged without treatment, the color, surface active substances, oil and grease cause aesthetic problems in the receiving environment and decrease the oxygenation capacity of the aquatic environment. The toxic and carcinogenic cyanide compounds contained in this type of wastewater and the contaminants such as heavy metals cause death in the receiving environment and permanent environmental damage. Therefore, such wastewater should not be discharged to receiving environments without proper and adequate treatment. Filtration [2], conventional chemical precipitation [3], Fenton [4], electro-coagulation/chemical [5], membrane separation [6], adsorption [7] and ion exchange [8] can be used for removal of heavy metals from metal plating wastewater [9, 10]. Conventional chemical precipitation is a simple and inexpensive method that can be used in high efficiency for the removal of heavy metals contained in such wastewater [11]. However, its important disadvantages are that too many chemicals are consumed, the precipitation efficiency is low, the chemical sludge in toxic structure is produced in large quantities as a result of the treatment and the cost of disposal of this sludge is high [12, 13]. The electrocoagulation process is more preferred because of its limiting disadvantages, which significantly affect the total operating cost and treatment efficiency of conventional chemical precipitation [9, 14]. The most important advantages of the electrocoagulation process are that it does not require chemical dosing, cheap materials such as iron, aluminum and steel can easily be used as electrodes, it creates small amounts of chemical sludge, the precipitation of this sludge is good, and it is a relatively simple and effective method. This process is based on the formation of metal hydroxide flocks as a result of the dissolution of the anode in the wastewater by electrolysis. The electrocoagulation process removes pollutants in the solution medium by one or more of the coagulation, adsorption, precipitation, flotation and partial oxidation mechanisms [15-19]. When aluminum, steel and iron electrodes are used
as sacrificial anodes in the electrocoagulation process, anodic dissolution occurs in the anode and hydrolyses. As a result, low-solubility metal hydroxides such as Al (OH)₃ and Fe (OH)₃ are produced in solution medium [20-22]. Metal ions such as Al³⁺, Fe²⁺, which dissolve from these electrodes, form metal-polymer complexes, adsorb and coagulate pollutants. Thus, it is assumed that the treatment mechanism in the electrocoagulation process takes place in 3 stages: (1) formation of metal ions of coagulant species with dissolving electrode, (2) destabilization of pollutants, particle suspension and breakage of emulsions, (3) collection of flocks [14, 23, 24]. In the treatment of wastewater in electrocoagulation process, various electrodes can be used with stainless steel, zinc, aluminum, magnesium or a combination of these with the exception of iron and aluminum [25, 26]. The oxidation reaction in the anode in the electrocoagulation process is given in Equation 1 and the reduction reactions in the cathode are presented in Equation 2. The formation of coagulant in solution is shown in Equation 3.

$M(s) \rightarrow M^{n+} + n \, e^-$ \hspace{1cm} (1)

$n(H_2O) + n \, e^- \rightarrow (n/2)H_2(g) + n \, (OH)^-$ \hspace{1cm} (2)

$M^{n+} + n \, (OH)^- \rightarrow M(OH)_n$ \hspace{1cm} (3)

Equation (1)-(3) shows some reactions in the electrocoagulation process. During the reactions, aluminum may form longer-chain aluminum hydroxides during dissolution depending on the contact time and pH [27]. In the literature, there are studies using different electrodes with real wastewater such as removal of copper (Cu), chromium (Cr) and nickel (Ni), aluminum electrodes [1], removal of aluminum electrodes [9], removal strontium using stainless steel and aluminium electrodes [25] and removal of copper (Cu), zinc (Zn) and chromium (Cr) with aluminum electrodes [19]. The wastewater used for this research is the actual wastewater produced by a plant that performs chrome, zinc and nickel plating. In the study, the efficiency of the electrocoagulation process in the treatment of metal plating industry wastewater and the success of pollutant removal were examined. In the experiment, the usability of two different electrodes, aluminum and steel, which can be found cheaply and abundantly was examined. The effects of current density, electrolysis time and initial pH values on pollutant removal were evaluated as operation variables. Response-Surface methodology and ANOVA test were used during the evaluation. KOL, copper, chromium (VI), nickel and zinc were selected as target parameters for the treatment of metal plating wastewater by electrocoagulation process (taking into account the character of the wastewater used in the study). Copper and chromium removal data which are suitable for the model were used.

![FIGURE 1](image)

**FIGURE 1**

Laboratory scale EC experiment setup (1: Sample reactor; 2: Electrodes; 3: Stirrer Bars; 4: Magnetic Stirrer; 5: Power Supply)

**MATERIALS AND METHODS**

**Characterization of Wastewater.** The metal plating wastewater used in this study was obtained from an industry engaged in different types of metal plating activities in the province of Konya. Wastewater samples were stored at +4 °C for up to 7 days after collected. Table 1 presents raw wastewater characterization.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pH</th>
<th>Conductivity/mS/cm</th>
<th>Temp./°C</th>
<th>COD/mg/L</th>
<th>TSS/mg/L</th>
<th>Nitrite/mg/L</th>
<th>Cr⁶⁺/mg/L</th>
<th>Nickel/mg/L</th>
<th>Iron/mg/L</th>
<th>Zinc/mg/L</th>
<th>Copper/mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.36</td>
<td>5.64</td>
<td>24.8</td>
<td>378</td>
<td>30</td>
<td>0.24</td>
<td>593</td>
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<td>876</td>
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</tr>
<tr>
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<td>±0.2</td>
<td>±0.4</td>
<td>±1</td>
<td>±25</td>
<td>±1.5</td>
<td>±0.07</td>
<td>±9</td>
<td>±6.2</td>
<td>±0.8</td>
<td>±8.3</td>
<td>±2.6</td>
</tr>
</tbody>
</table>

**Experimental Procedures and Developments.** Two electrodes with 3 cm spacing (an anode and a cathode) were placed perpendicular to the reactor. The electrocoagulation scheme is shown in Figure 1. A laboratory scale EC reactor was made of Plexiglas with a diameter of 9 cm and a length of 13 cm. Steel and aluminum electrodes consisting of an anode and a cathode, 6 cm wide, 11.5 cm high and 0.1 cm thick equal electrodes were prepared and used in the experiment. The total impact area of the electrodes was calculated as 85-90 cm². Direct current of the reactor was achieved by using Mervesan 305D II brand power supply. The volume of wastewater used is 500 mL. During the experimental
work, the wastewater in the reactor was mixed using a magnetic stirrer at a speed of 200 rpm. Prior to EC experiments, the surfaces of the electrodes were cleaned with acetone and maintained in a cleaning solution (100 mL of 35% HCl and 200 mL of 2.8% CaH₂N₂) for at least 5 minutes and then rinsed with distilled water and dried in a disinfector at 105 °C. The current density (16-48 mA/cm²), the reaction time (10-30 min) and pH (5-9), which affect the EC process, were optimized using the Response-Surface Method. NaCl was added to increase the value of electrical conductivity of the wastewater from 5.64 mS/cm above 10 mS/cm, so that experiments were carried out at constant conductivity. After a few hours of precipitation period after each experimental set, the purified samples were pipetted and analyzed according to Standard Methods. KOI, copper, chromium (VI), nickel and zinc analyses were performed in the samples taken from the clear phase.

Experimental Design and Statistical Analysis. The optimization process was carried out using the central composite experimental design in the Statgraphics Centurion XVI.I software program. With the experimental set created in the program, 15 discrete EC processes were carried out and the effects of current density (W₁; 16-48 mA/cm²), reaction time (W₂; 10-30 min) and initial pH (W₃; 5-9) changes on the removal efficiency of pollutant parameters were tried to be determined. Table 2 shows these parameters and levels.

Statistical analysis of the data and variance analysis (ANOVA) were performed with Statgraphics Centurion XVI.I software, which uses Fisher’s F test to estimate statistical significance. The suitability of the model can be verified according to the correlation coefficient (R²) by using the corresponding probability values of the same software. Depending on the effect of independent factors, three-dimensional graphics and related shape parcels were obtained.

RESULTS AND DISCUSSION

Statistical Analysis and Experimental Model Data. The optimization process was carried out with Statgraphics Centurion XVI.I software using the central composite experimental design matrix, which is the most common design under RSM (response surface methodology). The relationship between independent variables (current density, reaction time and pH) and dependent variable (removal efficiency) was determined by ANOVA test. The determination coefficient (R²) was used to determine the quality of the multi-term model. Whether the model was statistically significant was determined using the Fisher-F test. RSM can be used to solve the main variables (W₁, W₂ and W₃), interactions (W₁W₂, W₁W₃ and W₂W₃) and curvature effects (W₁², W₂² and W₃²). In the study, the parameters affecting the pollutant removal efficiency and the relations between these parameters were determined in the EC process using the Response Surface Method (Eq. 4).

\[ Y = \beta_0 + \beta_1 W_1 + \beta_2 W_2 + \beta_3 W_3 + \beta_{11} W_1^2 + \beta_{22} W_2^2 + \beta_{33} W_3^2 + \beta_{12} W_1 W_2 + \beta_{13} W_1 W_3 + \beta_{23} W_2 W_3 \]  

(4)

W₁, W₂ and W₃ show the coded levels of design variables (W₁: current density; W₂: electrolysis time and W₃: initial pH). β is a set of regression coefficients: intersection (β₀), linear (β₁; β₂; β₃), mutual interaction (β₁₂; β₁₃; β₂₃) and quadratic coefficients (β₁₁; β₂₂; β₃₃), and Y is estimated response (% removal efficiency) (Y₁;: copper removal; and Y₃: chromium removal).

Table 3 shows the experimental set-up and the removal efficiency of the heavy metals obtained by the experimental set and the removal efficiencies estimated by the developed model. In Equation Y₁ and Y₃, the prediction function given by the model to the aluminum electrode for copper removal efficiency is shown (Table 4). In Equation Y₂ and Y₄, the chromium removal equation in the steel electrode takes place. The positive and negative coefficients in the equations show synergistic and antagonistic effects, respectively. The removal of heavy metals is estimated using these equations.

As seen from the Equation Y₁, the increase in the current density and the reaction time increases the efficiency of the copper removal, while the increase in the initial pH value has the effect of reducing the copper removal efficiency. As seen from the Equation Y₂, the increase in the current density, the reaction time and the initial pH value have an effect on increasing the chromium removal efficiency. In Equation Y₃, the current density has a negative effect on the efficiency while the reaction time and pH value contribute positively. As seen from the Equation Y₄, the increase in the current density decreases the chromium removal efficiency, while the increase in the reaction time and the initial pH increases the copper removal efficiency (see Table 4-5).

<table>
<thead>
<tr>
<th>Coded factors</th>
<th>W₁: Current density (mA/cm²)</th>
<th>W₂: Electrolysis time (min)</th>
<th>W₃: initial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>16</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>32</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>30</td>
<td>9</td>
</tr>
</tbody>
</table>

TABLE 2

Independent variables and code levels for the EC process
TABLE 3
Experimental and predicted response of removal efficiency in EC process using Al and Steel electrodes

<table>
<thead>
<tr>
<th>Run</th>
<th>$W_1$</th>
<th>$W_2$</th>
<th>$W_3$</th>
<th>pH</th>
<th>$Y_1$: Copper removal (%)</th>
<th>$Y_2$: Chromium$^{6+}$ removal (%)</th>
<th>$Y_3$: Copper removal (%)</th>
<th>$Y_4$: Chromium$^{6+}$ removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>10</td>
<td>7</td>
<td></td>
<td>97.5875</td>
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<tr>
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<td>10</td>
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<td>98.5992</td>
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<td>98.2977</td>
<td>87.0152</td>
<td>85.3288</td>
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<td>97.8113</td>
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<td>9</td>
<td></td>
<td>98.5214</td>
<td>98.9786</td>
<td>75.2108</td>
<td>76.2226</td>
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<tr>
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<td>7</td>
<td></td>
<td>98.877</td>
<td>98.7601</td>
<td>78.5835</td>
<td>79.4829</td>
</tr>
<tr>
<td>14</td>
<td>32</td>
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<td>7</td>
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<td>98.8327</td>
<td>98.7601</td>
<td>80.2698</td>
<td>79.4829</td>
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<tr>
<td>15</td>
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<td>7</td>
<td></td>
<td>98.7704</td>
<td>98.7601</td>
<td>79.5953</td>
<td>79.4829</td>
</tr>
</tbody>
</table>

The Effect of Process Optimization and Application Variables on Copper and Chromium$^{6+}$ Removal Efficiency. Copper and chromium$^{6+}$ removal efficiencies with aluminum and steel electrodes were examined. Experimental data were tried to be interpreted by response surface methodology by taking into consideration the variables such as current density, pH and reaction time. The ANOVA results of the experimental study are shown in Table 7.

According to ANOVA results, current density ($W_3$), reaction time ($W_2$), combination of current density and reaction time ($W_1W_2$), combination of pH with current density ($W_1W_3$) and quadratic term of pH value ($W_2W_3$) are effective on heavy metal removal efficiency (p-value <0.05). In Figure 3, the response and surface graphs obtained for copper and chromium removal efficiencies are shown in three dimensions and in color. The relationship between current density and time for copper and chromium$^{5+}$ is explained by increasing the efficiency. It is seen in Figure 3b-d that the efficiency of chromium removal increases as the current density and time increase.

Table 6 shows the data for two electrodes for copper and chromium removal of the surface response quadratic model of the ANOVA test. As shown in Table 6, the linear coefficients of current density ($W_1$) and electrolysis time ($W_2$) values have a significant effect on the removal of copper in aluminum electrodes. In addition, electrolysis time ($W_2$) and pH ($W_3$) values are more effective on removal efficiency of aluminum electrode. In the same table, in the removal study with the steel electrode, the current density ($W_1$) and pH ($W_3$) for the copper and the time of electrolysis ($W_2$) and pH ($W_3$) for chromium significantly affect the removal efficiency. In the experiments carried out on the aluminum electrode, the quadratic terms of the current density ($W_1^2$) and electrolysis time ($W_2^2$) have a direct effect on the efficiency of copper and chromium removal. On the steel electrode, $W_1W_1$ coefficient affects the EC process in addition to the quadratic terms of the current density ($W_1^2$) and electrolysis time ($W_2^2$).

In the EC process, the effect of current density ($W_1$) variable (p> 0.05) is important in the removal of copper by treatment with aluminum electrode. However, the quadratic terms of electrolysis time ($W_2$), pH ($W_3$) and current density ($W_1W_2$) are more important in chromium removal. In Table 8, in the EC process using the steel electrode, the current density ($W_1$) and pH ($W_3$) has a significant effect on the removal of copper (p> 0.05). Besides, it is seen that the effect of initial pH ($W_1W_3$) values on copper removal is higher (p> 0.01). Quadratic terms of current density ($W_1^2$), electrolysis time ($W_2^2$), initial pH ($W_1W_3$) and two factor $W_1W_3$ are found to be important in the experimental model. In order to obtain maximum copper removal efficiency, the analysis was carried out under optimum operating conditions and the removal efficiencies were calculated (Table 7). Estimated removal efficiencies of the model in the optimum conditions were calculated as 99.47% copper, 85.57% chromium$^{6+}$ for aluminum electrode and 94.31% copper, 100% chromium$^{6+}$ for steel electrode.

Effects of consumption for economic evaluation. The two most important parameters in the electrocoagulation process are electrical energy consumption and electrode material consumption. These are shown by the operation cost (OC). The equation of the calculation is shown in equation (5) [29, 30].

\[
OC = a_{\text{ENC}} + b_{\text{ELC}}
\]

where $a_{\text{ENC}}$: electricity consumption (kWh / m$^3$) and $b_{\text{ELC}}$: electrode material consumption (kg/m$^3$). The cost of ENC and ELC are calculated according to the equation (6, 7).

\[
ENC = \frac{V \times \text{ELC}}{M \times I}
\]

(6)

\[
ELC = \frac{V \times \text{ENC}}{z \times I}
\]

(7)
In the equations for the EC process; U: applied voltage (V), i: current (A), t: electrocoagulation time (s), V: sample volume used for treatment (m³), M: molecular weight (Mₐ = 26.98 g mol⁻¹), Z: the number of electron transfer (Al:3) and F: Faraday constant (96485.34 C/mol). It is not necessary to calculate the electrode consumption due to the alloying and stainless properties of the steel electrode. Turkey market in 2018 (about 0.1 €/kWh), electrode (about 2 €/kg Al). The total consumption of the aluminum electrode during the removal of copper and chromium from the real metal plating industry wastewater was found to be 7.79 and 5.25 €/m³, respectively. Electricity consumption for the removal of copper and chromium during the use of steel electrodes was calculated as 3.44 and 1.44 €/m³, respectively.

**FIGURE 3**
Three-dimensional response surface graphs for copper and chromium (VI) at Al and Steel electrode (a) current density vs. time, (a, b) for Al electrode and (c, d) for steel electrode

**TABLE 4**
Regression equations by EC process using Al and Steel electrodes

<table>
<thead>
<tr>
<th>Copper removal (%)</th>
<th>Al</th>
<th>Steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y = 97.4429 + 0.0263727* W₁ + 0.103178* W₂ - 0.151427* W₃ + 0.076681* W₄ - 0.00648508* W₅ - 0.0129702* W₁ + 0.00391699* W₆ + 0.0145914* W₇<em>W₅ - 0.00551232</em> W₈*W₅</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y = -10.5679 - 2.58366* W₁ + 2.03502* W₂ + 24.5554* W₃ - 0.336792* W₄ - 0.0526589* W₅ + 1.12192* W₆ - 0.0444066* W₇* - 0.0320039* W₈* - 2.18215* W₉</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chromium removal (%)</th>
<th>Al</th>
<th>Steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y = 34.5366 + 11.3594* W₁ + 1.08418* W₂ + 5.42088* W₃ - 1.67073* W₄ - 0.132097* W₅ + 0.267004* W₆ + 0.00119449* W₇ - 0.0611298* W₈<em>W₉ - 0.47604</em> W₇</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y = -25.6588 - 12.1281* W₁ + 5.33066* W₂ + 25.2168* W₃ + 2.08154* W₄ - 0.00674536* W₅* W₂ - 0.0154581* W₆* W₈ - 0.0483383* W₇* - 0.479026* W₈* W₉ - 1.10995* W₉</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5**
ANOVA results for response surface of quadratic model for Al and Steel electrodes.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Model</th>
<th>R²</th>
<th>Adj.R²</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>p-value (Prob&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>Copper</td>
<td>76.70</td>
<td>34.77</td>
<td>4.01</td>
<td>0.34</td>
<td>1.82</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Chromium (VI)</td>
<td>92.10</td>
<td>77.9</td>
<td>279.38</td>
<td>28.59</td>
<td>6.48</td>
<td>0.02</td>
</tr>
<tr>
<td>Steel</td>
<td>Copper</td>
<td>91.37</td>
<td>75.86</td>
<td>745.70</td>
<td>75.71</td>
<td>5.88</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Chromium (VI)</td>
<td>99.86</td>
<td>99.63</td>
<td>622.96</td>
<td>69.12</td>
<td>420.02</td>
<td>0.001</td>
</tr>
</tbody>
</table>
### TABLE 6
ANOVA results for the response surface quadratic model for parameters at Al and Steel electrode

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Source</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F-Ratio</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_1</td>
<td>1,27254</td>
<td>1</td>
<td>1,27254</td>
<td>6.81</td>
<td>0.0477</td>
<td></td>
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<tr>
<td>W_2</td>
<td>0.681312</td>
<td>1</td>
<td>0.681312</td>
<td>3.65</td>
<td>0.1145</td>
<td></td>
</tr>
<tr>
<td>W_3</td>
<td>0.0189253</td>
<td>1</td>
<td>0.0189253</td>
<td>0.10</td>
<td>0.7632</td>
<td></td>
</tr>
</tbody>
</table>

| Copper | W_1 | 0.109873 | 1 | 0.109873 | 0.59 | 0.4778 |
|        | W_2 | 0.0378507 | 1 | 0.0378507 | 0.20 | 0.6715 |
|        | W_3 | 0.00605611 | 1 | 0.00605611 | 0.03 | 0.8642 |
|        | W_4 | 0.566504 | 1 | 0.566504 | 3.03 | 0.1421 |
|        | W_5 | 0.340656 | 1 | 0.340656 | 1.82 | 0.2349 |
|        | W_6 | 0.00179509 | 1 | 0.00179509 | 0.01 | 0.9257 |
| Total error | 0.934316 | 5 | 0.186863 |       |        |
| Total (corr.) | 4.01082 | 14 |         |       |        |

| Al | W_1 | 5.68749 | 1 | 5.68749 | 1.29 | 0.3075 |
| W_2 | 75.7716 | 1 | 75.7716 | 17.19 | 0.0089 |
| W_3 | 88.7391 | 1 | 88.7391 | 20.13 | 0.0065 |
| W_4 | 52.1763 | 1 | 52.1763 | 11.83 | 0.0184 |
| W_5 | 15.7046 | 1 | 15.7046 | 3.56 | 0.1178 |
| W_6 | 2.56648 | 1 | 2.56648 | 0.58 | 0.4799 |
| W_7 | 0.0526822 | 1 | 0.0526822 | 0.01 | 0.9172 |
| W_8 | 5.97897 | 1 | 5.97897 | 1.36 | 0.2967 |
| W_9 | 13.3877 | 1 | 13.3877 | 3.04 | 0.1419 |
| Total error | 22.0438 | 5 | 4.40875 |       |        |
| Total (corr.) | 279.381 | 14 |         |       |        |

| Chromium(VI) | W_1 | 86.7916 | 1 | 86.7916 | 6.75 | 0.0484 |
| W_2 | 84.3989 | 1 | 84.3989 | 6.56 | 0.0505 |
| W_3 | 126.572 | 1 | 126.572 | 9.84 | 0.0257 |
| W_4 | 2.12025 | 1 | 2.12025 | 0.16 | 0.7015 |
| W_5 | 2.49566 | 1 | 2.49566 | 0.19 | 0.6779 |
| W_6 | 45.3133 | 1 | 45.3133 | 3.52 | 0.1193 |
| W_7 | 72.8104 | 1 | 72.8104 | 5.66 | 0.0632 |
| W_8 | 1.6388 | 1 | 1.6388 | 0.13 | 0.7356 |
| W_9 | 281.31 | 1 | 281.31 | 21.88 | 0.0054 |
| Total error | 64.2848 | 5 | 12.857 |       |        |
| Total (corr.) | 745.7 | 14 |         |       |        |

| Copper | W_1 | 0.250818 | 1 | 0.250818 | 1.52 | 0.2718 |
|        | W_2 | 0.449889 | 1 | 0.449889 | 2.73 | 0.1592 |
|        | W_3 | 0.99731 | 1 | 0.99731 | 6.06 | 0.0571 |
|        | W_4 | 80.99 | 1 | 80.99 | 49.24 | 0.0000 |
|        | W_5 | 0.0409499 | 1 | 0.0409499 | 0.25 | 0.6391 |
|        | W_6 | 0.00860233 | 1 | 0.00860233 | 0.05 | 0.8282 |
|        | W_7 | 86.274 | 1 | 86.274 | 524.25 | 0.0000 |
|        | W_8 | 367.146 | 1 | 367.146 | 2230.99 | 0.0000 |
|        | W_9 | 71.6069 | 1 | 71.6069 | 435.12 | 0.0000 |
| Total error | 0.822832 | 5 | 0.164566 |       |        |
| Total (corr.) | 622.962 | 14 |         |       |        |

| Steel | W_1 | 25.08184 | 1 | 25.08184 | 1.52 | 0.2718 |
| W_2 | 44.9889 | 1 | 44.9889 | 2.73 | 0.1592 |
| W_3 | 99.731 | 1 | 99.731 | 6.06 | 0.0571 |
| W_4 | 80.99 | 1 | 80.99 | 49.24 | 0.0000 |
| W_5 | 0.0409499 | 1 | 0.0409499 | 0.25 | 0.6391 |
| W_6 | 0.00860233 | 1 | 0.00860233 | 0.05 | 0.8282 |
| W_7 | 86.274 | 1 | 86.274 | 524.25 | 0.0000 |
| W_8 | 367.146 | 1 | 367.146 | 2230.99 | 0.0000 |
| W_9 | 71.6069 | 1 | 71.6069 | 435.12 | 0.0000 |
| Total error | 0.822832 | 5 | 0.164566 |       |        |
| Total (corr.) | 622.962 | 14 |         |       |        |

### TABLE 7
Optimum operating values for Al and Steel electrodes

<table>
<thead>
<tr>
<th>Goal of model</th>
<th>Copper</th>
<th>Chromium(VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Al</td>
<td>Steel</td>
</tr>
<tr>
<td>Current density (mA/cm²)</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Reaction time (min)</td>
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<td>23</td>
</tr>
<tr>
<td>Initial pH</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Model prediction results (%)</td>
<td>99.47</td>
<td>94.31</td>
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<tr>
<td>Experimental results (%)</td>
<td>94.27</td>
<td>91.06</td>
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</table>

**Comparison of optimized experiment results with previous literature studies.** The current study demonstrates that the use of aluminum and steel electrodes in the treatment of an industrial wastewater will achieve very good removal efficiency. The comparison of the electrocoagulation process used in different industrial wastewater treatment and optimum values is given in Table 8. There are many research studies in the literature for the treatment of industrial wastewater. Few of these studies have the same scope as this article. Most of the previous studies have been conducted with a single electrode or on an aluminum-iron pair.

There are a lot of studies with only aluminum and steel pairs and there are a small number of studies with RSM application. In this study, cost optimization analysis was also performed. In this respect, it allows the determination of the cheapest application. Moreover, in this study, process efficiency is presented with the surface-response methodology. This study also presents the correlation between variable parameters and removal efficiencies.
TABLE 8
Comparison the results of this study with some studies

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Water types</th>
<th>Responses</th>
<th>Operating parameters</th>
<th>( R_e(%) )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, Al</td>
<td>real</td>
<td>Cu, Cr, Ni</td>
<td>( C_r ) (mg/L)</td>
<td>Conductivity (mS/cm)</td>
<td>( j ) (mA/cm²)</td>
</tr>
<tr>
<td>Steel, Al</td>
<td>syn-thetic</td>
<td>strontium</td>
<td>45, 44.5, 394</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Steel</td>
<td>real</td>
<td>Cr, Ni, Zn, Cu</td>
<td>93.2, 57.6, 20.4, 33.3</td>
<td>8.9</td>
<td>9.56</td>
</tr>
<tr>
<td>Steel</td>
<td>real</td>
<td>COD, TSS</td>
<td>2200, 768</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Al, Steel</td>
<td>real</td>
<td>nitrate-N</td>
<td>10-31.7</td>
<td>7.73 - 31.36</td>
<td>20</td>
</tr>
<tr>
<td>Steel, Fe</td>
<td>syn-thetic</td>
<td>8 reactive orange</td>
<td>300</td>
<td>3</td>
<td>110, 130</td>
</tr>
<tr>
<td>Fe-Fe</td>
<td>real</td>
<td>COD, color, Ni, Zn, total Cr,</td>
<td>475.8, 5983, 8.1, 149.25, 358</td>
<td>17.14</td>
<td>30</td>
</tr>
<tr>
<td>Fe-Al</td>
<td>real</td>
<td>COD²⁵, Cu⁺⁺⁺, and Ni⁺⁺</td>
<td>32350, 77.5, 23.1, 54.8</td>
<td>&gt;10.0</td>
<td>16° and 32° and 10°</td>
</tr>
<tr>
<td>Al, Steel</td>
<td>real</td>
<td>Cu, Cr</td>
<td>128.5, 593</td>
<td>5.64</td>
<td>48</td>
</tr>
</tbody>
</table>

\( C_r \): initial concentration; \( j \): current density; pHₐ: initial pH; t: reaction time; \( R_e \): removal efficiency

CONCLUSIONS

In this study, the treatment of wastewater containing toxic metal and produced from the metal plating industry using aluminum and steel electrode was investigated by using electrocoagulation process. In the optimization study using the response-surface method, maximum copper and chromium removal was found as a function of the current density, reaction time and initial pH value affecting the EC process. The results of ANOVA showed the suitability of the quadratic regression with experimental data. In copper and chromium⁶⁺ removal for aluminum and steel electrodes under optimum conditions 48 mA/cm² current density, 26 and 23 minutes reaction time and 9 and 7 initial pH value, and >91% of heavy metals removal efficiencies were obtained. In chromium⁶⁺ removal for aluminum and steel electrodes, 28 and 48 mA/cm² current density, 30 and 10 minutes electrolysis time and 5 and 9 pH values and 82.49% and 95.91% removal efficiencies were obtained. As a result of this study, it was found that especially copper and chromium metals were removed from the wastewater with aluminum and steel electrodes with high removal efficiencies and that the response-surface method is a powerful technique for determining the optimum operating conditions in optimization studies. In the literature, many studies and this study examined the electrocoagulation process is an effective process in pollutant removal. However, it should not be forgotten that the characterization of the actual wastewater and the type of pollutant parameter to be removed are of great importance.

ACKNOWLEDGEMENTS

The experimental part of this study conducted with the steel electrode was supported by S.U. the Scientific Research Coordinator (BAP No: 18401096). I would like to thank all my colleagues who did not spare their support during my study and the University that helped with the analysis.

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ANALYSIS OF FISH ABUNDANCE AND DISTRIBUTION PATTERN IN THE BEIBU GULF USING FISHERY ACOUSTIC MEASUREMENT COMBINED WITH ORDINARY KRIGING METHOD

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ABSTRACT

To evaluate the biomass distribution of fishery resources for hydroacoustic data, acoustic measurement was combined with ordinary kriging (OK) to perform data processing, which was supported with trawling data.

Acoustic data were collected during daytime by using a Simrad EY60 echosounder with 70- and 120-kHz split beam transducers. Afterward, the undesirable data were eliminated, data transformation was carried out, the semivariogram models were statistically compared and filtered and subsequently OK was performed. The goodness of fit of OK was also assessed with the determination coefficient (R2), mean error (ME), and root mean square error (RMSE).

The exponential model was the most suitable semivariogram model for the data, and the goodness of fit of OK was good (R2 = 0.8986, Pearson’s r = 0.9481).

The distribution pattern of fishery resources is probably less distributed from north to south and from east to west, which is consistent with the findings in the Summer between 1998 and 1999.

KEYWORDS:
Fishery acoustics, Biomass density, Data gap, Target size, Target depth, Ordinary kriging, Semivariogram model, Cross validation

INTRODUCTION

Fishery acoustics is increasingly applied in the field of fishery resources because of its intuitionistic, rapid, continuous and real-time monitoring features, which can effectively evaluate the biomass of fishery resources [1-6]. Fishery acoustics is a suitable method for estimating the density and biomass of pelagic planktivores [7], and can also obtain substantial information on fish size, density and biomass [8].

However, it is a challenging task to conduct large-scale survey due to funding shortage and environmental conditions. Therefore, the choice of sampling sites is limited and discrete. Moreover, data gaps and unreasonable sampling points can hardly reflect the spatial distribution characteristics of regional fishery resources [9], which significantly affect the reliability and accuracy of the evaluation results of fishery resources in large-scale survey. Consequently, precise data collection and presentation in a survey area are prerequisites in improving the accuracy of current fishery acoustics-based evaluation methods.

Spatial interpolation is a scientific and effective spatial analysis method that can predict the data of unknown points on the basis of known points in sufficient quantities. Consequently, the discrete points of measurement data become a continuous surface. It has been widely applied in multiple fields, such as meteorological analysis [10, 11], hydrological monitoring [12, 13], mining [14], environmental monitoring [15-17] and fishery resource assessment [3, 18]. However, the combination of fishery acoustics and ordinary kriging (OK) has been rarely applied in the assessment of fishery resource. The Beibu Gulf is one of the four traditional fishing grounds in China, and any changes in fishery resources in the Beibu Gulf have become a growing concern locally and internationally [19-23].

The present study was designed to (i) explore the correlation between target size and target depth, (ii) compare the fitness of the semivariogram models and the biomass distribution of fishery resources, (iii) predict the spatial distribution of fishery resources and test the reliability of this method, (iv) evaluate the feasibility of acoustics data combined with OK method and (v) assess the status and management of fishery resources.
**FIGURE 1**
Map of cruise track (+) and trawling sites (●) in the survey area (●).

| TABLE 1 |
| Main settings of the Simrad EY60 echosounder |
| Setting | 70 kHz transducer | 120 kHz transducer |
| Transducer type | ES70-7c | ES120-7c |
| Transducer gain | 27.0 dB | 27.0 dB |
| Transmitting power | 800W | 500W |
| Detection range | 600m | 300m |
| Ping interval | 1s | 1s |
| Pulse duration | 0.512 ms | 0.512 ms |
| Alongship 3dB beam width | 7.00° | 7.00° |
| Athwartship 3dB beam width | 7.00° | 7.00° |
| Min. ts value | -70 dB | -70 dB |
| Min. echolength | 0.8 | 0.8 |
| Max. echolength | 1.8 | 1.8 |
| Absorption coefficient | 0.018 dB/m | 0.045 dB/m |
| Sound speed | 1545 m/s | 1545 m/s |
| Two-way beam angle | -21.0 dB/steradian | -21.0 dB/steradian |

**MATERIALS AND METHODS**

**Study area.** The Beibu Gulf is a semi-enclosed sea surrounded by land territories of China, Vietnam and China’s Hainan Dao (Fig. 1) with a total area of 128,000 km². The width of the gulf is relatively narrow with the widest part of 180 nm (nautical mile). Several rivers converge into the Beibu Gulf including the Red River, Fangcheng River, Nanlujiang River, Qinjiang River, Dafengjiang River, Beilun River and Changhuajiang River [24]. Hydroacoustic assessment supplemented with trawling survey was performed on July 7–27, 2014 in the Beibu Gulf, northern South China Sea (Fig. 1). This area was selected as a representative part of the offshore fishery because it is one of the four traditional fishing grounds in China. The survey was conducted only in the area, that is, the right side of the dividing line where trawling is permitted in China. The cruise track was established by incomplete random design [25] around the trawling sites, which were isotermically scattered (Fig. 1).

**Data collection.** Acoustic data were collected using a Simrad EY60 echosounder with 70- and 120-kHz split-beam transducers (Table 1) and fixed vertically on the side of the middle portion of the ship immersed at 1.5 m into the water [26]. Acoustic data were acquired and stored continuously with an actual detection depth range of 10–100 m. The acoustic
equipment was calibrated prior to the survey by using 32.1- and 23.0-mm copper spheres in accordance with the standard procedures described in the Simrad ER60 reference manual [27]. Hydroacoustic assessment was conducted mainly during daytime for proper matching of the trawling and acoustic data.

Trawling was performed at a ship speed of 3.0–3.4 knots. A total of 38 tows were conducted at 6:00–18:00 in daylight for 1 h at each site. Biological samples were collected from trawl catches to calculate the actual parameters included in the biomass density formula. Fishes were identified based on species and standard length (L) measured to the nearest millimeter. Fish weight was directly measured to 1 g precision.

**Acoustic data analysis.** Echoview software (version 6.1) was adopted to process the acoustic data and remove the noise. An automatic algorithm was utilized to define the bottom line 1 m above the detected bottom. A surface line was added to each echogram at a distance of 2 m from the transducer to avoid the near-field (1 m) and wave disturbances. Only the data between these two lines were analyzed to exclude bottom echoes and integrate surface noise into fish backscattering measurements (Fig. 2).

The nautical area scattering coefficient (NASC) and the target strength (TS) were calculated over a distance of 1000 nm at a 1-nm interval between each unit by using Echoview. Only the backscattering data at 5–100 m were selected for data analyses; these data corresponded to the shallowest daylight occurrence of total fishes and depth of trawls. The bad data, i.e., the undesirable targets, were checked and eliminated. Biomass density (tons/n.mi²) was calculated using the following formula:

\[
\text{Biomass density} \ (\text{tons/n.mi}^2) = \left(\frac{\% \text{contribution}}{100}\right) \times \left(\frac{\text{NASC}}{4\pi\sigma}\right) \times \left(\frac{\text{weight}}{1000}\right),
\]

**FIGURE 2**

Echograms in vertical beam showing fish tracks (a) and fish schools (b).
where
\[ \bar{\sigma} = \sum_{\text{all species}} [\text{(%contribution/100)} \times 10^{-\text{(TS/10)}}]. \]

The mean weight and average backscattering cross-section (\( \sigma \)) values used in this study were calculated from the catch data and average TS (dB), respectively [25]. The fish track threshold was set to \(-70\) dB to avoid considerably small targets. Fish density (tons/m²) was calculated as 100% contribution, suggesting that all catches were calculated and selected in this study. TS distribution and trawling catches are necessary supplementary information in biomass density studies and reveals the target size and target depth, which can reflect different habitat levels of different sizes in this study.

Comparison of the semivariogram models. Semivariogram models are the fundamental components of geostatistical applications. To predict the biomass density, four semivariogram models including the spherical, exponential, Gaussian, and linear models were fitted [28] and statistically compared with the residual sum of squares (RSS), square of the correlation coefficient (\( r^2 \)), and proportion \( C/(Co + C) \) [29]. RSS provides an accurate measurement of how well a model fits the variogram data. The low reduced sums of squares indicate the good fit of a model. Additionally, \( r^2 \) provides an indication of how well a model fits the variogram data. The value of \( C/(Co + C) \) is 1.0 for a variogram with no nugget variance (where the curve passes through the origin) and 0 when no spatially dependent variation is present at the specified range. The most suitable semivariogram model was selected and utilized.

OK and cross validation. OK spatial interpolation is a simple prediction method that exhibits remarkable flexibility. OK assumes an unknown constant mean, and the data points must be sampled from a phenomenon that is continuous in space. The general formula of OK is as follows [30, 31]:
\[ \hat{Z}(x_0) = \sum_{i=1}^{N} \omega_i Z(x_i) \]

where \( Z(x_i) \) is the measured value at the \( i \)th location, is an unknown weight for the measured value at the \( i \)th location, \( \hat{x}_0 \) is the prediction location, and \( N \) is the number of measured values.

Data transformation is of significance for the assumption that the data are normally distributed. OK was adopted in this study to create fish density maps and prediction standard error maps in the Beibu Gulf after data transformation for normal distribution. The performance between acoustics alone and OK was statistically compared by using descriptive statistics.

Cross validation, a technique used to assess the accuracy of an interpolation model, leaves one point out and uses the rest to predict a value at that location in geostatistical analysis. The point is then added back into the dataset, and a different one is removed.

The pair of predicted and actual values can be compared to assess the performance of OK by determining the coefficient (\( R^2 \)), mean error (ME), and root mean square error (RMSE).

RESULTS

Correlation between target size and target depth. The mean TS value of 2012 fish-tracking targets was (49.73 ± 0.14) dB (Fig. 3a). The maximum and minimum values were −35 and −66 dB, respectively. The skewness and kurtosis of the TS distribution were 0.54 ± 0.05 and −0.01 ± 0.10, respectively. The standard deviation was 6.12.

F-test showed a correlation between target size and target depth (\( \rho < 0.05 \)), and Pearson’s \( r \) (0.65) identified the correlation as positive.

The three water layers, namely, 5–30 m as the surface layer (SL), 30–50 m as the middle layer (ML), and 50–78 m as the bottom layer (BL), were statistically compared in terms of TS scale and target size, and a vertical distribution was found (Fig. 3b). The layers followed the order of BL < ML < SL, when sorted by TS scale (difference between maximum and minimum), SL < ML < BL when sorted by mean TS, and ML < BL < SL when sorted by percentage.

Overview of catch. A total of 201100 fish individuals representing over 200 species were caught by trawling in the Beibu Gulf. The species of the catches were mainly small- and medium-sized fish located in the lower trophic levels of the food chain and cephalopods, such as Acropoma japonicum, Acropoma hanedai, Psenopsis anomala, Trachurus japonicus, Loligo edulis, and Loligo chinensis, etc. (Table 2).

The maximum quantity of catch was 28455 at site 31, and the quantity-concentrated area was in the common fishing zone around sites 33–38 in the south of the Beibu Gulf. The maximum weight was 333.00 kg at site 37, and the weight-concentrated area was also in the common fishing zone near sites 31–38 in the south of the Beibu Gulf (Fig. 4).

The maximum average weight of the catch was 0.049 kg in the northern part of the survey area, and the minimum average weight was 0.001 kg in the southern area (Fig. 5).

Comparison of the semivariogram models. Table 3 presented the results of fitting the semivariogram models to the 578 acoustic samples of biomass density and the error measurement values.

As illustrated in Table 3, the minimum values for RSS and the most remarkable values for \( C/(Co + C) \) were obtained in the exponential model. Thus, the exponential model was selected to obtain the OK model.
FIGURE 3
TS frequency distribution of the fish tracks (a) and the distribution of depth vs. TS (b), and the trend line is illustrated by color red in (b).

TABLE 2
Proportion of main species sorted by the tail and weight

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>% by tail</th>
<th>No.</th>
<th>Species</th>
<th>% by weight</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Acropoma japonicum</td>
<td>32.3719</td>
<td>1</td>
<td>Acropoma japonicum</td>
<td>10.3141</td>
</tr>
<tr>
<td>2</td>
<td>Acropoma hanedai</td>
<td>8.9617</td>
<td>2</td>
<td>Trachurus japonicus</td>
<td>8.6786</td>
</tr>
<tr>
<td>3</td>
<td>Pomadasys argenteus</td>
<td>4.4712</td>
<td>3</td>
<td>Parargyrops edita</td>
<td>7.2602</td>
</tr>
<tr>
<td>4</td>
<td>Parargyrops edita</td>
<td>4.1399</td>
<td>4</td>
<td>Decapterus maruadi</td>
<td>7.1688</td>
</tr>
<tr>
<td>5</td>
<td>Trachurus japonicus</td>
<td>3.8541</td>
<td>5</td>
<td>Psenopsis anomala</td>
<td>4.1893</td>
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<tr>
<td>6</td>
<td>Loligo edulis</td>
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<td>6</td>
<td>Loligo chinensis</td>
<td>3.8329</td>
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<tr>
<td>7</td>
<td>Decapterus maruadi</td>
<td>3.6208</td>
<td>7</td>
<td>Nemipterus bathybius</td>
<td>3.3306</td>
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<td>8</td>
<td>Leiognathus ruconius</td>
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<td>Apogonichthys etliot</td>
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<td>Leiognathus berbis</td>
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<tr>
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<td>Navodon xanthopterus</td>
<td>2.4061</td>
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<tr>
<td>11</td>
<td>Parapeneus fiorioides</td>
<td>1.3041</td>
<td>11</td>
<td>Psenopsis anomala</td>
<td>2.3798</td>
</tr>
<tr>
<td>12</td>
<td>Loligo chinensis</td>
<td>1.2802</td>
<td>12</td>
<td>Psenopsis anomala</td>
<td>1.7687</td>
</tr>
<tr>
<td>13</td>
<td>Priacanthus macracanthus</td>
<td>1.2268</td>
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<td>Priacanthus macracanthus</td>
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<tr>
<td>14</td>
<td>Setipinna taty</td>
<td>1.2184</td>
<td>14</td>
<td>Setipinna taty</td>
<td>1.5369</td>
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<tr>
<td>15</td>
<td>Solenocera crassicomis</td>
<td>1.1360</td>
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<tr>
<td>16</td>
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<td>Cephaloscyllium umbratile</td>
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<td>17</td>
<td>Apogonichthys striatus</td>
<td>1.1103</td>
<td>17</td>
<td>Setipinna taty</td>
<td>1.0554</td>
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<tr>
<td>18</td>
<td>Psenopsis anomala</td>
<td>0.9756</td>
<td>18</td>
<td>Muraenesox cinereus</td>
<td>0.8968</td>
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<tr>
<td>19</td>
<td>Argyrosomus argentatus</td>
<td>0.9701</td>
<td>19</td>
<td>Rhyhnochelum nyctom</td>
<td>0.8269</td>
</tr>
<tr>
<td>20</td>
<td>Caranx kalla</td>
<td>0.9501</td>
<td>20</td>
<td>Octopus striolatus</td>
<td>0.8188</td>
</tr>
<tr>
<td>21</td>
<td>Thrissa dussumieri</td>
<td>0.9257</td>
<td>21</td>
<td>Todarodes pacificus</td>
<td>0.7886</td>
</tr>
<tr>
<td>22</td>
<td>Leiognathus bindus</td>
<td>0.8740</td>
<td>22</td>
<td>Caranx kalla</td>
<td>0.6678</td>
</tr>
<tr>
<td>23</td>
<td>Metapeneaeopsis palmensis</td>
<td>0.7273</td>
<td>23</td>
<td>Gastrophus spadiceus</td>
<td>0.6521</td>
</tr>
<tr>
<td>24</td>
<td>Siganus oramin</td>
<td>0.6620</td>
<td>24</td>
<td>Carangoides equula</td>
<td>0.6482</td>
</tr>
<tr>
<td>25</td>
<td>Caridina japonica</td>
<td>0.6150</td>
<td>25</td>
<td>Argyrosomus macrocephalus</td>
<td>0.6313</td>
</tr>
</tbody>
</table>
FIGURE 4
The catch quantity and weight of each trawling site.

FIGURE 5
The average weight of each trawling site and the total average weight.

TABLE 3
Comparison of semivariogram models

<table>
<thead>
<tr>
<th>Model</th>
<th>Nugget variance($C_0$)</th>
<th>Structural variance sill ($C_0 + C$)</th>
<th>Range (A)</th>
<th>RSS</th>
<th>$r^2$</th>
<th>Proportion $C/ (C_0 + C)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical</td>
<td>0.0668</td>
<td>0.3276</td>
<td>1.0770</td>
<td>0.0172</td>
<td>0.852</td>
<td>0.796</td>
</tr>
<tr>
<td>Exponential</td>
<td>0.0328</td>
<td>0.3426</td>
<td>1.3110</td>
<td><strong>0.0140</strong></td>
<td><strong>0.879</strong></td>
<td><strong>0.904</strong></td>
</tr>
<tr>
<td>Gaussian</td>
<td>0.1013</td>
<td>0.3266</td>
<td>0.8937</td>
<td>0.0204</td>
<td>0.823</td>
<td>0.690</td>
</tr>
<tr>
<td>Linear</td>
<td>0.1530</td>
<td>0.3891</td>
<td>1.8994</td>
<td>0.0325</td>
<td>0.719</td>
<td>0.607</td>
</tr>
</tbody>
</table>
Prediction of spatial biomass density. The acoustic samples were subjected to natural log (base e) transformation. The p-value of K-S test was 0.200 > 0.05 (Fig. 6) and followed the normal distribution pattern.

OK was applied in the analysis of data prediction as a spatial interpolation method in the survey area. The cross-validation results between the measured and predicted data are illustrated in Fig. 7, wherein the determination coefficient ($R^2$) was 0.8986, Pearson’s r was 0.9481, ME was 0.0699, and RMSE was 3.8956, respectively.

The mean biomass density of the descriptive statistics on acoustics and OK were the same (Table 4). Acoustic measurement showed a higher standard deviation than that of OK, indicating that the latter was more stable than the former. Approximately 18 data gaps caused discontinuity in the investigation route (Fig. 8). This discontinuity increased the difficulty and inaccuracy of the biomass density evaluation in the acoustics approach alone.
TABLE 4
Comparison of biomass density based on acoustics data and OK

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acoustics</td>
<td>21.05</td>
<td>13.25</td>
<td>2.47</td>
<td>70.65</td>
</tr>
<tr>
<td>OK</td>
<td>21.44</td>
<td><strong>10.44</strong></td>
<td>3.86</td>
<td>62.18</td>
</tr>
</tbody>
</table>

FIGURE 8
Map of the biomass density analyzed by Echoview, the red rectangle marks data gap.

FIGURE 9
Map of the biomass density using the kriging interpolation, red indicates the highest density, whereas blue denotes the lowest density.
Spatial distribution was observed on the prediction map. Different colors denoted different density levels (Fig. 9). The red area in the north of the survey area was larger than that of the south, and the blue area in the west of the survey area was larger than that of the east. The distribution pattern of fishery resources was less distributed from north to south and from east to west. Red color reflected the highest density with an interval value of 43.38–62.18 tons/n.m², and blue color indicated the lowest density with an interval value of 3.86–10.25 tons/n.m². The top two levels were mostly concentrated in the northern area of the Beibu Gulf at sites 4, 7, 9, 10, 13, and 23.

The prediction standard error was within the acceptable range (Fig. 10). The mean value was 9.70 and the standard deviation was 5.57. Among different layers, layer 2 (4.47–8.70, 32%) had the largest percentage, whereas layer 6 (21.43–25.66, 2%) had the smallest proportion.

**DISCUSSION**

**Comparison and combination of fishery acoustics and OK.** Fishery acoustics is an advanced technology for resource investigation that can be applied in combination with catch data for resource assessment [32-35]. However, the acoustic sampling method exhibits several limitations that must be considered when interpreting or processing data [8]. To ensure the high accuracy of acoustic data, the undesirable data were eliminated in this investigation, which accounted for one-third of the total data. These data were mainly caused by the loss of GPS information, low signal-to-noise ratio and bubbles [36]. Consequently, discontinuity and data gaps occurred in the investigation route map due to the removal of bad data.

OK, which can create a statistical surface without gaps, plays a pivotal role by rationalizing and completing data through appropriate geographical statistical model predictions [28, 37]. In this study, OK exhibited better adaptability for biomass density based on acoustic technology than alternative interpolation methods, such as inverse distance weighting, local polynomial interpolation and radial basis function [3, 38-42]. Therefore, OK was applied in this investigation. Moreover, the fitness of OK in the present study was better than that in previous studies ($R^2 = 0.612$) because the interpolation model was optimized and the bad data were eliminated. The surface of biomass distribution made by OK is straightforward and reliable in revealing distribution characteristics. Also, rigorous data processing, such as data transformation for normal distribution, comparison of semivariogram models, and cross-validation, refined the acoustic data. Therefore, combination of fishery acoustics and OK is considered as an excellent trial for the analysis of fish abundance and distribution pattern in the case of funding limitations and poor environmental conditions in large-scale surveys.
**Status and management suggestion of fishery resources.** The biomass density of the survey area, calculated by OK, was higher and the range was smaller than (18.99 tons/n.mi$^2$) and (0.356–252.692 tons/n.mi$^2$) in the survey conducted in the Summer of 1998–1999 [43]. The regional distribution characteristics in the predicted surface map were the same, implying the relatively stable distribution pattern of fishery resources in the Beibu Gulf. With regard to fish size, a large TS value generally corresponds to a large size of the catch [44]. Judged by the correlation between target size and target depth, the vertical distribution pattern was obtained that large fish tended to live in deep waters, which was consistent with previous findings [45, 46]. Furthermore, the average weight measured by total catch was lower than (30–80 g) in the survey conducted in the Summer of 1998–1999 [43], indicating the high probability of miniaturized fishery resources in the Beibu Gulf. In the trawling catch, the proportion of low-trophic-level species at several sites was relatively high, thereby making the average weight as small as the minimum threshold.

The proportion of low-trophic-level species has been increased, and offshore ecosystem degradation is becoming considerably evident because of the excessive consumption of fishery resource, marine pollution, and overfishing [47-49]. Another observed phenomenon was that the biomass density was increased under such a complex living environment. The growth coefficient (K) was increased from 0.35 in 1962 to 0.50 in 2006, suggesting that a number of low-trophic-level species tended to be miniaturized with an increase in speed of growth and a decrease in time to mature [23]. Although the growth enhancement of low-trophic-level fish may be due to natural selection, this phenomenon is not beneficial for fishermen. Proper management, such as the extension of fishing moratorium and reasonable arrangement of fishermen’s employment, can help to protect and conserve the endangered and predatory species and benefit fishermen from recoveries of these high-trophic-level species. Perhaps, we can adopt modern technological strategies, such as transgenic technology, to strengthen the growth and development of high-trophic and high-value fish under the premise of ecological security. However, given the risks posed by gm technology, it should be treated with caution [50].

**CONCLUSIONS**

1) The correlation between target size and target depth indicates the vertical distribution pattern. Large fish tends to live in deep waters. The predicted surface map by OK demonstrates that the regional distribution characteristics is less distributed from north to south and from east to west.

2) Based on the TS value and the catch size, the proportion of low-trophic-level species at several sites is elevated, indicating the high probability of miniaturized fishery resources and the offshore ecosystem degradation in the Beibu Gulf.

3) Regarding the data processing, data transformation for normal distribution, comparison of semivariogram models, refined the acoustic data and cross-validation are performed to test the fitness of OK. The combination of fishery acoustics and OK is a feasible approach.

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IMPACT OF TYPE AND DOSE OF BIOCHAR AND HYDROCHAR ON GROWTH RESPONSE OF PHYTOPATHOGENIC AND ANTAGONISTIC SOIL RESIDENT FUNGI

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ABSTRACT

Biochar (BC) and hydrochar (HC) are solid by-products obtained through, respectively, pyrolysis and hydrothermal carbonization of biomass. Both materials are used as organic amendments allowing carbon sequestration in soil. Two BCs, from spruce pellets (BC_{SP}) and grapevine pruning residues (BC_{GV}), and two HCs, from urban pruning residues (HC_{UP}) and the organic fraction of solid urban waste (HC_{SU}), were tested at concentrations of 0.2 and 1% (w/v) on soil-resident fungi. The higher dose of BC samples significantly reduced mycelial growth of the phytopathogens *Rhizoctonia solani* and *Botrytis cinerea* (up to 53% for BC_{GV} on *B. cinerea* after 24 h from inoculation, compared to control), whereas, in general, the lower dose of both BCs inhibited *R. solani* for the whole experiment and *B. cinerea* only initially. HC samples evidenced a general lower toxicity on the two pathogens than BCs. In fact, only HC_{UP} at both concentrations reduced hyphal elongation of the sole *B. cinerea* for the entire experimental time (up to of 38% for both doses at 24 h from inoculation). All BC and HC treatments at the lower concentration did not influence or greatly stimulated the antagonists *Trichoderma harzianum* and *T. virens*. Differently, the higher dose of these materials caused some suppression at the initial stage or at each sampling by BC_{SP} on *T. harzianum* and HC_{UP} on *T. virens*. The whole results obtained indicate a plant protective activity of these materials with consequent less need for fungicidal treatments.

KEYWORDS:
Biochar, hydrochar, phytopathogenic fungi, antagonistic fungi, fungal growth

INTRODUCTION

Processes of pyrolysis and hydrothermal carbonization allow a sustainable reuse and safe disposal of waste biomasses of urban, industrial and agricultural origin. Solid by-products of the two technologies, i.e. biochar (BC) and hydrochar (HC), respectively, have various environmental and agricultural applications. When used as soil amendments, these materials contrast climate change acting as carbon sequestering agents in soil. Recent works demonstrated the positive impact of BC on soil quality, crop yield and soil microbial biomass [1-4]. Due to the very recent technology to produce HC, scarce information exists on HC influence on soil properties [2, 5], plant growth [3], and microbial community [4].

Although BC and HC are much more recalcitrant than their original feedstock, several studies revealed the presence in these materials of bioavailable fractions influencing microbial growth and activity [6]. In particular, BC was found to contain adsorbed volatile compounds [7], water soluble molecules and polycyclic aromatic hydrocarbons [8], and several metals potentially toxic to microorganisms [9]. HC too can contain heavy metals [10], polycyclic aromatic hydrocarbons [11] and easily degradable-highly dissolved organic C content [5].

Recent studies demonstrated that amendment of soil with BC could shift fungal taxa composition [12] and affect the growth of some groups of fungi, such as mycorrhizal fungi [13] and ligninolytic fungi [14]. Furthermore, BC seems capable to inhibit phytopathogenic fungi [15]. Much less information is present in the literature addressing the impact of HC on soil-borne fungi [16], and none on phytopathogenic fungi.

*Rhizoctonia solani* and *Botrytis cinerea* are well-known phytopathogens of great economic importance because of the wide range of cultivated host plants [17,18]. A successful suppression of these fungi is usually obtained using fungicides which, however, are dangerous for the environment and human health. In nature, fungal species of *Trichoderma* can play an important role in controlling phytopathogens acting also as plant symbionts and ubiquitous decomposers of plant debris [19]. *Trichoderma* spp. represent about 60% of all registered bio-fungicides worldwide [20]. The species *T. harzianum* and *T. virens* are very efficient both as antagonists [20] and as promoters of the growth of many cultivated plants [21].
This investigation evaluated the impact in vitro of different BC and HC treatments on the growth of the phytopathogens *Rhizoctonia solani* and *Botrytis cinerea* and the antagonists *T. harzianum* and *T. virens*.

**MATERIALS AND METHODS**

Two BCs, whose feedstocks were 100% high quality red spruce pellets (BC<sub>sp</sub>) and 100% grapevine pruning residues (BC<sub>GV</sub>), were provided by Blucumb s.r.l., Udine, Italy. Both BCs were produced using micro-gasification with a residence time of 3 h, a peak temperature of 550 °C and following dry cooling. Two HCs, whose feedstocks were urban pruning residues (HC<sub>up</sub>) and the organic fraction of solid urban wastes (HC<sub>su</sub>), were provided by Ingelgia Italia s.r.l., Lucca, Italy. Both HCs were produced using a hydrothermal carbonization chamber operating between 180-210 °C with pressures between 10 and 20 bar. BC and HC samples were air-dried, ground with mortar and pestle and 0.5-mm sieved. Some properties of BC and HC samples used are reported in Table 1.

Isolates of *Rhizoctonia solani* (CBS 101590), *Botrytis cinerea* (CBS 121.39), *Trichoderma harzianum* (CBS 120966) and *T. virens* (CBS 116947) were obtained from the Centraal Bureau voor Schimmelcultures (CBS-KNAW), Utrecht, The Netherlands. Fungi were grown on potato dextrose agar (PDA, 4% w/v) in Petri dishes in the dark at 20 °C ± 1 °C. Fungal inoculum was a 2-mm PDA disk overgrown by mycelium collected from the growing margin of the colony.

Aqueous solutions of PDA (4%, w/v) were not added (control) or added with each BC and HC, separately, at concentrations of 0.2% (BC<sub>sp</sub>L, BC<sub>GV</sub>L, HC<sub>up</sub>L, HC<sub>su</sub>L) and 1% (BC<sub>sp</sub>H, BC<sub>GV</sub>H, HC<sub>up</sub>H, HC<sub>su</sub>H). These suspensions were autoclaved at 121°C for 15 min. Then, aliquots of 20 mL were taken under agitation from each medium and poured into 9-cm-diameter Petri dishes and allowed to cool at room temperature. It was assumed that autoclaving did not alter BC and HC as their production temperatures were much higher than 121°C. The doses of both amendments were selected on the basis of recommended field application [22]. The fungus was then inoculated in the centre of the plate under axenic conditions and the dishes were randomly placed in an incubator in the dark at a constant temperature of 21 ± 1 °C. All experiments were replicated five times. Mycelial radial growth was monitored until the fungus reached approximately the border of the plate at least in one sample. All results were statistically analysed by one-way analysis of variance (ANOVA), and the means of treatments separated by the least significant differences (LSD) test.

**RESULTS**

*Rhizoctonia solani*. In almost all treatments, both BC types inhibited mycelial growth during the 120-h experimental period, with variations statistically significant (P ≤ 0.001) compared to control (Figs. 1a and 1b). In general, inhibitory effects of both types of BC increased with increasing dose. BC<sub>sp</sub>H and, especially, BC<sub>sp</sub>L caused a lower inhibition than BC<sub>GV</sub>H and BC<sub>GV</sub>L, respectively (Figure 1a and 1b). However, inhibitory effects of BC<sub>sp</sub> at both doses appeared to increase along the whole experiments (Fig. 1a), whereas the inhibition of BC<sub>GV</sub> decreased starting from 72 h (Fig. 1b).

Differently, HC did not show suppressing effects on this fungus, except HC<sub>su</sub>L after 48 and 72 h from inoculation (Fig. 1c and 1d). At the other sampling times, both HCs at the lower dose did not alter mycelial growth compared to control, whereas at the higher dose they stimulated hyphal elongation after 96 h from inoculation (up to 46% for HC<sub>up</sub>H at the last sampling time) (Figs. 1c and 1d).

*Botrytis cinerea*. The lower dose of both types of BC exerted significant depressive effects only at the first sampling (24 h). Successively, BC<sub>sp</sub>L had no effect on the fungus, whereas BC<sub>GV</sub>L caused a little stimulation of mycelial elongation (Figs. 2a and 2b). Differently, the higher dose of both BC, especially BC<sub>GV</sub>, caused significant (P ≤ 0.001 in most of samplings) depressive effects on the fungus throughout the experimental period (Figs. 2a and 2b). However, both BCs exerted the maximum inhibition after 24 h from inoculation, whereupon the effects decreased rapidly for BC<sub>sp</sub>H and gradually for BC<sub>GV</sub>H (Figs. 2a and 2b).

**TABLE 1**

Some properties of BC and HC samples used

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type – Feedstock</th>
<th>Ash (%)</th>
<th>pH*</th>
<th>EC* (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>Biochar – Red Spruce</td>
<td>9.87</td>
<td>9.1</td>
<td>390</td>
</tr>
<tr>
<td>BC&lt;sub&gt;GV&lt;/sub&gt;</td>
<td>Biochar – Vineyard Pruning Residues</td>
<td>13.57</td>
<td>9.9</td>
<td>223</td>
</tr>
<tr>
<td>HC&lt;sub&gt;up&lt;/sub&gt;</td>
<td>Hydrochar – Urban Pruning Residues</td>
<td>12.51</td>
<td>6.6</td>
<td>1026</td>
</tr>
<tr>
<td>HC&lt;sub&gt;su&lt;/sub&gt;</td>
<td>Hydrochar – Organic Fraction of Solid Urban Waste</td>
<td>35.73</td>
<td>7.7</td>
<td>1094</td>
</tr>
</tbody>
</table>

* 1:10 (w/v)
FIGURE 1
Effects of BC and HC treatments at concentrations of 0.2% (w/v, L) and 1% (w/v, H) on variations (%) of radial mycelial growth of Rhizoctonia solani, respect to PDA only (control, only error bars), as a function of time. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001, according to LSD test. Standard error of the mean (n = 5) is also indicated.

FIGURE 2
Effects of BC and HC treatments at concentrations of 0.2% (w/v, L) and 1% (w/v, H) on variations (%) in the radial mycelial growth of Botrytis cinerea, respect to PDA only (control, only error bars), as a function of time. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001, according to LSD test. Standard error of the mean (n = 5) is also indicated.

A marked reduction of mycelial growth was observed in all treatments with HC<sub>U</sub> at both doses (up to 38% reduction, compared to control, after 24h from inoculation) (Figs. 2c). In HC<sub>S</sub> treatments, fungal inhibition was noticeable at the first sampling (24 h) with a reduction of the mycelial radius of about 40% for both doses (Fig. 2d). No successive variations were observed with HC<sub>S</sub> on this fungus, compared to control (Fig. 2d).
**FIGURE 3**
Effects of BC and HC treatments at concentrations of 0.2% (w/v, L) and 1% (w/v, H) on variations (%) of radial mycelial growth of *Trichoderma harzianum*, respect to PDA only (control, only error bars), as a function of time. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001, according to LSD test. Standard error of the mean (n=5) is also indicated.

**FIGURE 4**
Effects of BC and HC treatments at concentrations of 0.2% (w/v, L) and 1% (w/v, H) on variations (%) of radial mycelial growth of *Trichoderma virens*, respect to PDA only (control, only error bars), as a function of time. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001, according to LSD test. Standard error of the mean (n=5) is also indicated.

*Trichoderma harzianum*. The lower dose of both types of BC and HC was irrelevant or slightly beneficial to the mycelial growth, whereas the higher dose of BC affected the fungus differently from that of HC (Fig. 3). In particular, BC30L showed a little stimulation only at the second sampling (8% increase at 48 h from inoculation), whereas BC30H caused a marked inhibition during the whole experiment (up to 37% decrease at 24 h from inoculation) (Fig. 3a). Initially, BC20L had no effect on the fungus, whereas at the successive two samplings it significantly promoted hyphal elongation (Fig. 3b). BC20H showed variable effects over time, with an evident fungal inhibition at 24 h from inoculation, a little inhibition at the second sampling and a significant stimulation at the end of experiments (Fig. 3b).

Both types of HC generally stimulated this
fungus at both concentrations, with maximum increase of mycelial radius of 22%, compared to control, in HC$_3$H treatment at 48 h from inoculation (Figs. 3c and 3d).

**Trichoderma virens.** Only the higher dose of both types of BC and only at the first sampling (48 h after inoculation) depressed ($P \leq 0.001$) fungal growth (Figs. 4a and 4b). Differently, at the two successive samplings, BC treatments, especially BC$_{C_{OV}}$, generally simulated fungal growth (Figs. 4a and 4b).

Similarly, HC treatments increased hyphal elongation of this fungus for the whole experiments, except HC$_{C_{OV}}$ that slightly inhibited the fungus (Figs. 4c and 4d). After 72 h from inoculation, HC$_{C_{S}}$H exerted the maximum stimulation of this fungus (20% increase of radial growth, compared to control) (Fig. 4d).

**DISCUSSION AND CONCLUSIONS**

**Phytopathogenic fungi.** Regardless of feedstock, BC samples at the higher dose demonstrated only an inhibitory activity towards both pathogenic species *R. solani* and *B. cinerea*. However, in the case of *B. cinerea*, the suppressing activity decreased throughout the experiments, suggesting the occurrence of some degradation of BC components in the medium or their absorption and detoxification by the fungus. This effect was not observed in the case of *R. solani* which showed different trends of inhibition in the course of trials. Loffredo and Traversa [23] found a progressive reduction of the suppressive activity of different allelochemical compounds on the phytopathogen *Sclerotinia sclerotiorum* and they attributed it to some degradation produced by the fungus. The inhibitory effects observed in our study are in agreement with the findings of Graber et al. [15] on *R. solani*. These authors, using BC samples from different types of feedstock and applied at various doses, reported maximum inhibition of *R. solani* at BC concentration of 1% [15]. In contrast, Gravel et al. [24] found that the addition in the potting medium (1:1 v/v) of a BC from a mixture of barks of various trees increased pot colonization by the plant pathogen *Pythium ultimum*. However, in the study of Gravel et al. [24], the different fungal species, the different BC dose and, especially, the different temperature adopted (475 °C) in BC production might account for the contrasting findings. It is known that feedstock and pyrolysis parameters largely affect the final characteristics of BC and consequently its biological action [25].

In this work, the lower dose of BC demonstrated a suppressing activity only towards *R. solani*, whereas the growth of *B. cinerea* was either not affected or slightly stimulated depending on the feedstock. Perhaps, the lack of effects of BC$_{C_{OV}}$ on *B. cinerea* depended on the too low dose or a possible fungal degradation or detoxification of some BC components. On the other hand, stimulation effects observed in BC$_{C_{OV}}$ treatment might be explained with the use by the fungus of BC as carbon source.

In general, HC treatments were less inhibitory on the two fungi than BC treatments. In fact, only HC$_{C_{OV}}$ at both doses significantly inhibited *B. cinerea* for the entire experimental time, whereas most of the other HC treatments were irrelevant on these fungi, or even stimulating at the higher concentration on *R. solani*. Also in the case of HC, the fungus might have benefited from the addition of this material to the medium by using some HC components as carbon source. Studies focusing on the impact of HC on microorganisms, especially fungi, are rather scarce when compared to similar investigations adopting BC. Although the mechanisms behind the effects of HC observed in this study are still not clarified, we assume that the chemical composition of this material has an important role. Rillig et al. [26] demonstrated that amendment of soil with HC obtained from beet root chips stimulated root colonization by arbuscular mycorrhizal fungus *Glomus intraradices*. These authors attributed the observed stimulation to some labile components present in HC [26].

**Antagonistic fungi.** The addition of both types of BC at the lower dose in the fungal medium caused either not significant effects or an evident stimulation of mycelial growth of the two fungi. These results are in agreement with the findings of Graber et al. [27] who demonstrated a significant increase of *Trichoderma* spp. biomass following the application in the potting medium of a BC from citrus wood. Furthermore, Hu et al. [28] demonstrated that when a BC from forest litter was applied to soil, it caused a change in the fungal populations of soil towards taxa of *Trichoderma* and *Paecilomyces*, thus suggesting that these fungi benefited from BC addition.

On the other hand, the higher dose of BC caused differentiated results as a function of the type of material. BC$_{C_{OV}}$ clearly inhibited *T. harzianum*, even if the effects gradually decreased over time. BC$_{C_{OV}}$ on *T. harzianum* and both BC samples on *T. virens*, caused similar effects, i.e., an initial inhibition followed by a relevant stimulation. Also in this case, we can assume that fungi were able to degrade BC components and use them as nourishment.

The two types of HC at each dose showed stimulating effects on both *T. virens* and *T. harzianum*, with the only exception of HC$_{C_{OV}}$ that inhibited the growth of *T. virens*. However, as in BC treatments, *T. virens* was able to reduce inhibition of HC$_{C_{OV}}$ during experiments. Unfortunately, no
other study on this matter can be found in the literature, therefore a comparison between our results and those of other researchers is not possible. Nevertheless, a general positive influence of BC on soil-resident fungi, especially arbuscular mycorrhiza, was reported [26]. Furthermore, Rex et al. [4] found that soil amendment with BC from Miscanthus giganteus straw increased the overall soil fungal biomass. Although our study does not clarify the mechanisms involved, we hypothesize that BC stimulation on these fungi might be due to both possible presence of stimulating compounds in it and/or decomposition of BC by fungi and use as carbon source. In comparison with BC, HC is more easily degradable because of fewer aromatic structures and a higher percentage of labile carbon present [29]. Furthermore, Trichoderma species are known decomposers of organic materials in soil, and for this reason they are also used as activators in the composting process [30].

In conclusion, this study showed that both BC and HC can exert relevant impact on the growth of both phytopathogenic fungi and biocontrol agents commonly present in soil. However, in addition to the fungal species considered, both the type and the dose of these materials largely influence the response of fungi. Our findings may contribute to elucidate the biological activity of these materials that are more and more adopted as soil amendments in modern sustainable agriculture. The correct choice of BC and HC types, or even the preparation of tailored BC and HC, may be helpful to improve soil fertility and, at the same time, to control plant diseases replacing, at least partially, pesticides with significant benefits for the environmental safety. Further comprehensive research on this matter is necessary in a world where soils are subject to progressive deterioration and food production is constantly challenged by the needs of increased population.

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ATMOSPHERIC VISIBILITY PREDICTION BASED ON NEURAL NETWORK AND CHAOTIC TIME SERIES ALGORITHMS

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ABSTRACT

In recent years, the problem of low visibility problems caused by air pollution has become increasingly serious. Accurate prediction of atmospheric visibility is more related to anthropogenic activities. In this study, visibility, environmental and meteorological data (2017–2018) from the Yinchuan area of China were selected as experimental data, and the LM-BP neural network, RBF neural network and chaotic time series algorithm were each used to predict atmospheric visibility. For the atmospheric visibility models based on the two neural networks, 1032 data elements were selected to establish the prediction model based on the chaotic time series algorithm. Experiments were performed to verify the feasibility of each model. The results showed that the prediction model based on the chaotic time series algorithm achieved better prediction results than the LM-BP model, the time overhead of the RBF prediction was much greater. However, in practical application, if only historical visibility data were used as the sample input, the chaotic time series algorithm could be used to predict visibility.

KEYWORDS:
Atmospheric visibility, LM-BP neural network, RBF neural network, chaotic time series algorithm, prediction model

INTRODUCTION

Atmospheric visibility is an important physical parameter reflecting air quality that has direct impact on human quality of life. In recent decades, many observational, statistical and numerical modeling studies have been conducted regarding the prediction of atmospheric visibility. Large-scale macroscopic statistical analysis has shown that atmospheric particulate matter (PM) directly or indirectly affects visibility. In fact, the characteristics of the spatiotemporal distribution of visibility are affected by geological dust particles, long-term relative humidity conditions, pollutant emissions and other factors [1-3]. Previous research on atmospheric visibility has focused largely on the correlation between visibility and meteorological and pollutant factors. It has been established that PM2.5, NO2, SO2 and other pollutants have significant correlation with visibility [4-7]. Moreover, meteorological conditions such as temperature, humidity, wind speed and rainfall have also been found important in relation to the transport, diffusion, subsidence and subtraction of atmospheric aerosol particles and thus to short-term change of visibility. Furthermore, long-term meteorological conditions and pollutant levels have been shown to have obvious effects on macroscale visibility trends [8-10].

Saadatseresht and Varshosaz [11] used artificial neural networks (ANNs) to study atmospheric visibility prediction. Clark et al. [12] proposed a visibility prediction model that incorporated humidity, aerosols and temperature as the main influencing factors. Based on Bayesian probabilities, Chmielicki and Raftery [13] used the Bayesian mean square method to predict the probability of visibility. In China, research has been undertaken on visibility during foggy days. Zhang et al. [14] established atmospheric visibility indicators based on meteorological elements such as atmospheric stability, water vapor content and suspended PM. Wu et al. [15] used numerical experiments to establish four methods for forecasting visibility in foggy areas. Chen et al. [16] used the WRF model to simulate atmospheric visibility. Moreover, Song et al. [17] analyzed the relationship between atmospheric visibility and three environmental and meteorological parameters: relative humidity, PM2.5 and PM10.

In this study, the improved LM-BP (Levenberg–Marquardt backward propagation) neural network and RBF (radial basis function) neural
network were used to establish atmospheric visibility prediction models. To train the networks, experimental data comprising atmospheric temperature, wind speed, pressure, humidity, PM2.5, PM10, SO2, O3, NO2 and particle average mass concentration were selected as inputs and atmospheric visibility was used as the output. Moreover, the chaotic time series algorithm was used to predict visibility using historical visibility data. Experiments were then performed to verify the feasibility of the three methods.

PRINCIPLES OF VISIBILITY PREDICTION MODELS

LM-BP neural network model. A BP neural network is a type of multilayered feedforward network that comprises input, middle and output layers. The signals received by the input layer are processed by the middle layer and finally output by the output layer. Each layer affects only the state of the next layer. Compared with the traditional BP algorithm, the gradient of the improved LM algorithm decreases much faster and the convergence performance is better. The LM algorithm is an iterative method for solving the least sum of nonlinear squares. As a basic method for solving nonlinear least squares problems, the LM algorithm can be regarded as a combination of the most rapid descent method and the Gaussian Newton method.

For an LM-BP neural network, the objective function is defined as:

$E(k)=\frac{1}{2}e^2(k)=\frac{1}{2}[y_c(k) - y_m(k)]^T$,

where $y_c(k)$ is the expected network output, $y_m(n)$ is the actual network output and $e(k)$ is the current error.

Let $\omega_k$ be the network weight vector of the $k$-th iteration. Then, according to the LM algorithm, the new weight vector of $\omega_{k+1}$ is calculated by:

$\omega_{k+1} = \omega_k - [J_k^TJ_k + \lambda_kI]^{-1}J_k^Te_k$ \hspace{1cm} (2)

$I_k = \begin{bmatrix} \frac{\partial e_k}{\partial \omega_1} & \frac{\partial e_k}{\partial \omega_2} & \cdots & \frac{\partial e_k}{\partial \omega_n} \\ \frac{\partial e_k}{\partial \omega_1} & \frac{\partial e_k}{\partial \omega_2} & \cdots & \frac{\partial e_k}{\partial \omega_n} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial e_k}{\partial \omega_1} & \frac{\partial e_k}{\partial \omega_2} & \cdots & \frac{\partial e_k}{\partial \omega_n} \end{bmatrix}$ \hspace{1cm} (3)

where $e_k$ is the error vector, $J_k$ is the Jacobian matrix of the network error versus the weight derivative, $I$ is the identity matrix and $\lambda_k$ is the scalar.

In this model, the factors of PM2.5, PM10, SO2, O3, NO2, atmospheric temperature, wind speed, pressure, humidity and particle average mass concentration were taken as inputs of the network and visibility was selected as the output. The structure of the prediction model based on the LM-BP neural network is shown in Fig. 1.

**FIGURE 1**
Structure of prediction model based on an LM-BP neural network
RBF Neural Network Model. An RBF neural network is a two-layered feedforward neural network that contains a hidden layer with an RBF and an output layer with linear neurons. The number of neurons of the hidden layer depends on the needs of the problem. The RBF is a non-negative nonlinear function with radial attenuation to the center point. The output layer responds differently to the different roles of the input modes. Therefore, the transfer function from the input space to the hidden layer space is nonlinear, but that from the hidden layer space to the output layer space is linear.

Although the transfer functions of the RBF neurons are varied, the most common form is the Gaussian function \( \text{radbas} \), in which the network input is the vector distance between the weight vector \( w \) and the input vector \( x \) multiplied by the threshold \( b \), i.e., \( d = \text{radbas}(\text{dist}(w, x) \cdot b) \). The RBF transfer function can be represented by:

\[
\text{radbas}(n) = e^{-n^2}.
\]

The radial basis neuron model is shown in Fig. 2.

The same 10 parameters shown in Fig. 1 were used as inputs and visibility was used as the output in constructing the prediction model. The prediction model based on the RBF neural network is shown in Fig. 3.

Chaotic time series prediction model. The chaotic system is sensitive to the initial value, which means a change of input can be reflected quickly in the output. Therefore, the chaotic model is a close representation of the real world that can provide realistic nonlinear modeling. In this study, a local multistep chaotic prediction method based on phase space reconstruction was used to establish a prediction of atmospheric visibility.
For a given time series, \( x_1, x_2, \ldots, x_N \), where \( N \) is the length of the sequence, the correlation dimension \( d \) of the time series can be calculated according to the Grassberger–Procaccia algorithm, and the embedding dimension \( m = 2d + 1 \) can be selected using Takens theorem. The FFT method can be used to analyze the signal from which the average orbit period \( m \) can be obtained. Based on \( m \), the time delay \( \tau \) can be calculated. Finally, the reconstructed phase space can be obtained, i.e., \( Y_i = (x_{i+1}, x_{i+2}, \ldots, x_{i+(m-1)\tau}) \), \( i = 1, 2, \ldots, M \), where \( M \) is the number of phase points in the reconstructed phase space and \( M = N - (m - 1)\tau \). Let the neighboring point of the center point \( Y_M \) be \( Y_{Mi} \), \( i = 1, 2, \ldots, q \), the distance between \( Y_{Mi} \) and \( Y_M \) be \( d_i \), and assume \( d_{\text{min}} \) as the minimum value of \( d_i \), the weight of the definition point \( Y_{ki} \) can be written as:

\[
P_i = \frac{\exp(-(d_i - d_{\text{min}}))}{\sum_{i=1}^{q} \exp(-(d_i - d_{\text{min}}))}.
\]  

Then, the first-order local linear fit is defined by:

\[
Y_{M+1} = a + bY_M, \quad i = 1, 2, \ldots, q,
\]  

where \( a \) and \( b \) are the real coefficients required for fitting, \( e \) is a \( q \)-dimensional vector, \( e = (1, 1, \ldots, 1)^T \) and \( Y_{M+1} \) is the phase point after \( Y_M \) evolution.

When the embedding dimension \( m = 1 \), the weighted least squares method is applied by:

\[
\sum_{i=1}^{q} P_{\beta} (x_{M+1} - a - bx_M)^2 = \text{min}.
\]

By solving the above equations, coefficients \( a \) and \( b \) can be calculated. Substituting \( a \) and \( b \) into a one-step prediction formula

\[
Y_{M+1} = ae + bY_M,
\]

the predicted value of the phase point after the evolution step \( Y_{M+1} \) can be obtained by:

\[
Y_{M+1} = \left( x_{M+1}, x_{M+1+\tau}, \ldots, x_{M+1+(m-1)\tau} \right),
\]

where the preceding \((m-1)\) elements in \( Y_{M+1} \) are known values in the original sequence and the \( m \)-th element \( x_{M+1+(m-1)\tau} \) is the one-step predicted value \( \hat{x}_{M+1} \) of the original sequence. This is a one-step prediction model of the weighted first-order local method. When needing to perform multistep forecasting, the predicted value can be adopted as the new value in the original time series and the above steps can be repeated.

In this study, continuous observational historical atmospheric visibility values are taken as the input, and the chaotic time series local method is used to predict the atmospheric visibility.

**ESTABLISHMENT OF PREDICTION MODELS**

**Observational data.** Many environmental and meteorological factors were observed in the Yinchuan area of China during 2017–2018, including PM_{2.5}, PM_{10}, SO_{2}, O_{3}, NO_{2}, atmospheric temperature, wind speed, air pressure, humidity, particle average mass concentration and visibility. Of these, PM_{2.5}, PM_{10}, SO_{2}, O_{3}, NO_{2} represent the pollution factors that have direct impact on atmospheric visibility. Atmospheric temperature, wind speed, air pressure and humidity are typical meteorological parameters that can affect visibility to some extent. The particle average mass concentration reflects the content of coarse particles in the air, which can also affect visibility. Table 1 lists some typical examples of the observed environmental and meteorological data.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Date</td>
<td>11.15</td>
<td>11.18</td>
<td>12.22</td>
<td>3.27</td>
<td>5.7</td>
<td>6.17</td>
<td>6.28</td>
<td>7.12</td>
<td>7.23</td>
</tr>
<tr>
<td>PM_{2.5} (ug/m)</td>
<td>31</td>
<td>41</td>
<td>41</td>
<td>34</td>
<td>61</td>
<td>52</td>
<td>20</td>
<td>25</td>
<td>32</td>
</tr>
<tr>
<td>PM_{10} (ug/m)</td>
<td>72</td>
<td>89</td>
<td>331</td>
<td>111</td>
<td>32</td>
<td>206</td>
<td>50</td>
<td>54</td>
<td>96</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>29</td>
<td>30</td>
<td>27</td>
<td>26</td>
<td>26</td>
<td>7</td>
<td>43</td>
<td>79</td>
<td>55</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>1</td>
<td>4</td>
<td>-1</td>
<td>6</td>
<td>15.9</td>
<td>28</td>
<td>28.6</td>
<td>21.7</td>
<td>29.7</td>
</tr>
<tr>
<td>Air pressure (kpa)</td>
<td>896.9</td>
<td>884.4</td>
<td>897.1</td>
<td>895.4</td>
<td>885.5</td>
<td>883.4</td>
<td>883.7</td>
<td>884.6</td>
<td>880.9</td>
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<tr>
<td>SO_{2} (ug/m³)</td>
<td>20</td>
<td>58</td>
<td>36</td>
<td>37</td>
<td>35</td>
<td>51</td>
<td>19</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>NO_{2} (ug/m³)</td>
<td>28</td>
<td>33</td>
<td>28</td>
<td>32</td>
<td>18</td>
<td>34</td>
<td>19</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Wind speed (degree)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Particle average mass concentration (mg/cm³)</td>
<td>0.085</td>
<td>0.086</td>
<td>0.298</td>
<td>0.126</td>
<td>0.348</td>
<td>0.144</td>
<td>0.073</td>
<td>0.057</td>
<td>0.081</td>
</tr>
<tr>
<td>Visibility (km)</td>
<td>15</td>
<td>11.27</td>
<td>7</td>
<td>11.4</td>
<td>3.9</td>
<td>9.3</td>
<td>16.6</td>
<td>19.1</td>
<td>16.7</td>
</tr>
</tbody>
</table>

9080
Establishing a neural network model. Using the PM$_{2.5}$, PM$_{10}$, SO$_2$, O$_3$, NO$_2$, atmospheric temperature, wind speed, air pressure, humidity and particle average mass concentration as inputs and historical data of visibility as the output, a neural network model can be established. When the training error or number of cycles reaches the required level, the network can be considered trained and then used subsequently to perform predictions. A flow chart of neural network model training and prediction is shown in Fig. 4.

Determination of LM-BP neural network structure. (1) Determination of the number of input neurons. In the neural network prediction model, it can be seen that there are 10 input nodes in the network, i.e., there are 10 neurons in the input layer.

(2) Determination of the number of neurons in the hidden layer. The number of neurons in the hidden layer is determined by:

$$ n = \sqrt{n_i + n_o + \alpha}, $$ (9)

where $n_i$ denotes the number of input neurons, $n_o$ is the number of output neurons and $\alpha$ is a constant in the range 1–10.

Table 2 lists the prediction errors for different numbers of hidden layer neurons using the LM-BP neural network.

---

**Flow chart of neural network model training and prediction**

---
TABLE 2
Prediction errors for different numbers of hidden layer neurons using the LM-BP neural network

<table>
<thead>
<tr>
<th>Hidden layer neurons number</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average error</td>
<td>2.6110</td>
<td>2.1889</td>
<td>1.9832</td>
<td>1.9836</td>
<td>1.9352</td>
<td>2.4605</td>
<td>2.0575</td>
</tr>
<tr>
<td>Root mean square error</td>
<td>3.4801</td>
<td>3.0187</td>
<td>2.6517</td>
<td>2.6866</td>
<td>2.6016</td>
<td>3.1917</td>
<td>2.7638</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.6585</td>
<td>0.8168</td>
<td>0.8472</td>
<td>0.8361</td>
<td>0.8241</td>
<td>0.775</td>
<td>0.6862</td>
</tr>
</tbody>
</table>

TABLE 3
Visibility predicting errors associated with different numbers of hidden layers

<table>
<thead>
<tr>
<th>Layer number</th>
<th>Visibility average error</th>
<th>Root mean square error</th>
<th>Correlation coefficient</th>
<th>Time consume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single hidden layer</td>
<td>2.0511</td>
<td>2.4105</td>
<td>0.8472</td>
<td>4.957 s</td>
</tr>
<tr>
<td>Double hidden layer</td>
<td>1.9832</td>
<td>2.6517</td>
<td>0.8392</td>
<td>5.413 s</td>
</tr>
<tr>
<td>Three hidden layer</td>
<td>2.0032</td>
<td>2.4845</td>
<td>0.8108</td>
<td>8.780 s</td>
</tr>
</tbody>
</table>

It can be seen from Table 2 that with an increase in the number of neurons in the hidden layer, the prediction results of the neural network become optimized before starting to worsen. When the number of neurons in the hidden layer is 15, the average error is 1.9832 km, root mean square error is smallest (2.6517 km) and correlation coefficient is a maximum. Therefore, in this study, the number of hidden layer neurons was designed as 15.

Having determined the number of hidden layer neurons as 15, different numbers of hidden layers (i.e., one, two or three) were selected to verify the prediction effect. Table 3 lists the visibility prediction errors associated with the different numbers of hidden layers.

As can be seen from Table 3, the prediction results become optimized as the number of hidden layers increases, although the time consumed also increases. In comparison with the single hidden layer, two hidden layers have a smaller average error, slightly larger root mean square error and similar correlation coefficient. The time consumed using two hidden layers is slightly longer than that for a single hidden layer but far less than for three hidden layers. For a neural network with three hidden layers, although the prediction results are satisfactory, the model is unsuitable for the prediction of large numbers of data because of the extended running time. Therefore, two hidden layers were chosen in this study to build the model network and the number of hidden layer neurons was 15.

(3) The output layer has a neuron that uses visibility as the output.

RESULTS AND DISCUSSION

The errors of the prediction results of the LM-BP and RBF neural networks and the chaotic time series algorithm are analyzed in this paper. The error mean value $\mu$ can be expressed as:

$$\mu = \frac{1}{n} \sum_{i=1}^{n} |P - Q|,$$  \hspace{1cm} (10)

where $n$ is the number of prediction data elements, $P$ is the prediction value and $Q$ is the actual value of visibility. The prediction error is defined by:

$$\text{error}_i = P_i - Q_i \hspace{1cm} (0 < i < n + 1).$$  \hspace{1cm} (11)

The standard deviation can be expressed as follows:

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (\text{error}_i - \mu)^2}.$$  \hspace{1cm} (12)

The root mean square error $R$ is given by:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} \text{error}_i^2}{N}}.$$  \hspace{1cm} (13)

The 95% confidence interval of the error can be calculated as follows:

$$P(\mu - 1.96 \frac{\sigma}{\sqrt{n}} < M < \mu + 1.96 \frac{\sigma}{\sqrt{n}}) = 0.95.$$  \hspace{1cm} (14)

The upper and lower limits of the 95% confidence interval are $P_T = \mu + 1.96 \frac{\sigma}{\sqrt{n}}$ and $P_L = \mu - 1.96 \frac{\sigma}{\sqrt{n}}$, respectively.

Analysis of visibility prediction based on the LM-BP neural network. In this study, 1032 data elements were selected for the LM-BP neural network, of which 1000 were used as training data and 32 were used as test data. The visibility prediction results based on the LM-BP neural network are shown in Fig. 5.

It can see that the results of the LM-BP neural network exhibit reasonable fitting with the predict-
ed values lying close to the actual values. In fact, the average error is reasonably small (1.9832 km), root mean square error is 2.6517 km and running time is 5.413 s. The correlation between the prediction values and actual values is shown in Fig. 6. The correlation coefficient \( R^2 \) of 0.69442 indicates that the LM-BP neural network method was reasonably satisfactory for predicting visibility.

**Analysis of visibility prediction based on the RBF neural network.** The RBF neural network also used 1032 data elements, of which 1000 were used as training data and 32 were used as test data. The visibility prediction results based on the RBF neural network are shown in Fig. 7.
It can be seen from Figs. 7 and 8 that the RBF neural network model produced a reasonable prediction. The average error of the visibility prediction is 1.6379 km, root mean square error is 2.1686 km, correlation coefficient ($R^2$) is 0.75738 and running time is 25.08 s.

Comparison of the above two prediction results reveals that the average error and root mean square error of the RBF neural network are smaller than that of the LM-BP neural network, and that the correlation coefficient of the RBF neural network is larger than that of the LM-BP neural network. Therefore, of the two models, the RBF neural network has better prediction capability. However, the time consumed running the RBF neural network was much larger than that of the LM-BP neural network, i.e., it was close to 5 times longer than the LM-BP neural network. Therefore, the RBF neural network could be suitable for predicting visibility when the data scale is small, but the LM-BP neural network should be used for predicting visibility when the data scale is large.

**Analysis of visibility prediction based on chaotic time series local method.** The chaotic time series local method used 1000 elements of visibility data input, of which 970 were used for training and 30 were used as prediction samples. The multistep prediction based on the chaotic time series local method is shown in Fig. 9.
The root mean square error of the multistep prediction of the chaotic time series local method is 3.0052 km and the correlation coefficient ($R^2$) is only 0.5766. It is clear that compared with the neural network methods the prediction error of the chaotic time series is larger. This is because the neural networks can comprehensively consider the relationship between the 10 inputs and 1 output. However, the chaotic time series method can only be predicted from a series of historical visibility data, which fails to exploit the other environmental and meteorological factors. However, an advantage of the chaotic time series method is that only historical visibility data are needed to predict future visibility.

**Error comparison of three prediction methods.** Table 4 presents a comparison of the errors for the above three prediction methods. The prediction result based on the RBF neural network is superior to the other two methods in terms of average error, standard deviation, root mean square error and confidence interval. The overall error of the chaos time series method is largest. The average error and standard deviation of the LM-BP neural network prediction algorithm are not overly large; however, because of the instability of the prediction, the prediction error is highly scattered, which means the prediction error range of the 95% confidence interval is the greatest. Therefore, it is concluded that the prediction ability of the RBF neural network is the most accurate.

**CONCLUSIONS**

This study used three methods to predict atmospheric visibility. For the LM-BP neural network and RBF neural network methods, the factors of PM$_{2.5}$, PM$_{10}$, SO$_2$, O$_3$, NO$_2$, atmospheric temperature, wind speed, air pressure and humidity, and particle average mass concentration in the atmosphere were used as inputs and atmospheric visibility was used as the output. In addition, the chaotic time series local method was selected to predict visibility using only historical visibility data. Experiments were performed to verify the feasibility of the three methods and the derived conclusions are as follows.
Both the LM-BP neural network and the RBF neural network produced reasonably satisfactory prediction results for atmospheric visibility. However, the average prediction error, standard deviation, root mean square error and confidence interval of the RBF neural network were better than both the LM-BP neural network and the chaotic time series method. Therefore, the prediction ability of the RBF method was considered best. However, the time consumed running the RBF neural network was much longer than that of the LM-BP neural network. Therefore, in practical application, the RBF neural network could be used for a small number of input samples, whereas the LM-BP neural network should be used for a large number of input samples.

The multistep prediction of the chaotic time series local method was found less effective than the neural network predictions, i.e., the average prediction error was larger, correlation coefficient was smaller and true prediction effect was worse than the two neural network predictions. However, if in practical application only historical visibility data were used as the input sample, the method could be suitable for predicting visibility.

In the prediction and analysis of this study, various environmental and meteorological factors that affect atmospheric visibility (i.e., PM$_{2.5}$, PM$_{10}$, SO$_2$, O$_3$, NO$_2$, atmospheric temperature, wind speed, air pressure, humidity, particle average mass concentration) were considered in establishing the prediction models, which is conducive to obtaining accurate prediction results and realizing the purpose of the prediction. In practical application, depending on the requirements and on the observational data available, the prediction method should be changed appropriately.

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STUDIES ON CORRELATION, REGRESSION AND HERITABILITY ESTIMATES IN M₃ GENERATION OF BREAD WHEAT (TRITICUM AESTIVUM L.)

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ABSTRACT

Development of new high yield wheat varieties is the main objective of the wheat improvement program. The information concerning the genetic variability, heritability and correlation analysis for different parameters is indispensable to propose effective wheat breeding program. (Table 1) which exhibited that genotypes performance variable for all the traits. A field experiment was carried out during 2016-17 in Randomized Complete Block Design (RCBD) with three replication and ten genotypes. The mean squares from analysis of variance revealed that all genotypes were significant difference at 1% probability level. NIA Sarang (mutant) recorded maximum mean performance for days to 50% heading (70.00 days), peduncle length (41.50 cm) and seed index (50.00 g) and next scorer for the days to 90% maturity (129.83) and spike length (13.33 cm), While NIA Amber (mutant) showed longer spike length (15.33 cm) more number of spikelet spike (24.33), more number of grains spike¹ (71.00 seeds), higher biological yield (49.00 g) and next ranked for days to 50% heading (67.00), tiller plant¹ (10.67), plant height (87.37 cm) and grain yield plant¹ (25.33 g), while TD-1 (check variety) recorded minimum (117.00 days) in 90% maturity, plant height (59.67 cm), peduncle length (30.33 cm) and maximum (11.33) tillers plant¹, grain yield plant¹ (25.67 g) and harvest index (53.47%). The high heritability (h² = 99.08%) were recorded for days to 50% heading (h² = 97.16%), days to 90% maturity (h² = 95.60%), tillers plant¹ (h² = 91.83%), plant height (h² = 99.08%), peduncle length (h² = 93.05%), spike length (h² = 93.05%), spikelets spike¹ (h² = 95.17%), grains spike¹ (h² = 96.02%), grain yield plant¹ (h² = 98.40%), biological yield plant¹ (h² = 97.17%), seed index (h² = 89.09%) and harvest index (h² = 93.27%). Correlation coefficient showed that significant and positive associations of spike length, spikelets spike¹, grains spike¹, biological yield and harvest index with grain yield plant¹. Regression analysis showed that a unit increase in spike length, spikelets spike¹, grains spike¹, biological yield and harvest index will simultaneously increase grain yield plant¹. Days to 90% maturity and plant height showed significant but negative association with harvest index. The regression analysis indicated that a unit increases in days to maturity and plant height cause reduction in harvest index. The present results indicated that Mutants NIA Sarang and NIA Amber could be used for developing new wheat varieties with improved yield traits with early maturity through simple selection from later generations.

KEYWORDS:
M₃ generations, correlation, regression, heritability, wheat

INTRODUCTION

Wheat in Pakistan and occupies a central position in agriculture. With respect to its contribution to the country, it contributes 10.0 and 2.1 percent value added in agriculture and in gross domestic product (G.D.P.) respectively. Talking about cultivated of wheat cultivation has declined of 9180 thousand hectares (ha) in 2016-2017 from previous year’s area of 9199 thousand hectares that displays a reduction of 0.2 percent. The wheat production stood at 26.1 million tons during 2017, demonstrating a decrease of 1.9 percent over the preceding year’s production (25.979 million tons). The decline in production was associated with extended winter season and exceptional rains during the months of April and May which caused damages to grain at harvesting time [1].

Promising genotypes with high yielding, good adaptation and agronomical desirable characteristics could reliably be exploited for commercial cultivation. The evaluation of the important traits and pattern of genetic variability of the existing germplasm [2]. While Akram et al. [3] identified positive and significant association for seed index with grains yield and [4] observed yield traits in wheat such a
tillering capacity had remarkable contribution to grain yield similarly spikes per meter squares, seeds spike\(^{-1}\) and seed index was contributed significantly to seed yield. The change in one results into a proportionate variation in another and it may be positive or negative positive correlation indicates that an increase in one character will bring simultaneous increase in another associated character. Whereas, negative correlation suggested character that an increase in one character causes decrease in the other associated character So that positive correlation between two desirable characters and negative correlation between a desirable and undesirable character is important [5, 6].

Selection on the basis of yield and its components has more accuracy [7]. The regression analysis is used to describe the association accuracy by means of an equation with a predictive value. The yield traits may be used a trustworthy selection criteria to improve grain yield [8, 9]. Heritability studies provide valuable genetic information to the breeders to envisage the gene’s interaction in segregation populations [4, 10]. Khan et al. [11] suggested that the higher the heritability and genetic advance, simple the selection procedure. Mutation breeding is one the methods to create the desired genetic variability through the use of physical or chemical mutagenesis [12]. Present studies were aimed to determine correlation, regression and heritability in M\(_3\) generation of wheat genotypes.

**MATERIALS AND METHODS**

The study was conducted aimed to estimate correlation, regression and heritability estimates in M\(_3\) populations of bread wheat. A field trial was carried out during 2016-2017 in Randomized Complete Block Design (RCBD) having three repeats at Nuclear Institute of Agriculture (NIA) Tandojam. Ten (four parents, four mutants and two check varieties) genotypes like NIA Sunehri, NIA Amber, NIA Sunder, NIA Sarang, NIA Sunehri (Mutant), NIA Amber (Mutant), NIA Sunder (Mutant) NIA Sarang (Mutant), TD-1 (Check variety) and Kiran-95 (Check Variety) were studied. The sowing was done by dibbling under the plant and row spacing of 20 and 30 cm, correspondingly. The following traits were investigated.

**Days to 50% head.** Total number of days taken from sowing to 50% heading was recorded as days to 50% heading.

**Days to 90% maturity.** Physically observed that the plants are 90% mature, the dates were recorded from sowing time to that time.

**Plant height (cm).** It was measured from tagged plants in centimeter from the surface of the soil to top of the plant at maturity stage.

**Peduncle length (cm).** Length of the peduncle was measured in centimeters from base of the spike to 1st node of the stem.

**Tillers plant\(^{-1}\).** The total tillers plant\(^{-1}\) was counted at the time of maturity. For this character, total fertile tillers from each plant in each replication were counted.

**Spike length (cm).** The main spikes from the tagged plants were measured in centimeters.

**Spikelets spike\(^{-1}\).** The spikelet of main spike was counted at the time of harvesting.

**Grains spike\(^{-1}\).** The spikes of tagged plant harvested and placed in laboratory. Single plants were threshed manually, cleaned and counted. The average was calculated.

**Grain yield plant\(^{-1}\)(g).** At the time of harvest each plant was threshed separately and cleaned. The grains were weighted on electronic balance (g) in laboratory.

**Seed index (1000 grain weight in grams).** Thousand seeds were counted and weighted in gram with the help of electronic balance in laboratory.

**Biological yield plant\(^{-1}\)(g).** The tagged plants were harvested at the maturity and single plants are weighted on electronic balance for measuring the biological yield plant\(^{-1}\) in grams.

**Harvest index (%).** The ratio of grain yield; over the biological yield is called harvest index. It was calculated by this equation which is as under:

\[
HI = \frac{\text{Grain yield per plant (g)}}{\text{Biological yield per plant (g)}} \times 100
\]

The pooled data were analyzed according to [13] for determined significant differences through ANOVA and correlation was calculated according to [14]. The heritability estimates were calculated as suggested by [15].

**RESULT AND DISCUSSION**

The breeding material comprised of ten genotypes like NIA Sunehri, NIA Amber, NIA Sunder, NIA Sarang, NIA Sunehri (mutant), NIA Amber (mutant), NIA Sunder (mutant), NIA Sarang (mutant) with two check varieties i.e. TD-1 and Kiran-95. To evaluate the best promising mutant lines and workout the correlation, regression and heritability
in M₃ generations in wheat genotypes. The results are described as under:

**Analysis of variance for morphological and yield traits.** The mean squares from analysis of variance for various qualitative traits of wheat genotypes corresponding to days to 50% heading, days to 90% maturity, tillers plant⁻¹, plant height, peduncle length, spike length, spikelet spike⁻¹, grains spike⁻¹, grain yield plant⁻¹, biological yield plant⁻¹, seed index and harvest index are presented in Table 1, which indicated that genotypes showed highly significant differences for all the characters.

Development of new high yield wheat varieties is the main objective of the wheat improvement program. The information concerning the genetic variability, heritability and correlation analysis for different parameters is indispensable to propose effective wheat breeding program. (Table 1) which exhibited that genotypes performance variable for all the traits. Our results are in conformity with [9, 10, 16] who also reported that genotypes were significant number of tillers plant⁻¹, plant height number of spikelets spike⁻¹ [17] who reported genotypes were significant for height of plant, total biomass yield, grains spike⁻¹, length of spike, weight of spike, days taken to heading, days taken to maturity, peduncle to height ratio, spike m² and grain yield. The grain yield was significantly and positively associated with total biomass yield and weight of spike.

**Mean performance of wheat genotypes for morphological and yield traits.** Mean performance of wheat genotypes for days to 50% heading, days to 90% maturity, tillers plant⁻¹, plant height (cm), peduncle length (cm) and spike length (cm) are presented in Figures (Fig. 1 to 6) other traits are presented in Tables 2.

Among the genotypes, NIA Sarang (mutant) takes maximum days to 50% heading was counted form NIA Amber. Kiran-95 (check variety) counted maximum days to 90% maturity followed by NIA Sarang (mutant). The minimum days to 90% maturity, plant height, peduncle length was recorded form TD-1 (check variety) and maximum number of tillers plant⁻¹. NIA Sunehri (mutant) recorded maximum plant height followed NIA Amber (mutant), NIA Sarang (mutant) recoded maximum peduncle length and spike length followed by NIA Sunehri (Mutant). Similar results were obtained by [16] they reported that among the parents AUP (5008), JBZ and TATARA and in F₁ like JBZ x WTN, JBZ x F. Sarhad, AUP (5008) x WTN were found better performing for yield and its contributing traits. Therefore these parental lines and F₂ populations may be used in future for the improvement of yield traits through simple selection.

The maximum spikelets spike⁻¹ was counted form the spikes of NIA Amber (Mutant) and NIA Sunehri (Mutant). The higher number of grains spikes⁻¹ was also counted form NIA Amber (mutant) whereas maximum grain yield plant⁻¹ and harvest index was produce by TD-1 followed by NIA Amber (mutant) and maximum grain yield plant⁻¹ was produced by NIA Amber (Table 2) and the bolder seeds was measure in NIA Sarang (mutant). Majeed [18] who reported that great variation was found in yield traits in M₁ progenies. The tagged plants when sown in progeny lines as M₂ and M₃ more or less sustained their supremacy over the mother for agronomic character. The majority of the mutant lines recorded uniform in performance for almost the traits studied. Eleven lines out of seventeen lines were found to be promising in respect of days to flower, plant height and other traits including grain yield.

Our results suggested that among the four mutant lines, NIA Sarang, NIA Sunehri perform better than other two lines like NIA Amber and NIA Sunder, so that these lines are suitable for development of new wheat verities in later generations through selection with improved yield traits and early maturity.

### TABLE 1

Means squares from analysis of variance of M₃ generations of wheat genotypes for various quantitative traits

<table>
<thead>
<tr>
<th>Characters</th>
<th>Replication (D.F.2)</th>
<th>Genotype (D.F.9)</th>
<th>Error (D.F.18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day to 50% heading</td>
<td>8.27</td>
<td>20.41**</td>
<td>0.37</td>
</tr>
<tr>
<td>Day to 90% maturity</td>
<td>0.40</td>
<td>84.59**</td>
<td>2.82</td>
</tr>
<tr>
<td>Tillers plant⁻¹</td>
<td>1.15</td>
<td>6.360**</td>
<td>0.408</td>
</tr>
<tr>
<td>Plant height</td>
<td>0.69</td>
<td>287.1**</td>
<td>2.02</td>
</tr>
<tr>
<td>Peduncle length</td>
<td>2.50</td>
<td>45.44**</td>
<td>2.41</td>
</tr>
<tr>
<td>Spike length</td>
<td>0.37</td>
<td>11.60**</td>
<td>0.62</td>
</tr>
<tr>
<td>Spikelets spike⁻¹</td>
<td>0.22</td>
<td>22.53**</td>
<td>0.83</td>
</tr>
<tr>
<td>Grains spike⁻¹</td>
<td>1.12</td>
<td>154.4**</td>
<td>4.6</td>
</tr>
<tr>
<td>Grain yield plant⁻¹</td>
<td>0.99</td>
<td>55.51**</td>
<td>1.19</td>
</tr>
<tr>
<td>Biological yield Plant⁻¹</td>
<td>4.80</td>
<td>149.8**</td>
<td>1.80</td>
</tr>
<tr>
<td>Seed index</td>
<td>7.03</td>
<td>54.43**</td>
<td>4.58</td>
</tr>
<tr>
<td>Harvest index</td>
<td>3.89</td>
<td>38.33**</td>
<td>1.97</td>
</tr>
</tbody>
</table>

**=Significant at 1% probability level
Wheat genotypes

FIGURE 1
Mean performance of wheat genotypes for days to 50% heading

Wheat genotypes

FIGURE 2
Mean performance of wheat genotypes for days to 90% maturity

Wheat genotypes

FIGURE 3
Mean performance of wheat genotypes for tillers plant⁻¹
FIGURE 4
Mean performance of wheat genotypes for plant height (cm)

FIGURE 5
Mean performance of wheat genotypes for peduncle length (cm)

FIGURE 6
Mean performance of wheat genotypes for spike length (cm)
Similarly [19] they reported high heritability in index was due to its association with days to 50% length, spike length, spikelets spike-1, grains spike-1, harvest index, moderate variability in spike length and seed index, significant but negative correlation with harvest index regression analysis indicates that unit increase days to 90% maturity was significant association with plant height and peduncle length while significant but negative correlation with harvest index estimated that improve-ment of studied traits are very easier with simple selection. Other researchers like [20, 21, 22] they also reported higher heritability for yield and yield contributing traits.

**Correlation, regression and coefficient of determination.** The significantly positive association of days to 50% heading with spike length and seed index, moderate variability in spike length and seed index was due to its association with days to 50% heading while regression coefficient (b) indicates that a unit increase days to 50% heading will results in an increase in spike length and seed index (Table 4). The days to 90% maturity was significant association with plant height and peduncle length while significant but negative correlation with harvest index regression analysis indicates that unit increase days to 90% maturity will increase in plant height and peduncle length but decrease in harvest index due to its negative correlation. Similarly our results are also confirm with [19] who reported that seed yield exhibited positive significant correlation with biological yield, number of spike-1 plant, harvest index, spike length, 1000 weight grain, plant height, peduncle length, number of spikelet’s spike-1 and number of grain spike-1 at both genotypic and phe-notypic level. Further [23] reported that significant and positive correlation for grain yield with dry matter, significant but negative correlation was observed between grain number spike and thousand grain weight.

**TABLE 2**
Mean performance of wheat genotypes for spikelets spike-1, grains spike-1, biological yield plant-1, seed index and harvest index

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Spikelets spike-1</th>
<th>Grains spike-1</th>
<th>Grain yield plant-1 (g)</th>
<th>Biological yield plant-1 (g)</th>
<th>Seed index (µg)</th>
<th>Harvest index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIA Sunehri</td>
<td>16.73</td>
<td>52.67</td>
<td>15.40</td>
<td>35.22</td>
<td>41.58</td>
<td>43.72</td>
</tr>
<tr>
<td>NIA Amber</td>
<td>17.23</td>
<td>54.23</td>
<td>12.45</td>
<td>26.88</td>
<td>38.38</td>
<td>46.32</td>
</tr>
<tr>
<td>NIA Sunder</td>
<td>16.77</td>
<td>53.63</td>
<td>15.02</td>
<td>32.38</td>
<td>45.55</td>
<td>46.37</td>
</tr>
<tr>
<td>NIA Saarang</td>
<td>17.27</td>
<td>53.47</td>
<td>17.25</td>
<td>35.67</td>
<td>48.12</td>
<td>48.37</td>
</tr>
<tr>
<td>NIA Sunehri (mutant)</td>
<td>21.67</td>
<td>67.67</td>
<td>21.00</td>
<td>39.67</td>
<td>46.83</td>
<td>52.94</td>
</tr>
<tr>
<td>NIA Amber (mutant)</td>
<td>24.33</td>
<td>71.00</td>
<td>25.33</td>
<td>49.00</td>
<td>36.53</td>
<td>51.70</td>
</tr>
<tr>
<td>NIA Sunder (mutant)</td>
<td>19.67</td>
<td>65.00</td>
<td>20.00</td>
<td>46.00</td>
<td>43.57</td>
<td>43.48</td>
</tr>
<tr>
<td>NIA Sarang (mutant)</td>
<td>17.13</td>
<td>55.93</td>
<td>17.88</td>
<td>39.00</td>
<td>50.00</td>
<td>45.85</td>
</tr>
<tr>
<td>TD-1 (check variety)</td>
<td>20.33</td>
<td>68.33</td>
<td>25.67</td>
<td>48.00</td>
<td>46.67</td>
<td>53.47</td>
</tr>
<tr>
<td>Kiran (check variety)</td>
<td>15.67</td>
<td>62.00</td>
<td>20.00</td>
<td>41.00</td>
<td>44.57</td>
<td>48.78</td>
</tr>
<tr>
<td>L.S.D. (5%)</td>
<td>0.31</td>
<td>0.92</td>
<td>0.25</td>
<td>0.71</td>
<td>0.27</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**TABLE 3**
Heritability estimates in broad sense (h².b.s.%.) of M₃ generations of wheat genotypes for various quantitative traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genetic variance (δ² g)</th>
<th>Phenotypic variance (δ² p)</th>
<th>Heritability (h².b.s.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to 50% heading</td>
<td>20.04</td>
<td>20.53</td>
<td>97.16</td>
</tr>
<tr>
<td>Days to 90% maturity</td>
<td>81.77</td>
<td>85.53</td>
<td>95.60</td>
</tr>
<tr>
<td>Tillers plant⁻¹</td>
<td>5.96</td>
<td>6.49</td>
<td>91.83</td>
</tr>
<tr>
<td>Plant height</td>
<td>285.08</td>
<td>287.70</td>
<td>99.08</td>
</tr>
<tr>
<td>Peduncle length</td>
<td>43.03</td>
<td>46.24</td>
<td>93.05</td>
</tr>
<tr>
<td>Spike length</td>
<td>10.98</td>
<td>11.80</td>
<td>93.05</td>
</tr>
<tr>
<td>Spikelets spike⁻¹</td>
<td>21.70</td>
<td>22.80</td>
<td>95.17</td>
</tr>
<tr>
<td>Grains spike⁻¹</td>
<td>149.70</td>
<td>155.90</td>
<td>96.02</td>
</tr>
<tr>
<td>Grain yield plant⁻¹</td>
<td>148.00</td>
<td>150.40</td>
<td>98.40</td>
</tr>
<tr>
<td>Biological yield plant⁻¹</td>
<td>54.32</td>
<td>55.90</td>
<td>97.17</td>
</tr>
<tr>
<td>Seed index</td>
<td>49.85</td>
<td>55.95</td>
<td>89.09</td>
</tr>
<tr>
<td>Harvest index</td>
<td>36.36</td>
<td>38.98</td>
<td>93.27</td>
</tr>
</tbody>
</table>

Heritability estimated in broad sense (h².b.s.%). The higher heritability (Table 3) recorded for days to 50% heading, days to 90% maturity, number of tillers plant⁻¹, plant height, peduncle length, spike length, spikelets spike⁻¹, grains spike⁻¹, harvest index and biological yield. Similarly [19] they reported high heritability in broad sense (h².b.s.%) was recorded for plant height, days to 50% heading, 1000-grain weight, days to 50% flowering, maturity days, number of grains spike⁻¹, seed yield plant⁻¹, peduncle length, harvest index and, biological yield. Further [10] they also reported highest heritability estimates were observed for plant height (98.27%) and grain yield (83.22%). High heritability estimates proposed that improvement of studied traits are very easier with simple selection. Other researchers like [20, 21, 22] they also reported higher heritability for yield and yield contributing traits.
results increase in spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\), grain yield plant\(^{-1}\), biological yield and harvest index. The regression coefficient (b) shows that unit increase in tillers plant\(^{-1}\) will result in increase in spikes length, spikelets spikes\(^{-1}\), grains spike\(^{-1}\), grain yield plant\(^{-1}\), biological yield and harvest index. Plant height was significant positive association with peduncle length and negative but significant with harvest index indicated that if plant height increased, peduncle length also increased but harvest index was decreased may be taller plant show the lodging. Same trend was also observed through regression analysis which exhibited a unit increase in plant height will increase in peduncle length and decrease in harvest index.

Other researcher like [24] who reported that positive and significant correlation was observed between tiller\(^{-1}\) plant and grain yield plant\(^{-1}\) (0.97**), spike length and spikelets spike\(^{-1}\) (0.58**), spike length and grain spike\(^{-1}\) (0.64**), spike length and grain yield plant\(^{-1}\) (0.55**) and spikelet spike\(^{-1}\) and grains spike\(^{-1}\) (0.55**), spikelet spike\(^{-1}\) and seed index *(0.5942**), spikelets spike\(^{-1}\) and grain yield per plant (0.48**), grains per spike and grain yield\(^{-1}\) plant (0.45**).

Spike length have significant and positive association with spikelets spike\(^{-1}\), grains spike\(^{-1}\), grain yield plant\(^{-1}\), biological yield plant\(^{-1}\) and harvest index which exhibited that spike length increase at mean time spikelets spike\(^{-1}\), grains spike\(^{-1}\), grain yield plant\(^{-1}\), biological yield plant\(^{-1}\) and harvest index will also increase and their regression analysis also manifested that a unit increase in spike length will also increase in spikelets per spike, grains per spike, grain yield per plant, biological yield per plant and harvest index. The significant and positive correlation was found among spikelets per spike with grains per spike, grain yield per plant and harvest index while regression coefficient (b) indicated that a unit increase in spikelets spike\(^{-1}\) will simultaneously increase in grains spike\(^{-1}\), grain yield plant\(^{-1}\) and harvest index.

Similarly [25, 26] they observed grains weight spike\(^{-1}\) was positively and significantly (P<0.05) correlation with grain yield ha\(^{-1}\). [24] their results indicated that grain yield showed positive and significant correlation with above ground biomass, straw yield, spike plant\(^{-1}\) and grains plant\(^{-1}\). Regression analysis showed that both above ground biomass and harvest index are reliable indicators to select promising genotypes in breeding wheat programs.

The inter-relationship of grains spike\(^{-1}\) with grains yield plant\(^{-1}\) and harvest index were significant and positive which demonstrated that that increase in grains spike\(^{-1}\) were increase in grain yield plant\(^{-1}\) and harvest index. Grains spike\(^{-1}\) and harvest index showed significant and positive correlation and grain yield plant\(^{-1}\) also showed positive and significant association with harvest index suggested that increase in grains spike\(^{-1}\), grain yield plant\(^{-1}\) may also result in increase in harvest index. While regression coefficient (b) revealed that a unit increase in grain spike\(^{-1}\), grain yield plant\(^{-1}\) will simultaneously increase in harvest index. These correlations suggested that grain yield plant\(^{-1}\) can be improved through simple selection. Mollasadeghi et al. [27] their results showed that the grain yield has a positive correlation (0.527) with harvest index, while the straw yield has a negative correlation with it (-0.366). The four traits including number of grain per spike (0.212), grain weight (0.408), seed index value (0.093) and biological yield (0.853) had the most positive correlation on grain yield.

From the present studies correlation and regression analysis suggested all the traits established the strong correlations with grain yield plant\(^{-1}\) hence yield can be improved through simple selection because yield contributing traits like tiller plant\(^{-1}\), spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\) and harvest index established significant and positive correlation, regression along with high heritability estimates so improvement in grain is yield is very easier through simple selection in later generations.

**CONCLUSIONS**

The mean squares from analysis of variance showed that genotypes were significant for all the traits like days to 50% heading, days to 90% maturity, tillers plant\(^{-1}\), plant height, peduncle length, spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\), grain yield plant\(^{-1}\), biological yield plant\(^{-1}\), seed index and harvest index. Among the M\(_{4}\) generations, NIA Sa-rang (Mutant) and NIA Amber (Mutant) performed better for days to 50% heading, spike length, spikelets spike\(^{-1}\), grains spike, grain yield plant\(^{-1}\). TD-1 recorded minimum days to 90% maturity, plant height, peduncle length and maximum tillers plant\(^{-1}\), grain yield plant\(^{-1}\) and harvest index. High heritability was recorded for all traits like days to 50% heading, days to 90% maturity, tillers plant\(^{-1}\), plant height, peduncle length, spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\), grain yield plant\(^{-1}\), biological yield plant\(^{-1}\), seed index and harvest index. Correlation coefficient showed that significant and positive association of spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\), biological yield plant\(^{-1}\), biological yield plant\(^{-1}\) and seed index and harvest index. Correlation coefficient showed that significant and positive association of spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\), biological yield and harvest index with grain yield plant\(^{-1}\). Regression analysis showed that a unit increase in spike length, spikelets spike\(^{-1}\), grains spike\(^{-1}\), biological yield plant\(^{-1}\) and harvest index simultaneously grain yield plant\(^{-1}\) will also increase. Days to 90% maturity and plant height showed significant but negative association with harvest index while regression analysis also indicated that a unit increase in days to maturity and plant caused reduction in harvest index.
TABLE 4
Correlation (r) regression (b) and coefficient of determination (r²) of M₃ generations of wheat genotypes for various quantitative traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>Correlation coefficient (r)</th>
<th>Regression coefficient (b)</th>
<th>Correlation of determination (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to 50% heading vs Spike length</td>
<td>0.55**</td>
<td>0.73</td>
<td>0.30</td>
</tr>
<tr>
<td>Days to 50% heading vs Seed index</td>
<td>0.40</td>
<td>0.24</td>
<td>0.16</td>
</tr>
<tr>
<td>Days 90% maturity vs Plant height</td>
<td>0.49**</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>Days to 90% maturity vs Peduncle length</td>
<td>0.46**</td>
<td>0.62</td>
<td>0.21</td>
</tr>
<tr>
<td>Days to 90% maturity vs harvest index</td>
<td>-0.52**</td>
<td>-0.75</td>
<td>0.27</td>
</tr>
<tr>
<td>Tiller plant⁻¹ vs Spike length</td>
<td>0.37*</td>
<td>0.28</td>
<td>0.13</td>
</tr>
<tr>
<td>Tiller plant⁻¹ vs Spikelets spike⁻¹</td>
<td>0.40*</td>
<td>0.22</td>
<td>0.16</td>
</tr>
<tr>
<td>Tiller plant⁻¹ vs Grain spike</td>
<td>0.70**</td>
<td>0.14</td>
<td>0.49</td>
</tr>
<tr>
<td>Tiller plant⁻¹ vs Grain yield plant⁻¹</td>
<td>0.78**</td>
<td>0.28</td>
<td>0.61</td>
</tr>
<tr>
<td>Tiller plant⁻¹ vs Biological yield Plant⁻¹</td>
<td>0.72**</td>
<td>0.15</td>
<td>0.52</td>
</tr>
<tr>
<td>Tiller plant⁻¹ vs Harvest index</td>
<td>0.52**</td>
<td>0.21</td>
<td>0.27</td>
</tr>
<tr>
<td>Plant height vs Peduncle length</td>
<td>0.92**</td>
<td>2.21</td>
<td>0.85</td>
</tr>
<tr>
<td>Plant height vs Harvest index</td>
<td>-0.37*</td>
<td>-0.97</td>
<td>0.14</td>
</tr>
<tr>
<td>Spike length vs Spikelets spike⁻¹</td>
<td>0.69**</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>Spike length vs Grains spike⁻¹</td>
<td>0.63**</td>
<td>0.17</td>
<td>0.40</td>
</tr>
<tr>
<td>Spike length vs Grain yield plant⁻¹</td>
<td>0.61**</td>
<td>0.28</td>
<td>0.37</td>
</tr>
<tr>
<td>Spike length vs Biological yield plant⁻¹</td>
<td>0.60**</td>
<td>0.17</td>
<td>0.36</td>
</tr>
<tr>
<td>Spike length vs Harvest index</td>
<td>0.43*</td>
<td>0.26</td>
<td>0.18</td>
</tr>
<tr>
<td>Spikelets spike⁻¹ vs Grains spike⁻¹</td>
<td>0.79**</td>
<td>0.30</td>
<td>0.62</td>
</tr>
<tr>
<td>Spikelets spike⁻¹ vs Grain yield plant⁻¹</td>
<td>0.68**</td>
<td>0.44</td>
<td>0.47</td>
</tr>
<tr>
<td>Spikelets spike⁻¹ vs Harvest index</td>
<td>0.54**</td>
<td>0.40</td>
<td>0.29</td>
</tr>
<tr>
<td>Grains spike⁻¹ vs Grain yield plant⁻¹</td>
<td>0.85**</td>
<td>1.43</td>
<td>0.73</td>
</tr>
<tr>
<td>Grains spike⁻¹ vs Harvest index</td>
<td>0.61**</td>
<td>1.19</td>
<td>0.37</td>
</tr>
<tr>
<td>Grain yield plant⁻¹ vs Harvest index</td>
<td>0.64**</td>
<td>0.74</td>
<td>0.41</td>
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</table>

**, * = Significant at 1% and 5% probability level respectively

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IN VITRO MULTIPLICATION PERFORMANCE OF LYSIMACHIA NUMMULARIA L. UNDER SALINITY STRESS

Muhammet Dogan*

Department of Biology, Kamil Ozdag Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Turkey

ABSTRACT

Salinity levels are a serious abiotic stress factor affecting the metabolic and physiological activities of plants. Therefore, it is very important to know the responses of plants against salt. In this study, the effects of different NaCl concentrations (0-100 mM) on the growth of Lysimachia nummularia L. by tissue culture techniques were investigated. In addition, 0.25 mg/L 6-benzylaminopurine (BAP), Kinetin (KIN) and Thidiazuron (TDZ) were added to Murashige and Skoog (MS) nutrient media and the interactions of NaCl toxicity and hormones were evaluated. With the NaCl toxicity, yellowing and browning were observed in the leaves of the regenerated shoots. Regeneration of shoots on the explants was adversely affected by an increase in NaCl concentrations in the culture medium. In the culture medium with BAP, KIN, and TDZ, the maximum number of shoots per explant was recorded as 3.66, 4.16, 11.22 shoots/explant at 25 mM NaCl application, respectively. In BAP (4.27 cm) and TDZ (1.49 cm) culture medium, the highest shoot lengths were obtained in the control group and in the culture medium added with KIN (4.73 cm) was obtained in 25 mM NaCl application. Minimum shoot numbers and shoot lengths were determined in 100 mM NaCl application in three hormone species. This paper is a comprehensive study of the effects of salt stress on in vitro plant production and development.

KEYWORDS:
NaCl stress, salinity, shoot regeneration, tissue culture

INTRODUCTION

Plant tissue culture techniques, which provide great advantages over traditional production methods, have been widely used in the production of agricultural and ornamental plants in recent years. In addition, this technique allows multiple productions of medically valuable plants and thus helps in mass production of phytochemicals in plants. With this technique, genetically improved-fortified plants can be obtained and the physiological growth-development performances of plants can be examined in detail [1-4]. Plants are exposed to many biotic and abiotic stress factors throughout their life cycle. These stress factors have negative effects on the growth and development of the plant, especially on metabolism and productivity. The most common abiotic stress factors are drought, lack of nutrients, salinity, extreme temperature, terrestrial and atmospheric pollution and radiation [5, 6].

Salinity is one of the important problems threatening sustainable agriculture and soil areas in many parts of the world. Most of the land in the world cannot be cultivated and a significant portion of the land that can be cultivated is negatively affected by the salt. Plants are severely affected by salt due to the spread and continual increase of the affected areas. In particular, this rate is increasing in the arid and semi-arid regions [7, 8].

Salt stress first causes ionic and osmotic stress in plants. Secondly, it causes oxidative stress, malnutrition, deterioration of membrane integrity, deterioration of the cell division process and inactivity in enzymatic metabolic processes. Thus, a decline in growth and development and a decrease in product quantity and deaths are observed in the plants. The levels of plants affected by salt vary depending on the plant species, the age of the plant, the duration of salt exposure and the salt concentration [9].

Knowing the reactions of plants against salt is very important for the development of salt tolerant plants. Tissue culture techniques are an effective method for monitoring and evaluating the effects of salt stress and other stress factors. It also helps in the detection and selection of salt-tolerant plants in culture conditions (in vitro selection) [10, 11]. In the present study, the effects of different concentrations of NaCl on in vitro micropropagation of Lysimachia nummularia L. were evaluated. In addition, the effects of different plant growth regulators against salt stress in culture conditions were investigated.

MATERIALS AND METHODS

L. nummularia, which is used as plant material, was obtained from aquarists in Konya. Before the surface sterilization process was applied, it was kept under the tap water flowing for 30 minutes. The upper body parts for surface sterilization of the plants were treated with commercial bleach (5.7% active
chlorine-NaOCl-ACE) for 10 min. 3 rinses for 5 min was applied. After surface sterilization, shoot tip explants were isolated under sterile conditions and cultured on Murashige and Skoog (MS) [12] medium supplemented with 3% (w/v) sucrose (Duchefa) and solidified with 0.65% (w/v) agar (Duchefa) without any growth regulators for 2 weeks for gaining contamination-free explants.

Each experimental treatments were run in six repeats. MS nutrients, 3% sucrose (Duchefa), 0.65% agar (Duchefa) and NaCl at different concentrations (0, 25, 50, 75 and 100 mM) were used in the preparation of culture media. 0.25 mg/L 6-benzylaminopurine (BAP), Kinetin (KIN) and Thidiazuron (TDZ) were added to the culture medium as plant growth regulators. The pH of all media adjusted to 5.7 ± 0.1 with 1N NaOH and 1N HCl before autoclaving and the medium was autoclaved at 118 kPa atmospheric pressure at 120°C for 20 min. Cultures containing shoot tip explants were incubated under white light-emitting diodes (LEDs) (1500 lux) at 24 ± 1 °C and 16 hours of light photoperiod for eight weeks. In the regeneration studies, the explants were taken once in culture medium and the trial was terminated after eight weeks.

All trials were established according to the trial design of random plots. The data were analyzed with SPSS 21 for Windows (IBM Corporation, Armonk, NY, USA) program and Duncan tests were performed for Post Hoc tests. Percentage data were applied to arcsine transformation [13].

RESULTS AND DISCUSSION

It is very important to clarify the stress-related mechanisms and develop tolerant species and varieties when the plants are faced with many stress factors in their life. It has also been suggested in recent years that some plant growth regulators such as salicylic acid, polyamines, abscisic acid and jasmonic acid can be used to increase salt tolerance in plants [14-16]. In this study, the shoot tip explants of L. nummularia were incubated for eight weeks in an MS nutrient medium containing 0.25 mg/L BAP, KIN and TDZ and different concentrations of NaCl. Color loss, yellowing, browning, and even deaths were observed in the regenerated shoots with respect to the effects of salinity rates (Figure 1). In the culture medium, yellowing and color loss in the NaCl effect was shown in Figure 1d for BAP, Figure 1e for KIN, and Figure 1f for TDZ. The views of the control group plants were given in Figure 1 a, b, c.

The color loss, browning, and yellowing in BAP and KIN media have been observed more clearly in the upper body and upper leaves. In plants in the culture medium supplemented with TDZ, the yellowing was in both the lower and upper trunks. According to the control group, regenerated shoots in NaCl exposure were weaker and the leaves were smaller. With high NaCl effect, the colors of some leaves turned into light green. According to these results, it can be said that NaCl stress decreases the chlorophyll content in L. nummularia. In relation to these results, Ozturk et al. [17] reported significant decreases in chlorophyll content of Pisum sativum cv Rona under long-term NaCl stress compared to control. Ma et al. [18] evaluated the photosynthetic pigment content of Dianthus superbus under salt stress and noted that salt stress decreased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content in D. superbus. Chlorophyll a content of 0.3, 0.6 and 0.9% of the salt applications decreased by 29.5%, 72.3% and 77.1%, respectively. Similarly, the decreases in the photosynthetic activity and in chlorophyll contents in Raphanus sativus have been reported under salt toxicity [19].

The effects of different NaCl concentrations on shoot regeneration. The release time of shoots on the explants in the culture medium under the influence of NaCl was different. While the first regenerated shoots were observed on the 12th day in the control group, the first exile in 25 mM application was observed on the 16th day. As can be seen from these results, an increase in NaCl concentrations in the culture medium adversely affected the regeneration of shoots on the explants. In the MS medium containing BAP and TDZ, shoot regeneration frequencies were recorded between 38.89-100%, while in the MS medium with KIN were recorded between 33.33-100% (Table 1). The highest shoot regeneration frequencies (100%) were obtained in the control group and 25 mM NaCl in the culture medium in the three hormone ratio. The lowest shoot regeneration rates were recorded in the culture medium where NaCl was used at the highest rate. Similarly, reductions in shoot regeneration of Chrysanthemum morifolium exposed to salt stress on tissue culture conditions were reported by Mohamad et al. [20].

The effects of different NaCl concentrations on the number of shoots per explant. The number of shoots of explants changed under increasing NaCl concentrations and statistically significant at p<0.05 (Table 2). Increased number of shoots declined with increasing NaCl dose in all applications. The mean shoot counts per explant were in culture media fortified with BAP as 2.51-3.88 shoots/explant, in culture media fortified with KIN as 2.56-4.16 shoots/explant and in culture media fortified with TDZ as 6.50-11.22 shoots/explant. In all applications, the maximum number of shoots was reached in the control group explants. In the MS medium containing BAP, KIN and TDZ, the highest number of shoots were recorded as 3.66, 4.16, 11.22 shoots/explant at 25 mM NaCl application, respectively. When all three hormones were compared, the highest number of shoots were obtained in MS medium containing
FIGURE 1
The effects of different NaCl concentrations on the regenerated shoots of *L. nummularia* at the end of eight weeks. View of shoots in BAP (a), KIN (b) and TDZ (c) culture media without NaCl (control group); BAP (d), KIN (e) and TDZ (f) culture media containing high concentrations of NaCl.
TDZ followed by KIN-containing MS medium. There were no statistically significant differences between the numbers of shoots in BAP and TDZ media with 25 mM NaCl and the number of shoots in the control group. Furthermore, there were no statistically significant differences between the number of shoots obtained in the KIN medium containing 25 and 50 mM NaCl and the number of shoots in the control group (p<0.05). These results showed that NaCl, which is used at low rates, did not show a statistically significant effect on the number of shoots. The lowest number of shoots were determined in culture medium containing 100 mM NaCl in the three hormone species. In the culture medium containing BAP, the lowest number of shoots was 2.51 (35.31% lower than the control) (Figure 2A), in culture medium containing KIN containing 2.56 (38.46% lower than control) (Figure 2B) and in culture medium containing TDZ 6.50 (38.46% lower than control) (Figure 2C). In accordance with these results, Ranjana et al. [21] explained that the number of shoots of *Saccharum officinarum* L. in tissue culture conditions decreased with NaCl effect. Decreases in the number of shoots under the influence of NaCl have been previously reported in *Azadirachta indica* [22].

Although the levels of hormones used were the same, the levels of exposure of the explants to NaCl differed. In general, higher shoot numbers were obtained in culture medium added with TDZ and fewer shoot numbers were obtained in culture medium added with BAP. In addition, TDZ has been shown to reduce the adverse effects of NaCl. This may be due to hormones affecting different metabolic, biochemical and physiological events on plants. These results prove that growth regulators have different effects on the explants. Similarly, in many studies, it was reported that hormone or hormone ratios showed different results on shoot regeneration frequency, shoot count per explant and shoot length data [23-27].

**The effects of different NaCl concentrations on shoot length.** Shoot lengths of explants under salt stress were shown in Table 3. Salt stress had also negative effects on shoot lengths as in the number of shoots. These effects were different in the environment including BAP, KIN and TDZ and were found between 3.41-4.27 cm, 3.52-4.73 cm and 0.95-1.49 cm, respectively. The longest shoots in NaCl exposure were obtained in the control group explants in MS medium containing BAP (4.27 cm) and TDZ (1.49 cm). In contrast, the longest shoots in KIN-containing MS medium were detected at a concentration of 25 mM NaCl. The length of shoots shortened under high NaCl effect. The shortest shoots were determined at a concentration of 100 mM NaCl. The shortest shoots were 3.41 cm in BAP-containing medium (20.14% lower than the control) (Figure 2D), 3.52 cm (24.30% lower than control) in KIN-containing MS medium (Figure 2E) and 0.95 cm in TDZ-containing MS medium (36.24% lower than control) (Figure 2D). The reducing effect of NaCl on shoot length reported in plants of *Solanum tuberosum* L. [28] and *Glycine max* L. [29]. This decrease in shoot length may be due to the fact that NaCl toxicity inhibits cell division and expansion [30].

Compared to all three types of hormones, the longest shoots were measured in culture media added with KIN hormone and the lowest shoots were measured in culture medium added with TDZ. Although NaCl had a negative effect on shoot length of plants, it had a negative effect on shoot elongation in *L. nummularia* in TDZ application according to BAP.
and KIN applications. In addition, the highest decrease in shoot lengths compared to control was recorded in TDZ-containing MS medium with 36.24%. Similarly, the negative effect of TDZ administration on shoot length was previously reported in Cassia sophera Linn [31], Cymbidium giganteum Wall. Ex Lindl [32] and Prunus fruticosa × Prunus lannesiana [33]. In addition, it was reported that TDZ effect was low when compared to BAP, KIN and 2iP on the length of shoot [34, 35].

**FIGURE 2**
The decreases in average shoot number (A, B, C) and shoot length (D, E, F) of L. nummularia exposed to NaCl concentrations in culture medium containing BAP, KIN and TDZ.
The effects of different NaCl concentrations on mean number of shoot length of L. nummularia in the culture medium

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>BAP (0.25 mg/L)</th>
<th>KIN (0.25 mg/L)</th>
<th>TDZ (0.25 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.27a</td>
<td>4.65a</td>
<td>1.49a</td>
</tr>
<tr>
<td>25</td>
<td>4.15a</td>
<td>4.73a</td>
<td>1.44b</td>
</tr>
<tr>
<td>50</td>
<td>3.84ab</td>
<td>4.58a</td>
<td>1.37a</td>
</tr>
<tr>
<td>75</td>
<td>3.79ab</td>
<td>4.11ab</td>
<td>1.12b</td>
</tr>
<tr>
<td>100</td>
<td>3.41b</td>
<td>3.52b</td>
<td>0.95b</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences (p<0.05)

CONCLUSIONS

Tissue culture applications are an important technique that allows the examination of factors such as growth, development and stress of plants. Therefore, this study was designed to determine the effects of NaCl stress in tissue culture conditions. The effects of NaCl in increasing concentrations on in vitro production L. nummularia were investigated. NaCl exposure led to yellowing and drying of regenerated shoots. The regeneration ability of the explants with high levels of NaCl decreased. Similarly, in NaCl treated media, significant decreases were determined in shoot length and the number of shoots compared to control. It has been noted that different hormones react differently to plant stresses. In general, the most shoots under the stress of NaCl was obtained in TDZ-containing MS medium, the longest shoots were obtained in KIN-containing MS medium. Because of the spread and increase of salt affected areas, it is important to know the reactions of plants against salt and to develop salt-resistant species. This paper is a comprehensive study including the effects of salt stress on in vitro growth and development. It may guide the development of salt-resistant L. nummularia and other plants in the future.

REFERENCES


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LOCAL PEOPLE OPINION ABOUT WIND FARMS IN TURKEY

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ABSTRACT

In contrast to fossil fuel energy, wind energy has been embraced by people. Because it is a new issue, people might have a limited knowledge about advantages and disadvantages. The aim of this study was to examine how perceived and evaluated the environmental impacts of wind farms by local people. For this purpose, a survey was conducted with 126 resident who lives in the area of wind farms, in Osmaniye, a province in Mediterranean region of Turkey. Respondents were expected to be informed about wind farms before construction. They stated that they were not disturbed by the wind noise (62%) and saw the whole wind system from their house (61,40). Some of them have indicated that the wind farms are not primarily concerned about the environmental impacts, while others have pointed out that they adversely affected the productivity in agricultural production. Local people think that wind farms can make economic contributions to their villages.

KEYWORDS:
Wind, environment, public, energy

INTRODUCTION

There is an ongoing debate about the fossil fuels and new energy sources for a decade. As a matter of fact, that fossil fuels will be depleted in near future. To be able to meet the increasing energy need, countries are trying to meet their energy need from renewable energy sources that are not disrupt the natural balance and pollute the environment. Renewable energy sources not only reduce countries’ external dependency but also increase their energy security. The sustainability of these sources will also increase the energy efficiency of countries. Renewable energy, clean energy or green energy – all of these terms are becoming more and more common. Solar, wind, hydro (water), biomass and biofuels and geothermal are mostly mentioned energy sources [1]. Through the improving technology and low cost, wind energy became more popular one. Wind energy has one of the fastest growing and rising technologies. Wind farms are spreading rapidly in all over the world because it occurs in the atmosphere spontaneously and continuously, wind turbines can be installed easily and developed technologically and investment costs are progressively cheaper than the other sources. A wind turbine is a device that converts the wind’s kinetic energy into electrical energy [2, 3, 4].

By the end of 2016, the worldwide wind power plants’ electricity production capacity increased by 12% compared to 2015, and rose up to 486,790 MW. With new installations, production capacities increased by 36,023 MW in 2013, 51,675 MW in 2014, 63,330 MW in 2015 and 54,642 MW in 2016 [5]. Overall, 52.5 GW of new wind power was installed across the globe in 2017, a slight decrease on the 2016 market of 54.6 GW, bringing total installed capacity up to 539 GW [6]. GWEC reported that 2017 marked a turning point for Turkey’s wind industry. Turkey added 766 MW in 2017, bringing the country’s total wind power capacity to 6,857 MW. In its Global Wind Report 2018, GWEC says new capacity in 2018 will remain at a similar level to the 52.5GW installed in 2017. Turkey’s national target for wind power is set at 20 GW by 2023 [6]. When compared, the Aegean and Marmara regions are becoming more benefited more significantly from wind energy. Wind power in Turkey is increasing rapidly but the desired level of installed capacity has not been reached while it has a very high potential [7]. Wind turbines provide a clean energy source, emitting no greenhouse gases and no waste product. Being environmentally friendly and green is a large advantage of wind turbines [8, 9, 10]. Although, it is known as cheap and clean energy; people are concern about some factors. These factors have been classified into three groups by [11]; perceptions of physical environmental factors, psycho-social factors and social and institutional factors. Some of these factors are; noise, visual and aesthetics impacts, turbine and farm size, ecological site characteristics, benefit and cost, familiarity, knowledge, social network influence, centralized/decentralized and local ownership.

Of course the public perceptions differ from country to country. In Turkey, there are not enough studies on public perception about wind farms. One
research was conducted by Bostan Budak and Eren [12] in Hatay province to analyze the public attitudes of wind energy and willingness to pay more for wind energy. A survey showed that the people have clearly positive attitudes toward wind energy and willing to pay more for it. Karaburun district of Izmir, in Aegean region, which maintain its rural characteristics, is facing a challenge about construction of wind farms [13]. It has a very high potential but there is a huge opposition toward wind farms by local people. Local people are affected negatively because pasture and agricultural areas are being allocated to wind farm constructions and expropriation of private agricultural land by emergent expropriation. Agricultural areas are turning to energy areas. In some villages, the economic activities are almost standstill. At the same time, through the construction stage, field cleaning-up process for turbine roads harmed natural and rural environment. After the construction phase, noise pollution has started negatively affecting daily life. All of these steps have attracted the interest of the environmentally conscious local people, and the process has moved to protest by the people. Many non-governmental organizations participated in the protest.

It is clear that there are anticipated and non-anticipated drawbacks and oppositions against wind farms both globally and in Turkey. Osmaniye, a province in Mediterranean region of Turkey which is the third region regarding wind farms. A research was conducted in this province to see local people attitude toward wind farms because there are not enough researches on the oppositions of wind farms in Turkey. The specific objectives of this paper are to identify the concerns, expectations and support of local people toward wind farms.

MATERIALS AND METHODS

This study was carried out in Osmaniye because it is one of the important province in Turkey in regard of wind power plants. At the time of the study, the population was 52,2175 in Osmaniye. Because of time and money constraints, researchers decided to work with the sample. Researchers have no prior idea about the target population so they used simple random sampling method to decide the sample size. If the target population (N<10,000) is finite, the following formula should be used [14].

\[ n = \frac{N \cdot p \cdot q}{Z^2 \cdot p \cdot q} \]

N = Population
n = Sampling size
z = 2.58 (z value in deriving 99% confidence level)
p = Proportion of sample (p=0.75)
q = (1-p) proportion of the population which has not the attribute in question
d = Accepted sampling error (±10%)

The sample size was found 125 with this formula (99% confidence level and 10% of sampling error). One more questionnaire was also filled out by respondents so totally 126 questionnaires were used in data analysis. The questionnaire was implemented face to face by researcher. Participants were selected randomly but the villages were selected purposively, depend on the number of wind farms and the distance to wind farms. Frequencies and percentage distributions were used about opinions and disadvantages of wind turbine. To determine if there is a difference between variables, the Kruskal-Wallis H test and the Mann-Whitney U test were used in this study.

The Kruskal-Wallis H test is a rank-based nonparametric test that can be used to determine if there are statistically significant differences between two or more groups of an independent variable on a continues or ordinal dependent variable. It is considered the nonparametric alternative to the one-way ANOVA. The Mann-Whitney U test is a nonparametric test allows two groups to be compared without making the assumption that values are normally distributed [15].

RESULTS

Demographic Characteristics. Almost 65% of the participants were male and the average household size was two. The respondents were predominantly age of 51 and over (54.8%) and mainly they were either pensioners (34.1%) or housewives (31.7%). Thirdy eight percent of them had a primary school diploma while 25% had a high school diploma. These villages are cooler in the summer months, for this reason that they are used as plateaus. Because of the cool weather 93% of the people living in these villages do not use air conditioners or ventilators. The average monthly income of approximately 55.8% of the families is between 1,000 - 1,500 TL. The electricity costs vary but the average cost is around 50 TL. They use wood-coal (94.5%) to warm up, although they think that the most harmful energy source to the environment is fossil fuels (99.2%).

Distance from wind farm. The distance between the wind farm and the respondents home might have an effect on people’s opinion, because of the visual appearance and the sound. The respondents were asked about the distance between wind farm and their home. There were five choices: ‘0–1 km,’ ‘1.1 to 2km,’ ‘2.1–3km,’ ‘3.1–4km’ and more than 4km. About 32% of those who participated in the survey expressed the distance of the houses from the wind farm between 0-1 km and 31% of them between 3-4 km. A cross tabulation analysis was conducted between the distance and the visual appearance (Table 1).
TABLE 1
Cross tabulation of the distance and the visual appearance

<table>
<thead>
<tr>
<th>Distance</th>
<th>Whole wind farm (%)</th>
<th>Part of the wind farm (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2.0 km</td>
<td>61,40</td>
<td>14,49</td>
<td>35,71</td>
</tr>
<tr>
<td>2.1-4.0 km</td>
<td>22,81</td>
<td>60,87</td>
<td>43,65</td>
</tr>
<tr>
<td>4.1 km and more</td>
<td>15,79</td>
<td>24,64</td>
<td>20,63</td>
</tr>
<tr>
<td>Total</td>
<td>45,24</td>
<td>54,76</td>
<td>100,00</td>
</tr>
</tbody>
</table>

$X^2 = 30.78, df = 2, p < 0.01$

While 44% of the respondents stated that they heard the noise of wind farms from their home and their garden, 36% said they did not hear anything. Sixty two percent of the respondents stated that they were not disturbed by the noise of wind farms. The percentage of those who see part of the wind farms from their home or garden is 54% and the remaining part (45%) declared that the see the whole wind farm.

Expectations, concerns and support of wind farms. People are expected to be informed about wind farms before establishment. In this study, 73% of the participants stated that they had not been informed before the construction of wind power plants. Only, 21.4% had some knowledge and 5.6% had little knowledge about the construction. To involve and inform the local people before construction of power plants may increase their acceptance level. Local people had also anticipation about the priority of the use of wind energy by them in case of emergency. Other expected issues were the discount on the wind power energy, contribution to the local economy and economic support to local people, like new jobs.

Other studies showed that, local people have concerns about the wind farms [16, 17, 18]. In Table 2, participants’ concerns about wind farms were presented. As seen from the table, respondents who live closer to wind farms, were not worried to much about some of the negative effects of wind farms. Most concerned issues were the loss of agricultural lands, cost of energy and loss of trees.

The participants in this research mainly support the construction of wind farms to produce energy. The most supported issue is the government’s implementation of programs (such as carbon tax) to reduce greenhouse gas emissions (Table 3). Strong public support is not enough for the development of wind energy; government policies are also important.

TABLE 2
Concerns about wind farms

<table>
<thead>
<tr>
<th>Concerns</th>
<th>Not very worried</th>
<th>Not worried</th>
<th>Not sure</th>
<th>A little worried</th>
<th>Very worried</th>
<th>Mean</th>
<th>St. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noise and vibration of wind turbines</td>
<td>83</td>
<td>65.9</td>
<td>7</td>
<td>5.6</td>
<td>23</td>
<td>18.3</td>
<td>6</td>
</tr>
<tr>
<td>Unattractive feature of landscape</td>
<td>103</td>
<td>81.7</td>
<td>2</td>
<td>1.6</td>
<td>6</td>
<td>4.8</td>
<td>11</td>
</tr>
<tr>
<td>The potential of killing birds</td>
<td>75</td>
<td>59.5</td>
<td>30</td>
<td>23.8</td>
<td>7</td>
<td>5.6</td>
<td>9</td>
</tr>
<tr>
<td>The potential of killing bats</td>
<td>76</td>
<td>60.3</td>
<td>29</td>
<td>23.0</td>
<td>6</td>
<td>4.8</td>
<td>9</td>
</tr>
<tr>
<td>The potential of damaging roads</td>
<td>95</td>
<td>75.4</td>
<td>15</td>
<td>11.9</td>
<td>8</td>
<td>6.3</td>
<td>7</td>
</tr>
<tr>
<td>Negative effect on immovable property owned by local people</td>
<td>86</td>
<td>68.3</td>
<td>13</td>
<td>10.3</td>
<td>6</td>
<td>4.8</td>
<td>14</td>
</tr>
<tr>
<td>Wind is an unreliable source of energy in terms of its continuity</td>
<td>60</td>
<td>47.6</td>
<td>44</td>
<td>34.9</td>
<td>6</td>
<td>4.8</td>
<td>11</td>
</tr>
<tr>
<td>Potential of its’ shadow flickering</td>
<td>52</td>
<td>41.3</td>
<td>55</td>
<td>43.7</td>
<td>7</td>
<td>5.6</td>
<td>8</td>
</tr>
<tr>
<td>Loss of agricultural lands</td>
<td>62</td>
<td>49.2</td>
<td>32</td>
<td>25.4</td>
<td>7</td>
<td>5.6</td>
<td>13</td>
</tr>
<tr>
<td>High cost of energy production</td>
<td>61</td>
<td>48.4</td>
<td>36</td>
<td>28.6</td>
<td>3</td>
<td>2.4</td>
<td>16</td>
</tr>
<tr>
<td>Disabling the use of communication tools (radio /TV/Internet /Mobile phone)</td>
<td>84</td>
<td>66.7</td>
<td>15</td>
<td>11.9</td>
<td>5</td>
<td>4.0</td>
<td>15</td>
</tr>
<tr>
<td>Disturbing radar signals</td>
<td>71</td>
<td>56.3</td>
<td>32</td>
<td>25.4</td>
<td>3</td>
<td>2.4</td>
<td>13</td>
</tr>
<tr>
<td>Waste and dust because of diggings</td>
<td>102</td>
<td>81.0</td>
<td>5</td>
<td>4.0</td>
<td>7</td>
<td>5.6</td>
<td>9</td>
</tr>
<tr>
<td>Solid waste</td>
<td>101</td>
<td>80.2</td>
<td>5</td>
<td>4.0</td>
<td>8</td>
<td>6.3</td>
<td>8</td>
</tr>
<tr>
<td>Power transmission lines</td>
<td>88</td>
<td>69.8</td>
<td>17</td>
<td>13.5</td>
<td>5</td>
<td>5.6</td>
<td>9</td>
</tr>
<tr>
<td>Reduce the implementation and effectiveness of agricultural drugs</td>
<td>69</td>
<td>54.8</td>
<td>31</td>
<td>24.6</td>
<td>4</td>
<td>3.2</td>
<td>11</td>
</tr>
<tr>
<td>Cutting of trees</td>
<td>57</td>
<td>45.2</td>
<td>16</td>
<td>12.7</td>
<td>5</td>
<td>4.0</td>
<td>26</td>
</tr>
</tbody>
</table>
**TABLE 3**

Support to wind farm

<table>
<thead>
<tr>
<th>Ideas</th>
<th>Strongly oppose</th>
<th>Somewhat oppose</th>
<th>Neither support, nor oppose</th>
<th>Somewhat support</th>
<th>Strongly support</th>
<th>Mean</th>
<th>St. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I support wind power plants.</td>
<td>21</td>
<td>16.7</td>
<td>16</td>
<td>12.7</td>
<td>20</td>
<td>15.9</td>
<td>1.57</td>
</tr>
<tr>
<td>I support the establishment of more turbines to generate energy from the wind.</td>
<td>20</td>
<td>15.9</td>
<td>18</td>
<td>14.3</td>
<td>8</td>
<td>6.3</td>
<td>1.56</td>
</tr>
<tr>
<td>I support the establishment of a new power plant.</td>
<td>21</td>
<td>16.7</td>
<td>16</td>
<td>12.7</td>
<td>9</td>
<td>7.1</td>
<td>1.56</td>
</tr>
<tr>
<td>I support the government to reduce taxes for the use of renewable energy resources.</td>
<td>17</td>
<td>13.5</td>
<td>16</td>
<td>12.7</td>
<td>11</td>
<td>8.7</td>
<td>1.50</td>
</tr>
<tr>
<td>I support the establishment of more high voltage lines for an increase in energy production from the wind.</td>
<td>29</td>
<td>23.0</td>
<td>17</td>
<td>13.5</td>
<td>15</td>
<td>11.9</td>
<td>1.63</td>
</tr>
<tr>
<td>I support the purchase of wind energy from electricity generation and distribution companies.</td>
<td>18</td>
<td>14.3</td>
<td>15</td>
<td>11.9</td>
<td>26</td>
<td>20.6</td>
<td>1.47</td>
</tr>
<tr>
<td>I support the government's implementation of programs (such as carbon tax) to reduce greenhouse gas emissions.</td>
<td>15</td>
<td>11.9</td>
<td>4</td>
<td>3.2</td>
<td>7</td>
<td>5.6</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Mann-Whitney and Kruskal-Wallis tests were conducted to investigate whether there is a statistically significant difference between the respondents’ opinion about the hazards of wind farms and their personal characteristics. It was found that there is a significant difference between the respondents’ opinion about the hazards of wind farms and their characteristics such as gender \( (U = 1147.00; p < 0.05) \), age \( (X^2 = 11.291; p < 0.05) \), occupation \( (X^2 = 16.283; p < 0.05) \), and education \( (X^2 = 11.745; p < 0.05) \).

Mann-Whitney test analysis showed that the women \( (78,57) \) were more concerned than the men \( (54,84) \) about the hazards of the wind farms. According to the Kruskal-Wallis test analysis, the group of age between 31-40 years \( (86,72) \) was more worried than the other groups of age. Also, housewives \( (78,94) \) were more worried than the other groups of profession and non-literate \( (74,59) \) were worried than the other groups of educational level.

Mann-Whitney and Kruskal-Wallis tests were conducted to investigate whether there is a difference between respondents’ opinions about the wind farm policies and their personal characteristics. It was found that there is a significant difference between the gender \( (U = 1082.00; p < 0.05) \), occupation \( (X^2 = 27.805; p < 0.05) \), education \( (X^2 = 11.745; p < 0.05) \) and the opinions about the wind farm policies. According to the Mann-Whitney test, men \( (72,98) \) supported wind farm policies more than women \( (47,02) \). The Kruskal-Wallis test showed that the civil servants \( (85,27) \) support government policies more than the other occupational groups.

**CONCLUSION AND DISCUSSION**

Turkey’s total energy consumption mainly \( (75\%) \) compensated from imported energy. As reported, 99.2% of natural gas and 93.9% of crude oil has been imported \([19], [20]\). On the other hand, Turkey has a significant potential in terms of renewable energy, which is ranked 16th with its wind energy capacity \([21]\). Wind energy is a rapidly growing energy source which has less environmental impact than the other energy sources. But investments are increasing slowly because of the relatively low Fit-in Tariff levels, in Turkey.

Of course, there are some adverse environmental effects of wind farms. The visual aspects and noise impacts were cited mostly in the literature \([22], [23], [24], [25]\). In this study, 62% of the respondents stated that they were not disturbed by the noise of wind farms. The respondents who live in the villages near the wind farms in Osmaniye province stated that they are not disturbed by the environmental effects of the wind farms. But, there is a concern about the possible impact of wind farms on their health and productivity in agriculture. Studies should be conducted to collect enough data about agricultural productivity and health issues.

Motosu \([26]\) found out that local residents oppose wind energy projects because no information was presented to the residents and no public participation was taken before the construction. In this study, 73% of the participants stated that they had not been informed before the construction of wind power plants. More information should be shared...
by local people before the construction of wind farm to increase the acceptance. Despite negative effects of wind farms, local people in Osmaniye are supporting wind farms for energy generation. They think wind farms will make economic contributions to their villages, such as new jobs.

REFERENCES


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THE SPATIAL AND TEMPORAL DISTRIBUTION OF ECOLOGICAL EFFICIENCIES IN XINJIANG, CHINA

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ABSTRACT

Eco-efficiency is an effective means to evaluate sustainable development. It is of great practical significance to study the ecological efficiency of the ecologically fragile arid region of Xinjiang in order to formulate scientific policies and measures of ecological environmental protection. In this study, variation in ecological efficiency was empirically analyzed for 14 cities of Xinjiang between 2001 to 2015 using a super-efficiency DEA-Tobit evaluation model. These analyses indicated that (1) the ecological efficiency of Xinjiang rose steadily every year between 2001 and 2015, except for a brief decline in 2009; (2) ecological efficiency was unbalanced among the prefectures, and ecological efficiencies were generally low; (3) analysis of environmental efficiency and resource efficiency led to the recognition of four developmental modes that represent Xinjiang prefectures: low energy consumption and low emissions, high energy consumption and low emissions, low energy consumption and high emissions, and high energy consumption and high emissions. By analyses of the Malmquist index (4), it was found that technical progress (TC) restricted the total factor productivity (TFP), while technical efficiency index (EC) promoted TFP. Finally (5), ecological efficiency was positively and significantly correlated with the utilization of foreign capital, urbanization rate, and average education degree, but significantly and negatively correlated with the marketization degree.

KEYWORDS:
Xinjiang, Ecological efficiency, Malmquist index, Tobit regression model

INTRODUCTION

Xinjiang is located on the northwest border of China and the hinterland of Eurasia. The desert area accounts for nearly 60% of China’s desert region. Xinjiang is the main part of China’s arid region and one of the world’s drought centers. The ecological environment is fragile. At the same time, Xinjiang is a province with large resources. It is rich in coal, oil, natural gas, and other mineral resources. The province mainly relies on the traditional development model of high input, high consumption and high pollution emissions. Therefore, excessive consumption of resources, environmental pollution, and ecological imbalance has become increasingly serious problems, which further restricts the sustainable development of Xinjiang. Therefore, how to scientifically measure the ecological level and efficiency of regional economic development is very important, and it is also the focus of policymakers at all levels.

In 1990, Schaltegger and Sturm first proposed the concept of ecological efficiency, that is, the ratio of increased value to increased environmental impact [1]. In 1992, the World Business Council for Sustainable Development (WBCSD) introduced the concept of ecological efficiency into economic activities using an economic perspective for the first time. This idea was defined as follows: “The ecological efficiency must provide a product or service which has a price competition advantages to satisfy human needs and ensure the quality of life, and can gradually reduce the ecological impact resources consumption intensity of the products or services, and the degree of reduction should be identical with the estimation of earth’s carrying capacity” [2]. Given this definition, ecological efficiency then becomes an important analytical tool to measure sustainable development. At present, the research on ecological efficiency mainly focuses on accounting methods and application research. There are three main accounting methods: single ratio [3-4], index system [5-6] and the model method [7-8]. Applied research is mainly conducted at three levels: enterprise [9-10], industry [11-12] and region [13-14]. Claude Fussier (1995) [15] introduced the concept of ecological efficiency to China for the first time. Li (2000) [16] introduced the idea of ecological efficiency when studying China’s environmental management mode, and the traditional data envelopment analysis method (DEA) is the method which is commonly used [17-18].

The evaluation of ecological efficiency in Xinjiang, China, is incomplete. Of the little research in the area, Jia [19] and Zhang [20] estimated the agro-ecological efficiency of Xinjiang. Jia [21]
investigated the ecological efficiency of the chlorine-alkali chemical industry in Xinjiang. Nevertheless, ecological efficiency investigations for Xinjiang are still lacking.

To realize the construction goal of a "big beautiful Xinjiang with blue sky, green land, and clear water", Xinjiang must establish the ecological civilization concept of "respecting nature, conforming to nature, and protecting nature", uphold the new development concept of high efficiency, energy conservation, environmental protection, and maintain the coordinated, sustainable and efficient development of economy and environment. The connotations and goals of ecological efficiency are: "minimize resource consumption, minimize environmental impact and maximize economic value". Therefore, the ecological efficiency index can be used to measure the status and degree of sustainable development. This article mainly uses the method of super-efficiency DEA - Malmquist - Tobit model. It utilises 15 consecutive years (from 2001 to 2015) of data from 14 cities of Xinjiang panel data as samples. The goal of the present study was to explore annual trends in ecological efficiencies, spatial distribution patterns, and identify potential influences on efficiencies. Besides, these analyses were used to propose improvement measures and provide a scientific basis for promoting the sustainable development of resource utilization and ecological environmental protection in Xinjiang.

RESEARCH METHODS AND DATA SOURCES

Research Methods. Super-efficiency DEA Model. The DEA-CCR model was used to evaluate the effectiveness of Decision-Making Units (DMUs) using the "multi-input and multi-output" model [22]. An improved CCR model termed the super-efficient DEA model was proposed by Andersen in 1993 [23]. The super-efficiency DEA model improves upon the critical problem of the traditional DEA model wherein multiple evaluation units are at the production frontier and cannot be further evaluated. Further, the improved model overcomes the issue with the CCR model wherein further evaluation and comparison of multiple DMUs is not possible, and thus, the improved model enables the comparison and sorting of effective DMUs. Moreover, the super-efficient DEA model aids in understanding the change in dynamic efficiency of decision units and the factors that influence changes in efficiencies.

The super-efficient DEA model is given as:

\[
\begin{align*}
\lambda & = \theta \lambda_t, \\
\lambda & > 0,
\end{align*}
\]

Here \( \theta \) represents the efficiency value of the decision-making unit; \( X \) and \( Y \) represent input and output variables, respectively, and \( \lambda \) represents the combined ratio in an effective decision-making unit that is used to judge the scale of the benefit of DMU. \( \sum \lambda < 1, \sum \lambda = 1, \) and \( \sum \lambda > 1 \) denotes an increase, invariance, and decreases in benefits of scale, respectively. \( S^- \) and \( S^+ \) represent relaxation and residual variables, respectively. When \( \theta < 1 \) the decision unit does not reach the optimal efficiency, whereas \( \theta > 1 \) indicates that the decision unit achieves optimal efficiency.

Malmquist index. The Swedish economist Malmquist [24] first proposed the Malmquist index in 1953. In 1982, Caves proposed that the Malmquist index represented the total factor production efficiency under multi-input and multi-output conditions, and integrated the index into the DEA model to calculate the total factor productivity of the production sector [25]. The basic principles of this implementation are as follows:

The Malmquist index from time interval \( t \) to \( t+1 \) can be expressed as [26]:

\[
\text{TFP} = \left[ \frac{\partial^n(x_{it}, \cdot y_{it}) - \partial^n(x_{it}, \cdot y_{it})}{\partial^n(x_{it}, \cdot y_{it}) - \partial^n(x_{it}, \cdot y_{it})} \right]^{\frac{1}{2}}
\]

\[
= \left[ \frac{\partial^n(x_{it}, \cdot y_{it}) - \partial^n(x_{it}, \cdot y_{it})}{\partial^n(x_{it}, \cdot y_{it}) - \partial^n(x_{it}, \cdot y_{it})} \right]^{\frac{1}{2}}
\]

\[
= \text{TC} \times \text{EC}
\]

\[
= \text{TC} \times \text{PE} \times \text{SE}
\]

where the variation in the Malmquist index is the total factor productivity (TFP) variation and represents the change in the degree of productivity for a decision unit from \( t \) to \( t+1 \). TFP > 1 indicates an increase in productivity, whereas TFP < 1 indicates a decrease in productivity. The TFP can be subdivided into technical change (TC) and efficiency change (EC). TC refers to the contribution of moving the production frontier to productivity, while EC is the contribution from changing technical efficiency to productivity between the period encompassed by \( t \) and \( t+1 \). EC can be further divided into PE (pure technical efficiency) and SE (scale efficiency).
Tobit Model. The ecological efficiency estimated by the DEA model is not only affected by the input and output indicators that are selected, but also by other factors. Coelliit [27] developed a two-step method based on the DEA to identify the factors that influence ecological efficiency and its degree of influence. The first step is to evaluate the efficiency of decision-making units by the DEA. Then, a regression model is established that uses the estimated efficiency value as the dependent variable, and influential factors as the independent variables. The orientation and intensity of the influential factors on ecological efficiencies are then determined by the coefficients of the independent variables.

The Tobit model can be written as:

\[ y_i^* = x_i^T \beta + \epsilon_i, \quad y_i^* \geq 0 \]
\[ y_i^* = 0, \quad y_i^* < 0 \]

Where \( x_i \) is the independent variable, \( y_i \) is the observed dependent variable, \( y_i^* \) is the latent variable, \( \beta \) is the correlation coefficient, \( \epsilon_i \) is the independent variable and the disturbance term is \( \epsilon_i \sim N(0, \sigma^2) \).

Selection of Evaluation Indicators and Data Source. The fundamental concept of increasing ecological efficiency is to maximize its value with minimal resource consumption and environmental pollution. Importantly, these objectives are consistent with the requirements for the inputs and outputs of the DEA method. In practice, profitability is usually defined as the output index while the cost is used as the input index.

In consideration of resource, environmental and economic factors, and concerning previous studies [13, 14]. Specifically, we selected several parameters as input indices including energy consumption, power consumption, water consumption, fixed assets investment, five resource consumption indices, exhaust emissions, wastewater discharge, and solid waste discharge from three kinds of environmental pollution parameters. The economic value was considered as the output index (Table 1). An ecological efficiency evaluation system was then constructed using these indices. The input and output data used in this study were acquired from The Statistical Yearbooks of Xinjiang Uygur Autonomous Region, The Yearbooks of Xinjiang Environmental Statistics and statistical yearbooks of the prefectures.

### RESULTS

Temporal variation in ecological efficiency. The ecological efficiency index is a comprehensive index considering the environment and resources. It can be considered to decompose ecological efficiency into environmental efficiency and resource efficiency for measurement analysis. According to relevant studies [28-29], environmental efficiency can be expressed by the ratio of economic output indicators to environmental emission indicators, and resource efficiency can be expressed by the ratio of economic input and output index. In this paper, DEA-solver PRO software and input-oriented super-efficiency DEA models were used to study the ecological efficiency level of Xinjiang from 2001 to 2015, and the ecological efficiency, resource efficiency, and environmental efficiency were calculated from 2001 to 2015. The results are shown in Figures 1 and 2.

Between 2001 and 2015, the overall ecological efficiency of Xinjiang trended upwards, with an average value of 1.0388. Ecological efficiency values exceeded the efficient production frontier in 10 out of the 15 years considered. Ecological efficiency briefly dropped in 2009 but peaked in 2010. Ecological efficiency values varied from 0.9881 in the 10th “five-year period” to 1.0643 in the 12th “five-year period”, exhibiting an overall increasing trend. From the beginning of the 11th “five-year period” in 2006 to the end of the 12th “five-year period” in 2015, the ecological efficiency remained higher than 1.0 and maintained at the effective production frontier except for the slight decline in 2009. These results suggest that Xinjiang gradually realized the coordinated development of economic growth, resource conservation and environmental protection through a series of policies and measures within the three five-year periods beginning in 2001.

To further investigate variation in ecological efficiency, the concept was decomposed into resource efficiency and environmental efficiency. The variation in resource efficiency between 2001 and 2015 was consistent with variation in ecological efficiency. Beginning in the 10th “five-year period” through the 12th “five-year period”, resource efficiency raised consistently with an average value of 1.0136, and its value was maintained at the effective production efficiency.
frontier. Environmental efficiency values show a fluctuating upward trend with time, and the increased range is large. Overall, the values increased by 2.35-fold from 2001 at the beginning of the 10th “five-year period” to 2015 at the end of the 12th “five-year period”, with an average rate of increase of 135.7%. These results indicate that Xinjiang achieved remarkable environmental governance between the 10th and 12th “five-year plan” through the continuous prevention and control of environmental pollution. Moreover, environmental efficiency greatly improved over this period and constituted a leap from low to high environmental efficiency.

**Spatial distribution of ecological efficiency in Xinjiang.** The ecological efficiency and its two components (resource efficiency and environmental efficiency) were estimated for 14 cities of Xinjiang using the input-oriented super-efficient DEA model, as implemented in the DEA-solver PRO software program.

As indicated in Table 2, there was an imbalance of ecological efficiency among regions. The mean ecological efficiency value for 14 prefectures was 1.02, where Karamay had the highest ecological efficiency (4.1888) that was 8.71-fold higher than the lowest ecological efficiency of Hami City (0.4807). However, the overall ecological efficiency values were low for the 14 prefectures. Only Karamay and Turpan City exhibited ecological efficiency values greater than 1.0, indicating that they had reached the efficient production frontier. The DMU’s ecological efficiency value intensity was subdivided into three categories [30] following Norman and Barry’s research and model output results. One of the components represents the area achieving the effective production frontier where the ecological efficiency was greater than 1.0. This included Karamay and Turpan City which accounted for 14.3% of the total. The second component included marginally non-efficient areas where the ecological efficiency ranged from 0.9 to 1, which included Urumqi and Bazhou. The last component included the inefficient areas where the ecological efficiency values were less than 0.9. Ten out of the 14 prefectures (71.4%) were separated into the inefficient area category and included Tacheng Prefecture, Bozhou, Changji Prefecture and Aksu Prefecture.
The ecological efficiency was decomposed into the resource efficiency and environmental efficiency, and regions were then classified based on these values as either greater than the average for high efficiency or below average for low efficiency. Following this framework, regional development could be divided into four types for the 14 cities, following the classification system proposed by Zhu and Liu [31]. The first development type was ‘low energy consumption and low emission’. An example of this type of region was Karamay, where the resource and environmental efficiencies were the highest, and energy saving and emission reductions were very efficient. The second development type was ‘high energy consumption and low emission’. This type of region was exemplified by Turpan where resource efficiency was low, but environmental efficiency was high. Though these areas achieved remarkable reductions in emissions, energy conservation still requires improvement. The third development type exhibited low energy consumption and high emissions and included areas like Urumqi, Tacheng Prefecture, Bazhou, and Aksu Prefecture, where resource efficiency was high, but environmental efficiency was low. These prefectures achieved remarkable success in energy conservation, but emission reduction should still be improved. The fourth development mode for prefectures was exemplified by high energy consumption and high emissions. A large portion of the cities (57% of the total prefectures) were classified as this type and included Bozhou, Kizl, Hami City, the Changji Prefecture, the Yili Prefecture, the Aletai Prefecture, the Kashi Prefecture, and the Hotan Prefecture. These areas were characterized by low resource and environmental efficiencies. Energy conservation and emission reductions in these regions require urgent improvement in the future.

Overall, the ecological efficiencies were unbalanced among regions in Xinjiang, which is primarily due to the developmental stage of Xinjiang. Many of these prefectures are still developing. GDP growth was primarily attributable to the development of high-input and high-pollution industries. Notably, only a few prefectures exhibited high ecological efficiency and achieved sound and sustainable development including, for example, the typical oil industry city of Karamay. After greater than 60 years of development, Karamay has entered a mature stage of industrial development and has incorporated sustainable development, as exemplified by low investment and low emissions. In contrast, Turpan suffers from severe drought and water shortages. Due to the remarkable implementation of water-saving measures, sewage discharge in Turpan was the lowest among all other regions of Xinjiang, comprising a total of 0.38 tons/10,000 yuan of industrial GDP. Consequently, the ecological efficiency of Turpan remained at the effective production frontier. In Urumqi and Bazhou, where industry dominated their economies, their eco-efficiency values were

### TABLE 2
Eco-efficiency and its components within Xinjiang cities between 2001 and 2015.

<table>
<thead>
<tr>
<th>Region/district</th>
<th>Ecological efficiency</th>
<th>Resource efficiency</th>
<th>Environmental efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urumqi</td>
<td>0.9628</td>
<td>0.9628</td>
<td>0.6326</td>
</tr>
<tr>
<td>Karamay</td>
<td>4.1888</td>
<td>2.3401</td>
<td>3.8802</td>
</tr>
<tr>
<td>Turpan City</td>
<td>1.5927</td>
<td>0.6147</td>
<td>1.5927</td>
</tr>
<tr>
<td>Hami City</td>
<td>0.4807</td>
<td>0.3806</td>
<td>0.4662</td>
</tr>
<tr>
<td>Changji Prefecture*</td>
<td>0.7351</td>
<td>0.5316</td>
<td>0.7054</td>
</tr>
<tr>
<td>Yili Prefecture *</td>
<td>0.5482</td>
<td>0.5482</td>
<td>0.4225</td>
</tr>
<tr>
<td>Tacheng Prefecture</td>
<td>0.8841</td>
<td>0.8039</td>
<td>0.8841</td>
</tr>
<tr>
<td>Altay Prefecture</td>
<td>0.6893</td>
<td>0.5023</td>
<td>0.6893</td>
</tr>
<tr>
<td>Bozhou *</td>
<td>0.8627</td>
<td>0.6215</td>
<td>0.8627</td>
</tr>
<tr>
<td>Aksu Prefecture</td>
<td>0.9085</td>
<td>0.7521</td>
<td>0.9085</td>
</tr>
<tr>
<td>Kizl *</td>
<td>0.7600</td>
<td>0.7305</td>
<td>0.7600</td>
</tr>
<tr>
<td>Kashi Prefecture</td>
<td>0.5965</td>
<td>0.4801</td>
<td>0.5965</td>
</tr>
<tr>
<td>Hotan Prefecture</td>
<td>0.5568</td>
<td>0.4641</td>
<td>0.5568</td>
</tr>
<tr>
<td>Mean</td>
<td>1.0202</td>
<td>0.7266</td>
<td>0.9624</td>
</tr>
</tbody>
</table>


### TABLE 3
Developmental characteristics for 14 Xinjiang cities between 2001 and 2015.

<table>
<thead>
<tr>
<th>Group</th>
<th>Characteristic</th>
<th>Mode</th>
<th>District</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group one</td>
<td>High resource efficiency</td>
<td>Low energy consumption and low emission mode</td>
<td>Karamay</td>
</tr>
<tr>
<td>Group two</td>
<td>Low resource efficiency</td>
<td>High energy consumption and low emission mode</td>
<td>Turpan City</td>
</tr>
<tr>
<td>Group three</td>
<td>High resource efficiency</td>
<td>Low energy consumption and high emission mode</td>
<td>Urumqi, Tacheng Prefecture, Bazhou, Aksu Prefecture</td>
</tr>
<tr>
<td>Group four</td>
<td>Low resource efficiency</td>
<td>High energy consumption and high emission model</td>
<td>Hami City, Changji Prefecture, Yili Prefecture, Altay Prefecture, Bozhou, Kizl, Kashi Prefecture, Hotan Prefecture</td>
</tr>
</tbody>
</table>
close to 1.00. Accordingly, the GDP of Urumqi and Bazhou ranked first and third, respectively, among prefectures of Xinjiang in 2015. The energy consumption was low, but the pollutant emission was high, which affects the ecological efficiency value. However, most prefectures exhibited high energy consumption and high pollution. Thus, considerable scientific and technological research attention, in addition to investment in environmental protection, should be given towards Xinjiang to achieve increased energy conservation and emission reductions, and thereby narrowing the regional gap in ecological efficiencies and achieve overall sustainable development.

**Dynamic Analysis of Ecological Efficiency.**

To better clarify the ecological efficiency trend in Xinjiang, the Malmquist index model was used to calculate the ecological efficiency variations in 14 prefectures from 2001 to 2015. Firstly, the Malmquist index and its decompositions of the average ecological efficiency of different cities were analyzed, including the comprehensive technical efficiency (EC), technical progress (TC), pure technical efficiency (PE), scale efficiency (SE) and total factor productivity (TFP). The following chart is represented by English abbreviation, and the results are shown in Table 4.

From 2001 to 2015, the average TFP of various prefectures and cities was 0.940, showing an average downward trend with an average decline range of 6.0%. TFP of all prefectures and cities was less than 1, showing a downward trend. Among them, Kizl had the largest decline range of 18%, contributing most to the TFP index decline of the whole region.

According to the analysis of the changes of TFP decomposition indexes, the average TC index was 0.925, showing a downward trend on average, with an average annual decline rate of 7.5%. The TC index of various prefectures and cities was less than 1, showing a downward trend, especially in Kizl, with the largest decline rate of 18%. And average EC was 1.015, with an annual growth rate of 1.5%, besides the EC was slightly decreased in Urumqi (decreased 0.7%), throughout the rest of the city were on the rise, further EC was decomposed into pure technical efficiency (PE) and scale efficiency (SE), the former increased by 1.5%, the latter held steady at 1.0. It can be seen that only the technical progress index (TC) showed a downward trend and kept pace with the TFP index, which was the main influence and constraint factor of TFP, while the technical efficiency index (EC) played a promoting role.

**FACTORS INFLUENCING REGIONAL ECOLOGICAL EFFICIENCY**

There are few current theoretical and empirical studies regarding the factors that affect ecological efficiency. Here, six indices were selected for empirical analysis based on previous studies [32-33] and case studies in Xinjiang, including (1) economic development level that was measured by per capita GDP; (2) industrial structure that was estimated by the proportion of tertiary industry; (3) utilization of foreign capital that was calculated by the proportion of foreign investment; (4) urbanization rate that was inferred by the ratio of urban population to total population; (5) average education level that was computed by the number of primary, secondary, and university students in the region multiplied by their time of study; and (6) degree of marketization that was measured by the ratio of non-public officials to employees. The above metrics were considered as both independent and dependent variables. Tobit regression analysis was then conducted with the ecological efficiency values and the six indicators in the Stata software program.

<table>
<thead>
<tr>
<th>District</th>
<th>EC</th>
<th>TC</th>
<th>PE</th>
<th>SE</th>
<th>TFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urumqi</td>
<td>0.993</td>
<td>0.952</td>
<td>0.993</td>
<td>1.000</td>
<td>0.946</td>
</tr>
<tr>
<td>Karamay</td>
<td>1.000</td>
<td>0.945</td>
<td>1.000</td>
<td>1.000</td>
<td>0.945</td>
</tr>
<tr>
<td>Turpan city</td>
<td>1.035</td>
<td>0.927</td>
<td>1.035</td>
<td>1.000</td>
<td>0.959</td>
</tr>
<tr>
<td>Hami City</td>
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<td>1.015</td>
<td>1.000</td>
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In particular, ecological efficiency was maintained at between the 10th and 12th “five-year plan” periods. Ecological efficiency rose steadily increased between 2001 and 2015, except for a brief development of Xinjiang should be encouraged.

Investment that is beneficial to the sustainable development of non-public enterprises should be sacrificed for the sake of GDP. Fourth, foreign investment that is beneficial to the sustainable development of non-public enterprises should be stimulated, and environmental benefits should not be sacrificed.

This is a process that is in line with the urbanization rate and education level generally played a considerable role in promoting ecological efficiency for a prefecture. However, marketization appeared to significantly inhibit ecological efficiency. Based on the above results and considering the practical realities of Xinjiang, the following methods are recommended to improve ecological efficiency in Xinjiang and ensure the coordinated sustainable development of environmental protection was gradually achieved in Xinjiang after 2001 through a series of policies and measures implemented in the three “five-year plans”.

Ecological efficiency varied among regions of Xinjiang over this period, with the highest value being 8.71-fold higher than the lowest value. Regional ecological efficiency is generally low, and only two regions reach the effective frontier. The overall ecological efficiency is in an ineffective state.

The development of various regions within Xinjiang could be divided into four types: 1) low energy consumption and low emissions, 2) high energy consumption and low emissions, 3) low energy consumption and high emissions, and 4) high energy consumption and high emissions.

Through the Malmquist index decomposition, it was found that technical progress (TC) restricted TFP, while technical efficiency index (EC) promoted TFP.

Foreign capital utilization, urbanization rate and average education level all were significantly and positively correlated with ecological efficiency. Additionally, the marketization degree was significantly and negatively correlated to ecological efficiency.

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<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Coefficient</th>
<th>Standard deviation</th>
<th>Z statistic</th>
<th>P value</th>
<th>Significance</th>
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<td>-29.058</td>
<td>2e-16</td>
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<td>-0.106</td>
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<tr>
<td>The proportion of tertiary industry</td>
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<td>0.101</td>
<td>1.422</td>
<td>0.155</td>
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<td>The proportion of foreign investment</td>
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<td>4.476</td>
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<td>The proportion of urban population</td>
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<td>0.194</td>
<td>-6.082</td>
<td>1.19e-09</td>
<td>***</td>
</tr>
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</table>

***, * represent statistical significance at the 0.001, and 0.05 levels, respectively.

**CONCLUSIONS AND SUGGESTIONS**

The ecological efficiency of Xinjiang generally increased between 2001 and 2015, except for a brief decline in 2009. Ecological efficiency rose steadily between the 10th and 12th “five-year plan” periods. In particular, ecological efficiency was maintained at the efficient production frontier level in 2006 at the beginning of the 11th “five-year period”. These results indicate that the coordinated development of economic growth, resource conservation, and environmental protection was gradually achieved in Xinjiang after 2001 through a series of policies and measures implemented in the three “five-year plans”.

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**REFERENCES**


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SURROGATES FOR PREDICTING TASTE AND ODOR COMPOUNDS IN EUTROPHICATION SHALLOW LAKE

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ABSTRACT

Black bloom has an offensive odor (caused by, e.g., dimethyl sulfide, DMS; dimethyl disulfide, DMDS; dimethyl trisulfide, DMTS and so on) and disastrous consequences for natural limnic ecosystems worldwide. However, research on black bloom and its taste and odor (T&O) compounds has been limited by the difficulty of predicting the time and location of black bloom events. Therefore, the determination of T&O compounds in each stage of black bloom are critical.

In this study, potential surrogates were assessed using laboratory column experiments. Dissolved oxygen (DO), pH, electric potential (Eh), total nitrogen (TN), ammonium nitrogen (NH4+-N), total phosphorus (TP), soluble reactive phosphorus (SRP), total organic carbon (TOC) and so on are selected as initial surrogates. We also tested the validity of these models using an independent data set that was previously collected from Taihu Lake in 2015.

Different surrogates contributed different significant to the T&O predictive models. For instance, the concentrations of DMTS were related to cyanobacteria biomass, temperature and other environmental factors. In comparing the concentrations of the T&O compounds observed in 2015 with those concentrations predicted from our models, we found that most of the predicted data points fell within the 90% confidence intervals of the observed values.

This result supported the validity of these models in the studied system. These models, basing on laboratory collected environmental data, will be sometime extent for water resource management of eutrophic shallow lake in evaluating the probability of T&O incidence.

KEYWORDS:
Taste and odor compounds, surrogates, predictive models

GRAPHICAL ABSTRACT

The production of T&O compounds during the decomposition of cyanobacteria
INTRODUCTION

Cyanobacteria blooms, widely reported in summer or even throughout the year, tend to assemble in high density along the shoreline of eutrophic lakes under meteorological and hydraulic conditions [1-4]. For instance, the world-shaking water pollution issue known as black blooms occur in Lake Taihu and is purported to be a result of overcrowded cyanobacteria [5, 6]. Particularly, in the summer of 2007, an odorous tap water crisis that affected two million residents for several days occurred in Wuxi, China, due to the occurrence of black blooms [6, 7]. It has been suggested that β-cyclocitrinal, β-ionone and volatile organic sulfur compounds (VOSCs), such as DMS, DMDS and DMTS, are the major compounds responsible for the strong offensive odor of black blooms [8-10]. Thus, considerable economic and social benefit would accrue if the development of unwanted T&O contamination could be predicted before full outbreaks occur.

Fortunately, a few studies suggest that it is feasible to predict the occurrence of off-flavors using certain closely related environmental factors that can be measured by simple and low-cost methods [11-13]. Clearly, it is of practical value to use models with easily detectable parameters to predict the occurrence of T&O events a few days before they occur, which is undoubtedly important for the implementation of appropriate and timely measures by departments responsible for the management of water quality. In order to apply the models in eutrophication shallow Lakes, validation is important. Surrogate identification starts in the pre-validation step when potential surrogates are proposed, while in the validation monitoring step these proposed surrogates are tested and verified. The verified surrogates are then employed for monitoring the real time performance of the treatment systems in the operational monitoring step. The production and occurrence of objectionable T&O compounds are known to be correlated with various biological and environmental factors, such as phytoplankton, nutrient concentrations and their ratios [11, 14], water temperature, pH and dissolved oxygen [15-17]. Nevertheless, only a few studies have reported models constructed to identify the key surrogates determining the T&O events. Identification of surrogates is especially important for T&O compounds since they can be difficult to quantify at low concentrations. And suitable surrogate needs to be easily and cheaply detected in the system. Most importantly, the surrogates have highly correlated to the change in T&O concentration [18-20].

The use of surrogates to monitor pollutant compounds has been previously reported by researchers working on different wastewater systems. Hence, we attempted to identify the key environmental surrogates responsible for the development of the T&O compounds and to optimize the predictive models so that water resource managers can take appropriate and timely measures to prevent unwanted T&O outbreaks. In this study, a microcosm system was conducted to investigate the response of T&O compounds production to environmental surrogates, e.g., cyanobacteria biomass, temperature, nutrients and so on. The dynamics of the T&O compounds, diverse physicochemical parameters were monitored during the experiment process. The purpose of the present research was to utilize the data gathered in laboratory test to construct a series of models to predict the quantities of the T&O compounds likely to develop in the lake.

MATERIALS AND METHODS

Laboratory column test. Lake Taihu is a typical shallow eutrophic lake in China. Surface sediments were collected from Zhushan Bay by a Petersen Grab sampler. Upon return to the laboratory, fresh sediment samples were sieved (1 mm mesh) to remove plant residues and shell fragments. The overlying water was collected from the same site. Cyanobacteria materials were also harvested from a surface water bloom dominated by Microcystis spp., using a plankton net (64μm-mesh size).

Experiment materials were fresh sediments, filtered lake water and cyanobacteria. The microcosm system was performed in 60 L glass beakers of 20 cm in diameter and 200 cm in height, and the beakers were side-wrapped in black plastic to avoid light. At first, 8kg of fresh sediment were put into the bottom of the vessel, then 30L of filtered lake water was added into each beaker slowly, ultimately cyanobacteria samples were added. Three cyanobacteria biomass of 7500 g/cm²(L), 12500 g/cm²(M) and 25000 g/cm²(H) were set to represent the extent of eutrophication in the field. There were three treatments: (a) sediment + 7500 g/cm² cyanobacteria + water, (b) sediment + 12500 g/cm² cyanobacteria + water, (c) sediment + 25000 g/cm² cyanobacteria + water [21-23]. Samples were obtained according to the experimental progress.

Field challenge test. The study was conducted in Taihu Lake, a freshwater shallow lake with an area of 2338 km² in China. At our sampling sites, the average depth was 1.9 m, and the maximum depth was 2.6 m. The lake supplies the water needs of the region, supporting approximately 10 million residents [24]. In recent decades, Taihu Lake has experienced serious pollution and anthropogenic eutrophication [25], severely influencing the normal life of citizens. For example, a 2007 incident in Wuxi City involving malodorous drinking water seriously influenced the water usage of approximately two million citizens for nearly one week [26]. The lake survey was conducted in July 2015.
Water samples were collected from 12 sampling sites, each water sample from each site was a mixture of two sub-samples: one from 0.5 m below the surface and one from 0.5 m above the bottom.

**Analytical methods and data analyses.** The samples for odorant determination were stored in the dark at 4°C and analyzed within 7 days. The samples for nutrients and organic carbon determination were stored in the dark at 0–4°C and −20°C, respectively.

Eight possible surrogates were initially selected based on freshwater lake quality. These surrogates were then tested in field challenge test and laboratory test. Based on the literature, during the decay of cyanobacteria, the odor smell was emitted with extremely low dissolved oxygen (DO) and electric potential (Eh), which not only endanger the safety of the drinking water supply but is also harmful to lake ecosystems. This phenomenon causes the release of microcystins, sulfide, methane, odoriferous substances, and nutrients. Then surrogates were selected for initial testing: dissolved oxygen (DO), pH, electric potential (Eh), total nitrogen (TN), ammonium nitrogen (NH₄⁺-N), total phosphorus (TP), soluable reactive phosphorus (SRP) and total organic carbon (TOC). All samples collected were stored on ice or in the fridge and then delivered to accredited laboratory for analysis. Standard methods were employed to quantify surrogate parameters, DO, pH, Ec, TN, NH₄⁺-N, TP, SRP, and TOC [27].

The DMS, DMDS, DMTS, β-Cyclocitril and β-ionone levels were determined by HS-SPME-GC/MS according to previous studies [15, 21-22]. The sample was stirred at 500 r/min with a magnetic hot stirrer at 65°C. The SPME fiber was heated at 270°C for 30 min in the injection port of the chromatographic system. The outer needle of the SPME fiber was passed through the septum, and the fiber extended into the headspace for 30 min. After extraction, the fiber was retracted into the needle, then the needle was removed from the septum and inserted directly into the GC-injection port of the GC–MS instrument. The fiber was held in the GC-injection port for 5 min, and the fiber was then retracted into the needle. The needle was removed from the GC–injection port and used for the next sample.

Odorous compound analysis was performed using GC-MS (Agilent 7890AGC, 5975MSD, USA) with a J&W Scientific column DB-5 MS (30 m × 0.25 mm ID × 0.25 μm film, Agilent, USA). The GC was operated under the following conditions: injection temperature, 250°C; carrier gas flow rate, 1.43 ml/min (helium); column temperature program, 60°C (1 min). It was heated from 60°C to 220°C at a rate of 10°C/min in the splitless mode. The EI-MS conditions were as follows: ion-source temperature, 230°C; ionizing voltage, 70 eV; scan range, m/z 40–350 amu; cycle time, 0.5 s. For the selection of monitoring (SIM) mode, m/z 62 and 47 for DMS, m/z 94 and 79 for DMDS, m/z 126 and 111 for DMTS, m/z 152 and 137 for β-cyclocitril, m/z 177 and 135 for β-ionone were monitored.

**Model Development.** Based on our previous studies [27-30], DMS, DMDS, DMTS, β-cyclocitril and β-ionone were monitored. Variance analysis was conducted for the main T&O compounds (DMS, DMDS, DMTS, β-cyclocitril and β-ionone). The analyses were conducted using SAS/INSIGHT of SAS statistical software version 9.1 on the environmental parameters collected during the Laboratory column test. Each model used the concentration of T&O compound as the dependent variable (Y) and the other environmental parameters as the independent variables (X).

Then, the parameters with the smallest F statistical value and an insignificant p-value were deleted using the backward elimination procedure. The quality and fitness of the models for predicting the predominant T&O compounds were further evaluated using a residual scatter plot for each model. If the points were randomly dispersed around the zero line, a linear regression model was considered appropriate for the data; otherwise, a non-linear model was considered more appropriate.

Finally, it is necessary to test the applicability of the predictive models developed. We calculated the T&O values according to the models using the surrogate data and compared these predicted T&O values with the T&O values collected from Taihu Lake in 2015.

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**RESULTS AND DISCUSSION**

**Assessment of suitability of the tested surrogates.** Taste and odor (T&O) problems, which have adversely affected the quality of water supplied to millions of residents, have repeatedly occurred in Taihu Lake (e.g., a serious odor accident occurred in 2007). Because these accidents are difficult for water resource managers to forecast in a timely manner, there is an urgent need to develop optimum models to predict these T&O problems. We attempted to identify the key environmental factors responsible for the development of the T&O compounds and to optimize the predictive models so that water resource managers can take appropriate and timely measures to prevent unwanted T&O outbreaks. Models can be seen in Table 1.

The DMS level was positively related to the levels of the TN and TP, and negatively related SRP, DO, pH and Ec. The TP was strongly associated with the DMS with the greatest relative weight (0.2691), followed by the pH, Ec, SRP, TN and DO (0.1853, 0.1590, 0.1586, 0.1295 and 0.0986, respectively). The environmental factors in the mod-
explained 81.11% of the variations in the DMS levels during the experiment.

The DMDS level was positively related to the levels of the TP, and negatively related TN, pH and T. The TP was strongly associated with the DMDS with the greatest relative weight (0.3582), followed by the TN, T and pH (0.2301, 0.2109 and 0.2008, respectively). The environmental factors in the models explained 78.30% of the variations in the DMDS levels during the experiment.

The DMTS level was positively related to the levels of the TN, cyanobacteria density and T, and negatively related pH and Ec. The Ec was strongly associated with the DMTS with the greatest relative weight (0.2784), followed by the TN, T, D and pH (0.2385, 0.1742, 0.1709 and 0.1380, respectively). The environmental factors in the models explained 69.60% of the variations in the DMTS levels during the experiment.

The β-cyclocitrinal level was positively related to the levels of the TN, cyanobacteria density and T, and negatively related Ec. The Ec was strongly associated with the β-cyclocitrinal with the greatest relative weight (0.3789), followed by the D, TN and T (0.2813, 0.1729 and 0.1668, respectively). The environmental factors in the models explained 71.70% of the variations in the β-cyclocitrinal levels during the experiment.

The β-ionone level was positively related to the levels of the TP and T, and negatively related SRP and pH. The TP was strongly associated with the β-ionone with the greatest relative weight (0.4556), followed by the SRP, T and pH (0.2975, 0.1729245 and 0.1225, respectively). The environmental factors in the models explained 83.10% of the variations in the β-ionone levels during the experiment.

The correlation between the nutrients and odorous compounds were developed by linear correlation analysis. In our manuscript, we constructed models for the dominant T&O compounds using multiple linear regression analyses. For example, there were strong positive correlations between DMS, DMDS, DMTS, β-cyclocitrinal and TN. Also, there were strong positive correlations between DMS, DMDS, β-ionone and TP. In Taihu Lake, the growth of cyanobacteria was N- and P-limited during the growing season, and TN and TP are known to favor cyanobacteria blooms. Thus, the nutrients can indirectly regulate the production of taste and odor compounds. It has also been found that nitrogen can directly or indirectly affect the concentrations of taste and odor compounds. The present results indicate that the production of odorous compounds were closely associated with nitrogen and phosphorus. However, there were strong negative correlations between DMS, DMDS, DMTS and pH. As we know, pH decreased after the decomposition of cyanobacteria, with the minimum values concurrently observed followed by gradual increases.

Similar to the results of many previous studies, the current results showed that abiotic factors are important for predicting the occurrence of the T&O compounds in Taihu Lake. Notably, most of the models developed in the present study included nitrogen (e.g., Eqs. (1), (2), (3) and (4)) and phosphorus (e.g., Eqs. (1), (2) and (5)). A previous investigation closely associated the production of the T&O compounds in Taihu Lake with the nitrogen levels [31]. The nitrogen could affect the concentrations of the T&O compounds directly or indirectly [15, 32]. Additionally, the models developed in our study included temperature (e.g., Eqs. (2), (3), (4) and (5)) and phosphorus (e.g., Eqs. (1), (2) and (5)) closely associated the production of the T&O compounds. Black water blooms frequently occur in the higher temperatures of late spring or early summer [23]. This is probably due to the fact that temperature can stimulate the activity of bacteria and some biochemical processes [31]. The more increase in incubation temperature, the higher the concentration of odor compounds will produce. Regardless of the mechanisms involved, it should be stressed that the levels of environment temperature were closely related to the levels of the T&O compounds in Taihu Lake.

The impact of different parameters on the performance of predictive models. Diagnosis and test for the models. In the scatter plots of the residuals (Fig.1), a random dispersal of the data points

<table>
<thead>
<tr>
<th>Index</th>
<th>Models</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS</td>
<td>DMS=665.274+0.827TN+8.911TP-6.802SRP-12.298DO-89.284pH-0.029Ec   (1)</td>
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<tr>
<td>DMDS</td>
<td>DMDS=13.105-0.035 TN +0.284TP-2.321pH-0.124T                 (2)</td>
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<td>DMTS</td>
<td>DMTS=21.500+0.094TN-4.112pH-0.003Ec+7.044×10^3D + 0.264 T     (3)</td>
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<td>β-cyclocitrinal</td>
<td>β-cyclocitrinal=−1490.520+14.987TN-0.932Ec+0.025D+55.448T   (4)</td>
<td>0.7170</td>
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<tr>
<td>β-ionone</td>
<td>β-ionone=49.938 +2.325 TP -1.962SRP-9.110pH+0.470T   (5)</td>
<td>0.8310</td>
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</table>

* DMS (ng/L), DMDS (ng/L), DMTS (ng/L), β-cyclocitrinal (ng/L), β-ionone (ng/L), TN (mg/L), TP (mg/L), SRP (mg/L), D refers to cyanobacteria density, T refers to temperature.
around the zero line would indicate that the developed models can be considered appropriate. Fortunately, the data points in most of the plots in Fig. 1 were randomly distributed. Although some of the plots displayed scatter points slightly away from the zero line, they did not show a clear tendency to be curved (e.g., U-shaped and inverted U). Therefore, the scatter plots provided little evidence to question the appropriateness of the linear regression models, and the models for the T&O compounds were to be considered valid.

In the present study, the T&O compounds in the water column were closely related to both the algal biomass and environmental factors. Temperature has been proven to be a main factor affecting the production of T&O compounds in wetland soil and wastewater treatment plants [33, 34]. This is probably due to the fact that temperature can stimulate the activity of bacteria and some biochemical processes [33]. Our results indicated that temperature also affected the production of T&O compounds. Black blooms frequently occur at the high temperature of late spring or early summer [23]. Previous studies have reported cyanobacteria contain about 1% sulfur-containing amino acid [35]. The high temperature can accelerate the decomposition of sulfur-containing amino acids such as methionine in the agglomerative dead algae, thereby causing the release of the T&O compounds in black blooms. Our results agreed with a study carried out by Du et al. (2012), which suggested that high temperature caused a rapid degradation of methionine and an increase of T&O compounds under anaerobic conditions [33].

Generally, highly significant positive correlations were observed between released T&O compounds and nutrients. The concentrations of TP and showed significant correlations with DMS, DMDS and \( \beta \)-ionone. Further analysis showed that the correlation between TP and T&O compounds was higher than the correlation between TN and T&O compounds, which indicated that the decomposition

![FIGURE 1](image-url)

Residual scatter plots for T&O compound models
of algae had more effects on phosphorus in water than on nitrogen. Yang et al. (2008) suggested that the taste and odor incident caused by the black water “agglomerate” in Lake Taihu was associated with the unusually high concentrations of ammonia levels [26]. The great heterogeneity is a characteristic of huge lake. Regardless of the mechanisms involved, it should be stressed that the levels of the various forms of nutrients were closely related to the levels of the T&O compounds in Taihu Lake.

Application of surrogates for monitoring. The test results with the independent data set collected from Taihu Lake in 2015 showed that most of the values predicted with the models fell within the 90% confidence intervals of the observed values (Table 2). Table 2 presents the concentration of four T&O compounds, the observed value and predictive results had good accordence. The significance (p value) between observed value and predictive value for DMS, DMDS, DMTS, β-cyclocitrinal and β-ionone are as follows: 0.965, 0.971, 0.952, 0.852, 0.863. The timing and intensity of these odors were well predicted with a few deviations. Therefore, models developed here could predict well the occurrences and levels of the corresponding odors in Taihu Lake.

The use of the independent data set to test the accuracy of the models showed that the occurrence and intensity of these T&O compounds were satisfactorily predicted, though there were several deviations. In a body of water as large and spatially heterogeneous as Taihu Lake, the environmental and climatic conditions may substantially vary both spatially and temporally. In addition, it is known that the volatility of the compounds producing the off-flavors causes their concentrations to vary easily in aquatic environments. The above phenomena could certainly result in discrepancies between the predicted and observed values. Nevertheless, the models developed in the present study accurately predicted the levels of the T&O compounds in Taihu, which supplies water for drinking, industry and agriculture to millions of people. Therefore, these models, basing on easily collected environmental data, are of practical value to water resource managers for evaluating the probability of T&O incidents in Taihu Lake.

Previous researchers reported that the dynamics of odor compounds strongly depend on the local environmental conditions and vary from system to system. Even for the same T&O compound, different models were generated in different reservoirs of the same region; thus, it is difficult to obtain a universal model applicable to all ecosystems. It was not possible to test our models in all systems due to lack of enough data, as we mentioned above. Nevertheless, models developed here have good utilities in such a huge lake based on the quite informative data in different algal growth seasons for different fractions of the T&O compounds. We believe that

### Table 2

<table>
<thead>
<tr>
<th>Observed value</th>
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<th>Observed value</th>
<th>Predictive value</th>
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* the odor threshold values (OTTV) of DMS (ng/L), DMDS (ng/L), DMTS (ng/L), β-cyclocitrinal (ng/L), β-ionone (ng/L) are as follows: 300, 5, 10, 500, 7.
the models in the present study should provide insights into developing a general model applicable to a variety of lake systems, especially other shallow eutrophic lakes with Microcystis blooms.

ACKNOWLEDGEMENTS

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SYNTAXONOMIC CHECKLIST OF THE VEGETATION OF TURKEY; THE HIGH-RANK SYNTAXA (ANATOLIAVEGCHECKLIST-AVC)

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ABSTRACT

A revision was undertaken of the high-rank syntaxa of the vegetation of Turkey. All published synecological and phytosociological relevés available were collected in order to create a comprehensive list of previously proposed syntaxonomic units. These units were evaluated for their floristic and ecological distinctness, clarity of geographic distribution, and compliance with the nomenclature code from the viewpoint of the Braun-Blanquet syntaxonomy for vascular plants. Accepted units were compiled into these systems of classes, orders, and alliances (AnatoliaVegChecklist-AVC) for communities dominated by vascular plants. Arboreal, riparian woodland, steppic, halophytic and brackish, sand dune, chasmophytic, ruderal, and aquatic vegetation types include 30 classes, 1 subclass, 49 orders, 5 suborders, 107 alliances, and 9 suballiances.

KEYWORDS: AnatoliaVegChecklist, phytosociology, syntaxonomic checklist, Turkey

INTRODUCTION

Turkey is situated at a confluence of three continent and phytogeographical regions which are Euro-Siberian, Mediterranean and Irano-Turanian. As a result, the climatological, topographical, geographical, and geomorphological diversity leads to high biodiversity and endemism in plant life.

In the present study, the aim was to review all of the syntaxonomical units of the vegetation of Turkey. During the preparation of the publication ‘Vegetation of Turkey’ (hereafter referred to as “AnatoliaVegChecklist”), hundreds of published syntaxonomical concepts of Anatolian phytosociological classes, orders, and alliances were assessed. The syntaxonomy of the vegetation of Turkey has been studied since the 1960s by both native and foreign scientists (e.g., Pierre Quétel, Marcel Barbéro, Yıldırım Akman, Osman Ketenci, Meclit Vural, Ender Yurdakul, Latif Kurt, Ergin Hamzaoglu, Mahmut Kiline, Hamdi Güray Kutbay, etc.). Many syntaxa, including some that create phytosociological problems, were therefore described within the vegetation of Turkey. The aim of this review was to provide a broad overview of all of the high syntaxonomical units of the vegetation of Turkey.

The main purpose of this paper is to perform nomenclatural changes and discuss nomenclatural issues in arboreal, riparian woodland, steppic, halophytic and brackish, sand dune, chasmophytic, ruderal, and aquatic vegetation types and to revise the system of high-rank syntaxa of the vegetation of Turkey.

MATERIALS AND METHODS

In order to facilitate the consultation of the AnatoliaVegChecklist-AVC, syntaxonomic units were grouped into syntaxonomic criteria [1, 2, 3]. To achieve this syntaxonomic synthesis, we examined a vast number of vegetation studies. These units were evaluated by experts for their floristic and ecological distinctness, clarity of geographic distribution, and compliance with the rules of the International Code of Phytosociological Nomenclature [4]. Accepted units were compiled into these systems of classes, orders, and alliances (AnatoliaVegChecklist-AVC) for communities dominated by vascular plants.

RESULTS

AnatoliaVegChecklist includes 30 classes, 1 subclass, 49 orders, 5 suborders, 107 alliances, and 9 suballiances. The rank, full valid name, any synonyms, and incorrect names are provided for each syntaxon. The short declaration highlights the physiognomy, synecology, syndynamics, and distribution of the plant communities that belong to the syntaxon.

Arboreal Vegetation. Turkey’s forests cover approximately 21 million hectares of land. In other words, one-fifth of Turkey’s surface area is covered
with forest. Half of this forest area is thorny, and the other half is unfertile (degraded) forest. Forestlands are mostly concentrated in the mountainous areas of the Mediterranean, Aegean, Marmara, and Black Sea coasts as well as the Southeastern Taurus mountain range in Turkey. Most parts of Central, Eastern, and Southeastern Anatolia do not have forests.

Plants covering the northern part of Anatolia today are a continuation of Boreal Flora, and those of the Aegean, Mediterranean, Central, and Eastern Anatolia Regions are of Tethys Flora. These floras have been subjected to changes in climatic, edaphic, and biotic factors since their beginning. In particular, palynological studies [5, 6] show that the Central Anatolia Region was greatly affected by the Würm glaciation. Following the glaciation that started 10,000 years ago, forest vegetation started to cover the region gradually, from the coasts inland. Pre-steppe vegetation comprised of pine (Pinus), fir (Abies), and cedar (Cedrus) species and a forest of deciduous oak trees and junipers (Juniperus) had become dominant.

However, this forestation in Anatolia quickly stopped spreading after the development of agriculture in the Neolithic Age and the commencement of grazing. Settled social life based on agriculture began around ten thousand years ago in some parts of Anatolia and led to human exploitation of plants and damage to forests. Consequently, the footprints of those once-thick forests of Anatolia are no longer found there today. For instance, while the main trees around Lake Van 3500 years ago were beech (Fagus), lime (Tilia), chestnut (Castanea), hornbeam (Carpinus), pine (Pinus), spruce (Picea), fir (Abies), and cedar (Cedrus), none of them are seen in that area today [7].

Since 1960 is related to Turkey's arboreal vegetation has performed numerous studies and examined all the literature in this study [8, 9, 10, 11, 12].

**Mediterranean Woodlands**

**MdW: 1. Class: QUERCETEA ILICIS** Braun-Blaq. in Braun-Blaq., Roussine & Nègre 1952  
**Syn.:** Quercetia ilicis Br.-Bl. in Br.-Bl., Emberger & Molinier 1947 (art. 8), Quercetia ilicis Br.-Bl. in Br.-Bl., Roussine & Nègre 1952 (art. 22), Euphorbieta dendroidis Zohary & Orshan 1966 (art. 8), Pistacio lentisci-Rhamnetea alaterni Julve 1993 (syntax. syn.)

Vegetation communities that include prevalently evergreen and sclerophyllous forests, maquis, and garrigues. They are found throughout the Mediterranean macrobioclimate, but are associated exclusively with the mesotemperate thermotype in the Temperate macrobioclimate. They have no particular soil requirements.

**MdW: 1.1. Order: QUERCETALIA ILICIS** Braun-Blaq. ex Molin. 1934  
**Syn.:** Quercetalia ilicis Braun-Blaq. 1931; Quercetalia ilicis Braun-Blaq. ex Molin. 1934 em. Rivas Mart. 1975.

**MdW: 1.1.1. Alliance: Quercion ilicis** Braun-Blaq. ex Molin.

**Sync.:** Quercion ilicis Braun-Blaq. 1931; Quercion ilicis Braun-Blaq. ex Molin. 1934 em. Rivas Mart. 1975.

**MdW: 1.2. Order: PISTACIO LENTISCI-RHAMNETALIA ALATERNI** Rivas Mart. 1975  

**MdW: 1.2.1. Alliance: Oleo sylvestris-Ceratonion siliguae** Braun-Blaq. ex Guin. & Drouineau 1944  


**MdW: 1.2.3. Alliance: Gonocytis pterocladii-Pinion brutai** Barbéro, Chalabi, Nahal & Quézel 1976

**MdW: 1.2.4. Alliance: Ptosimopappo bracteat-Qercion microphyllae** Barbéro, Chalabi, Nahal & Quézel 1976

**MdW: 1.2.5. Alliance: Quercion calliprini-Juniperion excelsae** Barbéro & Quézel 1979

**MdW: 1.2.6. Alliance: Andrachno-Quercion cocciferae** Barbéro & Quézel 1979

**Central Anatolian Woodlands**

**CAW: 1. Class: QUERCETEA PUBESCENTIS** Doign-Kraft ex Scamoni & H.Passarge 1959

Thermophilous woodlands with deciduous oaks of eastern sub-Mediterranean regions.

**CAW: 1.1. Order: QUERCETALIA PUBESCENTIS** Doign Kraft 1955

**CAW: 1.2. Order: QUERCO CERRIDIS-CARPINETALIA ORIENTALIS** Akman, Barbéro & Quézel 1980

**CAW: 1.2.1. Alliance: Quercion frainetto** Horvat 1954

**CAW: 1.2.2. Alliance: Carpinobetula-Acerion hyrcani** Quézel, Barbéro & Akman 1978

**CAW: 1.2.3. Alliance: Pino-Cistion aurifolii** Akman, Barbéro & Quézel 1978

**CAW: 1.2.4. Alliance: Quercion anatolicae** Akman, Barbéro & Quézel 1979

**CAW: 1.2.5. Alliance: Staphyleo pinnatae-Buxion sempervirentis** Quézel, Barbéro & Akman
1980

CAW: 1.2.6. Alliance: Cisto laurifolii-Pinion pallasianae Akman, Barbéro and Quétel 1978


CAW: 1.3.1. Alliance: Abieto ciliciaceae- Cedrion libani Quétel, Barbéro & Akman 1978

CAW: 1.3.2. Alliance: Loniceroc nunnulari- iafolioae-Cedrion libani Quétel, Barbéro & Akman 1978

CAW: 1.3.3. Alliance: Ostryo carpinifoliaeQuercion pseudocerridis Quétel, Barbéro & Akman 1978

CAW: 1.3.4. Alliance: Adenocapculo complicat i-Pinion pallasianae Quétel, Barbéro & Akman 1978

CAW: 1.3.5. Alliance: Geranio libani- Cedrion libani Quétel, Barbéro & Akman 1978

North Anatolian Woodlands


Syn: Querco-Fagetales Br.-Bl. & Vlieger in Vlieger 1937 (original name, art. 41b), Quercetalia roborisi-sessili fo rae Br.-Bl. & Tüxen 1943 (art. 8), Quercetalia roborisi-sessili fo rae Br.-Bl. & Tüxen ex Br.-Bl. 1950 (syntax. syn.), Quercetalia pubescentis Do ing 1955 (art. 8), Quercetalia roboripetraeae Br.- Bl. & Tüxen ex Oberdorfer 1957 (art. 31), Quercetalia pubescentis Do in g ex Scamoni & Passarge 1959 (syntax. syn.), Quercetalia pubescenti-petraeae Jakucs 1960 (syntax. syn.), Carpino-Fagetalia Jakucs 1967 (syntax. syn.), Fraxino-Fagetalia Moor 1975 (syntax. syn.)

Mesophilous and thermophilous forest vegetation of the Temperate macrobioclimate that grows in the mesotemperate and supratemperate thero- types, occasionally extending as far as the Mediterranean macrobioclimate. It has no specific substrate requirements.


NAW: 1.1.1. Alliance: Crataego pentagynaec-Fagion orientalis Quétel, Barbéro & Akman 1980

NAW: 1.1.2. Alliance: Castaneo sativa- Carpinion orientalis Quétel, Barbéro & Akman 1980

NAW: 1.1.3. Alliance: Alnion barbaeae Quétel, Barbéro & Akman, 1980

NAW: 1.2. Order: PINO SYLVESTRIS- PICEETALIA ORIENTALIS Quétel, Barbéro & Akman 1980

NAW: 1.2.1. Alliance: Veronicetum pederuncu laris-Fagion orientalis Quétel, Barbéro & Akman 1980

NAW: 1.2.2. Alliance: Geranio ibericiPinion sylvestris Quétel, Barbéro & Akman 1980

NAW: 1.3. Order: FAGETALIA SYLVATICAe Pawl. 1928


NAW: 1.3.1. Alliance: Fagion sylvatica Lu- quet 1926


Circumboreal acidophile resinous forests on oligotrophic soil.

NAW: 2.1. Order: VACCINIO-PICEETALIA ORIENTALIS Br.-Bl. 1939

Riparian Woodlands


Syn: Salici purpureae-Populenea nigrae Ri vas-Martinez & Cantó ex Rivas-Martinez, Bascones, T.E.Diaz, Fernández-Gonzalez & Lodi 1991 (art. 27a), Salici-Populenea nigrae Rivas-Martinez & Cantó ex Rivas-Martinez 1987 (art. 5), Populetea albae Br.-Bl. 1962 (art. 2b), Alno- Populetea Fukarek & Fabijanic 1958 (art. 2b)

Meso-hygrophilous, riparian, deciduous forests that grow along watercourses and alluvial plains in the Euro-siberian and Mediterranean regions.


RpW: 1.2. Order: PLATANETALIA ORIEN- TALIS Knapp 1959

RpW: 1.2.1. Alliance: Platanion orientalis Karpati & Karpati 1961

RpW: 1.3. Order: POPULETALIA ALBAE Braun-Blanq. ex Tchou 1949

RpW: 1.3.1. Alliance: Populion albae BraunBlanq. ex Tchou 1949


Syn: Rhamno-Prunetea Rivas Goday & Borja 1961 (art. 3b), Crataege-Prunetea Tüxen 1962 (art. 3a), Sambucetea Doing 1962 (art. 8), Urtico-

Shrubland and mantle communities that are dynamically related to the deciduous forests of the *Querco-Fagetea* class.

**Rwp:** 2.1. **Order:** PRUNETALIA SPINOSAE

**Tüxen 1952**

**Steppe Vegetation.** One of the most important vegetation types in Turkey is steppe, which spreads across Central Anatolia and extends toward east and southeast Anatolia. It also occurs at high altitudes of Black Sea and Taurus mountains.

Central Anatolian steppe has anthropogenic characteristics and is accepted as a secondary vegetation type. There are many studies indicating that steppe vegetation has anthropogenic characteristics [13, 14, 15, 16, 17]. Although Anatolian steppe shows a unique physiognomy; hemicyryptophytic grasses (*Bromus tomentellus, Festuca valesiaca, Koeleria cristata, Stipa lessingiana, S. holosericeus*) and cushion-forming chamaephytes (*Astragalus angustifolius, A. microcephalus, Onobrychis cornuta*, etc.) are dominant with respect to its peculiar ecology, however; it sometimes includes trees and shrubs.

Steppe formations in Turkey can be physiognomically placed into groups [18, 19, 20, 21]:

**Malacophyll steppes:** Steppes in which broad-leaved plants are dominant. It is thought that they can be derived from *Gramineae* steppes as a result of overgrazing in relatively moist conditions and deep soils.

**Gramineae steppes:** Gramineae species are dominant; today it is very rare.

**Tragagantchic steppes:** Steppes in which cushion-forming spiny species like *Astragalus* and *Acantholimon* are dominant.

**Salty steppes:** Steppes in which species of *Chenopodiaceae* and *Plumbaginaceae* families are dominant, particularly occupying salt pans around Salt and Sevye Lake.

Central Anatolian steppe vegetation also shows altitudinal variations. For this reason, steppe vegetation between 800–1200 m is called “plain steppe” and steppe vegetation above 1200 m is called “montane steppe”. Steppe vegetation of Anatolia is within the border of the Irano-Turanian phytogeographical region.

Since 1950, numerous studies surveying Turkey’s steppe vegetation have been carried out and are all analyzed in this study.

**StpVg:** 1. **Class:** DAPHNO OLEOIDIS-FESTUCETEA VARiae Quézel 1964

Xeric oremediterranean grasslands and cushion-tragagantchic scrub on calcareous and ultramafic substrates of the Anatolia, the Hellenic mainland, and the Aegean region.

**StpVg:** 1.0.1. **Alliance:** Hyperico-Verbascion

**Akman & Ketenoglu 1978**

**StpVg:** 1.1. **Order:** DAPHNO OLEOIDIS-FESTUCETALIA VARiae Quézel 1964

**StpVg:** 1.1.1. **Alliance:** Stipo pulcherrimo-Morinion persicæ Quézel 1964

**StpVg:** 1.1.2. **Alliance:** Festucion chalco-phaeæ Tåth 1982

**StpVg:** 1.2. **Order:** DRABO-ANDROSACETALIA Quézel 1973

**StpVg:** 1.2.1. **Alliance:** Agropyro-Stachion Quelzel 1973

**StpVg:** 1.3. **Order:** TRIFOLIO ANATOLICI-POLYGONETALIA ARENASTRI Quézel 1973

**StpVg:** 1.3.1. **Alliance:** Thlaspiophagion papillosi Kürschner, Parolli & Raab-Straube 1998

**StpVg:** 1.3.2. **Alliance:** Bolanthion frankenioidis Quézel em. Kürschner, Parolli & Raab-Straube 1998

**StpVg:** 1.3.3. **Alliance:** Trifolio-Polygonion Quézel 1973

**StpVg:** 2. **Class:** ALCHEMILLO-CAMPANULETALIA TRIDENTATA Quézel & Düzenli 1979

**StpVg:** 2.1. **Order:** ALCHEMILLO-CAMPANULETALIA TRIDENTATAE Quézel & Düzenli 1979

**StpVg:** 2.1.1. **Alliance:** Stachydion macranthae Quézel & Düzenli 1979

**StpVg:** 2.1.2. **Alliance:** Oxytropion albanae Quézel & Düzenli 1979

**StpVg:** 3. **Class:** ASTRAGALO-BROMETEA Quézel, 1973

Steppe, rocky steppes, and sandy grasslands of the sub-continental temperate and sub-boreal regions.

**StpVg:** 3.0.0.1. **Alliance:** Astragal o erythrotaeni-Gundelion armatae Kay & Ketenoglu 2010

**StpVg:** 3.0.0.2. **Alliance:** Tanaceto orientalii-Astragaliun gummiferi Kay & Ketenoglu 2010

**StpVg:** 3.1. **Order:** ONOBRYCHIDO AR-MENI-THYMETALIA LEUCOSTOMI Akman, Ketenoglu & Quézel, 1985

**StpVg:** 3.1.0.1. **Alliance:** Cousinia iconici-Artemision santonici Geven, Adigüzel and Vural 2010
StpVg: 3.1.0.2. Alliance: Euphorbio armeni- 
Oligochaetion divaricatae Tash 1991
StpVg: 3.1.1. Suborder: ONOBRYCHIDO 
ARMENIAE-THYMENALIA LEUCOSTOMI 
Akman et al. 1991
StpVg: 3.1.1.1. Alliance: Alysso lepidoto-
stellati-Astragalion condensati Aydoğdu, 
Ketençoğlu & Hamzaoğlu 1999
StpVg: 3.1.1.2. Alliance: Convulvulo assyr-
ici-Helianthemion cani Ketençoğlu, Aydoğdu, Kurt, 
Hamzaoğlu, Tuğ & Aslantürk 2008
StpVg: 3.1.1.3 Alliance: Convulvulo 
holosericeo-Agion salicifoliae Akman, Ketençoğlu, 
Quzel & Demirörs 1984.
StpVg: 3.1.1.3.1. Suballiance: Ebeno hirs-
sutae-Thymion leucostomii Hamzaoğlu, Aydoğdu, 
Kurt & Cansaran 2004
StpVg: 3.1.1.4. Alliance: Salvia tchi-
hatcheffii-Hedyasarion varii Akman, Ketençoğlu, 
Quzel & Demirörs 1984
StpVg: 3.1.1.5. Alliance: Phlomido armeni-
acae-Astragalon microcephali Akman, Ketençoğlu, 
Quzel & Demirörs 1984
StpVg: 3.1.1.5.1. Suballiance: Astragalion 
lyci Ketençoğlu, Quzel & Demirörs 1984
StpVg: 3.1.1.6. Alliance: Phlomido nissolii-
Onobrychion tournefortii Kurt 2002
StpVg: 3.1.1.7. Alliance: Astragalo karama-
sici-Gypsophyton eriocalycis Ketençoğlu, Quzel, 
Akman & Aydoğdu 1983
StpVg: 3.1.1.7.1. Suballiance: Asperuleni 
bormuelleri Ketençoğlu, Quzel, Akman & Ay-
doğdu 1983
StpVg: 3.1.1.7.2. Suballiance: Helichryso-
Thymion cappadocii Ketençoğlu, Aydoğdu, Kurt, 
Akman & Hamzaoğlu 2000
StpVg: 3.1.1.8. Alliance: Achilleo wilhelmsii-
Artmision santonici Aydoğdu, Kurt, Hamzaoğlu, 
StpVg: 3.1.1.8.1. Suballiance: Artemision 
santoniciii Ketençoğlu, Quzel, Akman & Aydoğdu 
1983
StpVg: 3.1.1.9. Alliance: Arenario-
Astragalion plumosi Akman 1990
StpVg: 3.1.1.10. Alliance: Minuartion junci-
perino-pestalozzae Ketençoğlu, Serin, Kurt & Ak-
man 1996
StpVg: 3.1.1.11. Alliance: Thymo subisophyl-
lii-Alysison virgatii Akman, Quzel, Aydoğdu, 
Ketençoğlu, Kurt & Evren 1994
StpVg: 3.1.1.12. Alliance: Genisto involucra-
tae-Marrubion micranthi Akman, Vural, Quzel, 
Ketençoğlu, Serin & Barbéro 1996
StpVg: 3.1.2. Suborder: ASPERULO 
PHRYGIAE-THYMENALIA CHAUBARDII 
Akman Quzel, Barbéro, Ketençoğlu & Aydoğdu 
1991
StpVg: 3.1.2.1. Alliance: Siderito phrygiae-
Centaurion maculicipis Akman, Quzel, Barbéro, 
Ketençoğlu & Aydoğdu 1991
StpVg: 3.1.2.2. Alliance: Verbasco phrygiae-
Astragalion flavescens Akman, Quzel, Barbéro, 
Ketençoğlu & Aydoğdu 1991
StpVg: 3.1.2.3. Alliance: Astragalo 
askeriensis-Onobrychidion pisidici Akman, 
Quzel, Barbéro, Ketençoğlu & Aydoğdu 1991
StpVg: 3.1.2.4. Alliance: Micromero phrygi-
ae-Olympodiadion caespitosi Akman, Quzel, 
Barbéro, Ketençoğlu & Aydoğan 1991
StpVg: 3.2. Order: FESTUCO OREOPHIL-
EAE-VERNICETALIA ORIENTALIS Hamzaoğlu 
2006
StpVg: 3.2.0.1. Alliance: Festuco oreophilae-
Veronicen orientalis Hamzaoğlu 2006
StpVg: 3.2.0.2. Alliance: Tanacetou acheran-
i-Thymion pubescens Hamzaoğlu 2006
StpVg: 3.2.0.3. Alliance: Astragalo aurei-
Festucion canecusiae Hamzaoğlu 2006
StpVg: 3.3. Order: ASTRAGALO-
BROMETALIA Quzel 1973
StpVg: 3.3.0.1. Alliance: Tanacion prateriti 
Quzel 1973
StpVg: 3.3.0.2. Alliance: Agrophyro-Stachion 
Quzel 1973
StpVg: 3.3.0.3. Alliance: Thurion capitatae 
Quzel 1973
StpVg: 3.3.0.4. Alliance: Paronychion lyci-
cae Quzel 1973
StpVg: 3.3.0.5. Alliance: Silenion oreades 
Quzel 1973
StpVg: 3.4. Order: HYPERICO-
THYMENALIA SKORPILII Akman, Quzel, 
Ketençoğlu, Yurdakul & Demirörs 1987
StpVg: 3.4.0.1. Alliance: Sileno-Astragalon 
densifoli Akman, Ketençoğlu, Yurdakul 
& Demirörs 1987
StpVg: 3.4.0.1.1. Suballiance: Globulario 
cordifoliae-Dianthemion leucopae Akman, Quzel, 
Barbéro, Aydoğdu, Demirörs & Ekim 1988
StpVg: 3.4.0.2. Alliance: Pedicularo-
Asterion alpinii Akman, Ketençoğlu, Yur-
dakul & Demirörs 1987
StpVg: 3.4.0.3. Alliance: Festuco cypeliaca-
Verbascion occidentale Akman, Quzel, Ketençoğlu, 
Yurdakul & Demirörs 1987
StpVg: 4. Class: POLYGONO ARENASTRI-
POETEA ANNUAE Rivas Mart. 1975 corr. Rivas 
Mart., Báscones, T.E.Díaz, Fern.Gonz. & Loidi 
1991
Syn: Polygono-Poetea annuae Rivas-Martinez in Géhu 1973 nom. inval. (art. 2d, 3b), 
Polygono avicularis-Poetea annuae Rivas-Martinez 
1975 (art. 43), Polygono-Poetea annuae Rivas-
Martinez 1975 nom. superfl. (art. 23), Coronopo-
Polygometea avicularis Lohmeyer 1970 nom. inval. 
(art. 2b, 3b, 3), (‘Coronopo-‘ art. 41b), 
Plantaginetea majoris Tüxen & Preising ex Von 
Rochow 1951 (‘... maioris’ art. 41a) (syntax. syn.) 
[Plantaginetea majoris Tüxen & Preising in Tüxen 
1949 nom. nud. (art. 2b, 8) p.p. (‘... maioris’ art.
Nitrophilous pioneer vegetation of small therophites and hemicyryptophytes that grow on tamped and nitrified soils subjected to trampling: paths, roadsides, crevices of paved roads.


StpVg: 4.1.1. Alliance: Polygono arenstri-Coronopodion squamati Braun-Blanq. ex G. Singh 1969
Syn.: Polygonion avicularis Br.-Bl. 1931.

StpVg: 5. Class: SECAFINETA ORIENTALIA Zohary 1949

StpVg: 5.1. Order: SECAFINETA ORIENTALIA Zohary 1949
StpVg: 5.1.1. Alliance: Secalinion cereale vegetale Zohary 1949

StpVg: 5.1.2. Alliance: Caucaulion Tüxen 1950

StpVg: 6. Class: JUNCETA TRIFID Tüxen in Klika et Hadac 1944

Acidophilous grasslands in the alpine belt of the nemoral zone of Europe, the Caucasus, and in the boreo-arctic and arctic zones of Northern Europe and Greenland.

StpVg: 6.1. Order: TRIFOLIETALIA PARNASSI Quezel 1964

StpVg: 7. Class: ALCHEMILLO RETINERVIS-SIBBALDIETALIA PAVIFLORA Vural 1994


StpVg: 7.1.1. Alliance: Agrostio laizae-Sibbalどion paviflorae Vural 1994

StpVg: 7.1.2. Alliance: Lilio pontici-Anemonion narcissiflorae Vural 1994

StpVg: 7.1.3. Alliance: Centaureo appendicigeae-Senecion taraxacifolii Vural 1994

StpVg: 7.1.4. Alliance: Vaccinio myrtilli-Rhododendron caucasicum Vural 1994

StpVg: 7.2. Order: SWERTIO IBERICA-NADETALIA STRICTAE Vural 1994

StpVg: 7.2.1. Alliance: Swertio ibericae-nardion strictae Vural 1994

StpVg: 8. Class: MOLINIO-ARRHENATHERETALIA Tüxen 1937
Syn.: Molinieto-Arrhenatheretale Tüxen 1937 (original name), Molinio-Juncoetra Br.-Bl. in Br.-Bl., Emberger & Moliniert 1947 (art. 8), Ar-rhenatheretra Br.-Bl in Br.-Bl., Emberger & Molinier 1947 (art. 8), Plantagineta majoris Tüxen & Preising ex Von Rochow 1951 (syntax. syn.), Molinio-Juncoeta Br.-Bl. ex A. & O. Bolo’s 1950 (syntax. syn.), Agrostio stoloniferae-Arrhenatheretra de Foucault 1989 (art. 29)

Hygrophilous and mesophilous, often mared and irrigated meadows that are typical of deep, damp soils and are mainly distributed in the Temperate macrobioclimates, and to a lesser extent in the Mediterranean macrobioclimates.


StpVg: 8.2. Order: ARRENATHERETALIA ELATIORIS Tüxen 1931

Halophytic and Brackish Swamp Vegetation. Plant communities of arid lands are the most typical indicator of soil and climatic conditions. Although there is no relation between them, halophytic plants growing in such lands are of a socioecolog group formed based on similarities in how they survive in certain environmental conditions [22].

Halophytic steppes of Turkey were initially studied by Birand [23], and similar studies have continued since [24, 25]. In the enclosed basin of Salt Lake, one of the most saline basins of Turkey, syntaxonomic units were revealed with comprehensive studies by Aydogdu et al. [26, 27]. Vegetative similarities in arid lands are derived from the accumulation of the limiting factors sodium chloride (NaCl) and sodium sulfate (Na2SO4) on the surface of saline soils in amounts high enough to hamper the growth of many plant species. The most important saline basins of Turkey are Salt Lake, Seyve Lake, Burdur Lake, Akı Lake, and İğdır Lowland. In addition, there are numerous large and small inland saline regions. However, a majority of these have been destroyed by the development of agricultural fields, overgrazing, and building settlements. In terms of phytogeography, inland saline marshes are included in the Aral-Caspian sub-group of the Asian inland marshes group.

In areas of saline, alkaline, and saline-alkaline soils, briefly called arid soil, mean annual precipitation is 250–300 mm and the mean yearly temperature is 10–12°C. The natural vegetation consists of Salicornia sp., Limonium sp., Atriplex sp., and other halophytic grass and shrubs. Such areas generally display a concave topography with smooth slopes, surrounded by alluvial soils without any outflow. The existence of concave topography in arid soils causes underground waters containing high amounts of salt to rise and cause flooding, and because of excessive evaporation, salt accumulates on the soil surface. Salt is seen in forms of white crystals along the soil profile, particularly on the
surface. In regions mostly utilized as corrupt pasture, it is not possible to sustain agriculture without soil rehabilitation because of the high salinity. Halophytic steppe vegetation is physionomically and socio-ecologically in different zones from the center to the periphery.

Research related to Turkey's halophytic and brackish swamp vegetation gained momentum in the 2000s and was particularly concentrated on the large salt basins such as Salt Lake.

**HlpVg**: 1. **Class**: *SALICORNIEA FRUTICOSAE*. Braun-Blanq. et Txs. ex A. Bolos y Vayreda et O. de Bolos in A. Bolos y Vayreda 1950

Mediterranean and thermo-atlantic perennial saltmarsh herblands and scrub.

**HlpVg**: 1. **Order**: *SALICORNIALIA FRUTICOSAE*. Braun-Blanq. 1933

**HlpVg**: 1. **Class**: Salicornion fruticosae Braun-Blanq. 1933

**HlpVg**: 1. **Order**: *HALOSTACHETALIA* (Grossheim) E. Topa. 1938

**HlpVg**: 1. **Class**: Lepidio caespitosi-Limonion iconici Aydoğdu, Hamzaoglu & Kurt 2002

**HlpVg**: 1. **Class**: *IMULO AUCHERANAE-ELYMION SALSI* Aydoğdu, Hamzaoglu, Kurt 2002

**HlpVg**: 1. **Class**: Alhagi-Suaedion microphyllae Tath ve İstanbulluoğlu 1987

**HlpVg**: 2. **Class**: *ARTHROCNEMETEAE FRUTICOSI*. R. Tüxen & Oberd.1958

Scrubby formations of woody glassworts (*Arthrocenenum*), seablites (*Suaeda*), Halimione, Halocnemum or Limoniastrum of saltmarshes and of their immediate vicinity.

**HlpVg**: 2. **Order**: *ARTHROCNEMETALLIA FRUTICOSI* (Br.-Bl. 1931) R. Tüxen & Oberd. 1958

**HlpVg**: 2. **Class**: Zygophyliion albi Géhù et al. 1989

**HlpVg**: 3. **Class**: *JUNCETEA MARITIMI*. Braun-Blanq. in Braun-Blanq., Roussine & Nègre 1952

**Syn**: Juncetalia maritimae Br.-Bl. ex Tüxen & Oberdorfer 1958 (art. 31), Astereteca tripolium Westhoff & Beeftink in Beeftink 1962 (syntax. syn.)

Mediterranean perennial salty and brackish grasslands.

**HlpVg**: 3. **Order**: *JUNCEALIA MARITIMI-M*. Braun-Blanq. ex Horvatić 1934

**HlpVg**: 3. **Class**: Juncion maritimii Braun-Blanq. ex Horvatić 1934

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**Sand Dune Vegetation.** Sand dunes are called “coastal dunes” if they are of marine origin, and “inland sand dunes” if originated inland from residual lakes and inland seas or rocks. Coastal dunes are moving dunes by nature; they are saline and highly permeable. Inland sand dunes are non-saline and less permeable. In Turkey, there are 24 coastal dune formations scattered along 8,333 km of coastline that differ in terms of direction, form, climate, habitat, and vegetation; the length of these dunes is 922.1 km while the surface area is 38,557 ha. In Turkey, sand dunes are mostly on the Mediterranean coast based on length, width, and surface area.

Research related to Turkey's sand dune vegetation is concentrated on the Mediterranean and Black Sea coasts [28, 29].

**SdVg**: 1. **Class**: *EUPHORBIO-AMMOPHILETEA ARUNDINACEA*. (Br.-Bl. 1952) J.-M. Géhù 1988 corr. Géhù in Bardat et al. 2004

**SdVg**: 1. **Order**: *AMMOPHILETALIA*. Br.-Bl. (1931) 1933 em. J.-M. Géhù 1988

**SdVg**: 1. **Class**: Euphorbio-Ammophilon arundinaceae J.-M. Géhù 1987

**SdVg**: 1. **Subclass**: Sporobelenion arenarii J.-M. Géhù 1987

**SdVg**: 1. **Subclass**: Sporobolo-Elymenion farcii J.-M. Géhù 1987

**SdVg**: 1. **Subclass**: Ammophilion arundinaceae Rivas Mart. & J.-M. Géhù 1980

**SdVg**: 1. **Subclass**: Elymion gigantei Morariu 1957

**SdVg**: 1. **Order**: *HELICHRYSO-CRUCIANELLETALIA*. Géhù, Rivas Mart., R. Tüxen 1973

**SdVg**: 1. **Class**: Sileno thymifoliae-Jurion kilae Géhù & Uslu 1989

**SdVg**: 2. **Class**: Cakiletea MARITIMA Tüxen & Preising ex Br.-Bl. & Tüxen 1952

Annual halonitrophile vegetation of sea lodges, tidal flats, salt meadows, and coastal cliffs (bird nesting areas).

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**Chasmophytic Vegetation (Vegetation of Rock Crevices and Seres).** Although rocky areas are nearly everywhere, they are protected from human impact because of difficulty of access. Plant diversity increases depending on humidity, rock stability, and availability of suitable places for seed germination. Rock surfaces form a barrier to certain vegetation because of several factors, including low water-holding capacity relative to the surrounding habitat, scarcity of nutrients, difficulty of attachment and germination of seeds, and greater exposure to wind and sun. Because of these characteristics, rocky areas generally have very unique vegetation.
The vegetation covering moving and firm rocky surfaces and cracks forms varying floristic compositions, and accordingly, varying syntaxa depending on the status and properties of the respective bedrock. However, there is insufficient research on Turkey’s chasmophytic vegetation, particularly concentrated on the Taurus mountain range and western Anatolia.

ChVg: 1. Class: ASPLENIETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl. 1934) Oberd. 1977

Syn: [Asplenietea rupestriae Br.-Bl. in Meier & Br.-Bl. 1934 (art. 34)]

Non-nitrophilous, perennial vegetation dominated by chasmophytes and occasionally by ch thoughts, that grows in the crevices of rocks, cliffs, and walls. Communities with a holartic distribution.


ChVg: 1.1.1. Order: SILENETALIA ODONTHOPETALEA Quézel 1973

ChVg: 1.1.1.1. Alliance: Aubretiopro olympicae Quézel & Pamukcuğulu 1970

ChVg: 1.1.1.2. Alliance: Silenion odontopetralae Quézel 1973

ChVg: 1.1.1.3. Alliance: Onosmion mutabilis Quézel 1973

ChVg: 1.1.1.4. Alliance: Campanulion isauricae Hein, Kürschners & Parolly 1998

ChVg: 1.1.1.5. Alliance: Drabion acaulis Hein, Kürschners & Parolly 1998

ChVg: 1.1.1.6. Alliance: Campanulion cymbalarae Hein, Kürschners & Parolly 1998

ChVg: 1.1.2. Order: ANDROSACETALIA VANDELII Braun-Blanq. in H.Meier & Braun-Blanq. 1934

ChVg: 2. Class: ADIANTEETEA Br.-Bl. in Br.-Bl., Roussine & Nègre 1952

Relict chamaephytes and chasmophytic vegetation in the shaded and water-splashed habitats of the Mediterranean, the Atlantic islands, North Africa, and the Middle East.

ChVg: 3. Class: HELDREICHETEA Quézel ex Parolly 1995

ChVg: 3.1. Order: LAMIETALIA CYMBALARIFOLII Parolly 1995

ChVg: 3.1.1. Suborder: LAMIENALIA CYMBALARIFOLII Parolly 1995

ChVg: 3.1.1.1. Alliance: Scrophularion deperatae Parolly 1995

ChVg: 3.2. Order: HELDREICHETALIA Quézel ex Parolly 1995

ChVg: 3.2.1. Suborder: LAMIENALIA GLANDULOSIDENTIS Parolly 1995

ChVg: 3.2.1.1. Alliance: Scrophularion myriophyllae Parolly 1995

ChVg: 3.2.2. Suborder: LAMIENALIA ERI- OCEPHALI Parolly 1995

ChVg: 3.2.2.1. Alliance: Scrophularion rimarium Parolly 1995

ChVg: 3.2.2.2. Alliance: Jurinellion moschus Parolly 1995

Ruderal Vegetation. Ruderal vegetation spreads across vast places, mainly in settlements. Ruderal species can be recognized as species that are able to grow in severely disturbed habitats. Comprehensive vegetation analyses have not yet been conducted on ruderal vegetation of Turkey. Therefore, the vegetation of such areas is briefly given below.

RdVg: 1. Class: THLASPIETEA RU- TUNDUFOLII Br.-Bl.1948


Perennial vegetation that grows on screes, loose debris substrata, slope deposits, moraines, and stream terraces in the Boreal, Temperate and Mediterranean macrobioclimates.

RdVg: 1.1. Order: ANDROSACETALIA ALPINAEB Braun-Blanq. in Braun-Blanq. & H. Jenny 1926

RdVg: 1.1.1. Alliance: Murbeckiellion huetii Onipchenko 2002

RdVg: 1.1.2. Alliance: Allosuro crisp-Athyriion alpestris Nordh. 1936

RdVg: 1.2. Order: EPILIOBETALIA FLEISCHERI Moor 1958

RdVg: 2. Class: SISYMBRIETEA OFFICINALIS Gutte & Hilbig 1975


Thermomediterranean loam, strongly nitrophilous at the feet of walls and sites sprinkled with urine.

RdVg: 2.1. Order: CHENOPODIETALIA MURALIS Braun-Blanq. in Braun-Blanq., Gajewski, Wraber & Walas 1936

RdVg: 3. Class: STELLARIEETEA MEDIACE Tüxen, W. Lohmeyer & Preising ex von Rochow 1951

Annual vegetation, nitrophilous, commensal of annual crops or weeds.

RdVg: 3.1. Order: CHENOPODIETALIA ALBIs Tüxen & W. Lohmeyer ex von Rochow 1951
**RdVg: 3.1.1.** Alliance: *Diplotaxis erucoidis* Braun-Blanq., Gajewski, Wraber & Walas 1936

**RdVg: 3.2.** Order: *SISYMBRIETALIA OFFICINALIS* I.Tüxen ex Matuszsk. 1962

**RdVg: 3.2.1.** Alliance: *Hardeion marini* Braun-Blanq. in Braun-Blanq., Gajewski, Wraber & Walas 1936

**RdVg: 4.** Class: *ARTEMISIETEA VULGARIS* W. Lohmeyer, Preising & Tüxen ex von Rochow 1951

**Syn:** Artemisietae vulgaris Lohmeyer, Preising & Tüxen in Tüxen 1950 (art. 8), Onopordetalia Br.-Bl. 1964 (art. 8), Onopordetalia Br.-Bl. 1967 (syntax. syn.), Agropyretalia repentinis Oberdorfer, Müller & Görs in Oberdorfer, Görs, Korneck, Lohmeyer, Müller, Philipp & Seibert 1967 (art. 8), Agropyretalia pungentis Göhr 1968 (syntax. syn.), Agropyretalia intermedio-repentinis Müller & Görs 1969 (syntax. syn.), Onopordetalia acantho-nervosi Rivas-Martínez 1975 (syntax. syn.)

Perennial pioneer synanthropic ruderal and nitrogenous herbaceous vegetation that grows on soils rich in organic matter, in Euro-Siberian and Mediterranean regions.

**RdVg: 4.1.** Order: *ONOPORDETALIA ACANTHII* Braun-Blanq. & Tüxen ex Klika in Klika & Hadač 1944

**RdVg: 4.1.1.** Alliance: *Onopordion acanthii* Braun-Blanq. in Braun-Blanq., Gajewski, Wraber & Walas 1936

**Aquatic Vegetation.** Aquatic habitats, including lakes and marshes, cover an area of approximately 10,000 km² in Turkey. There are 250 lakes of varying sizes in Turkey, up to the altitudes of 3,000 m in certain geographic regions. Lakes of Van, Tuz, Beysheir, and Eğirdir are the largest; they constitute 65% of the total lake area in Turkey.

Research related to Turkey’s wetland vegetation has focused especially on Western Anatolia.

**AqVg: 1.** Class: *POTAMETEA PECINATI* Klika in Klika & Novák 1941

Rooted herbaceous, perennial, fresh (possibly subaeratic), mesotrophic to eutrophic, common to stagnant.

**AqVg: 1.1.** Order: *POTAMETALIA PECINATIONIS* W.Koch 1926

**AqVg: 1.1.1.** Alliance: *Potamion luzcentis* Vollmar 1947

**AqVg: 1.1.2.** Alliance: *Nymphaeion albae* Oberd. 1957

**AqVg: 2.** Class: *PHRAGMITETALIA AUSTRALIS-MAGNOCARICETEA ELATAE* Klika in Klika & V.Novák 1941

**Syn:** Phragmitetalia Tüxen & Preising 1942; Bolboschoenetalia maritimae Tüxen & Vicherek ex Tüxen & Hülbusch 1971.

The vegetation of the edges of ponds, lakes, rivers, and marshes on mesotrophic ground with eutrophy, sometimes peaty.

**AqVg: 2.1.** Order: *PHRAGMITETALIA AUSTRALIS* W.Koch 1926


**AqVg: 2.1.1.** Alliance: *Phragmition communis* W.Koch 1926

**AqVg: 3.** Class: *GLYCERIO FLUITANTIS-NASTURTITEA OFFICINALIS* Géhu & Géhu-Frank 1987

**AqVg: 3.1.** Order: *NASTURTIO OFFICINALIS-GLYCERETALIA FLUITANTIS* Pignatti 1953

**AqVg: 3.1.1.** Alliance: *Glycerio fluitantis-Sparganiion neglecti* Braun-Blanq. & G.Sissingh in Boer 1942

**AqVg: 3.2.** Order: *SCIRPETALIA COMPACTI* Rivas Mart., M.J.Castro, Castrov. & Valdés Berm. 1980.

**Syn.:** Bolboschoenetalia maritimae Heijn in Holub, Heijn, Moravec & Neuhausl 1967

**AqVg: 3.2.1.** Alliance: *Scirpion compactolittoralis* Rivas Mart. in Rivas Mart., M.J.Castro, Castrov. & Valdés Berm. 1980

**AqVg: 3.2.2.** Alliance: *Typhion laxmannii* Losev & V. Golub 1988

**AqVg: 4.** Class: *LEMNETEA MINORIS* O. Bolós & Masclans 1955

Annual vegetation of free-floating plants on the surface or below the surface (pleustophytes), rarely subaeratic, stagnant to weakly common freshwaters, with a preference for sheltered biotopes.

**AqVg: 4.1.** Order: *LEMNETALIA MINORIS* O. Bolós & Masclans 1955

**Syn.:** Lemnetalia Tüxen 1955; Hydrocharitetalia Rübel 1933; Ceratophylletalia Hartog & Segal 1964; Utricularietalia Hartog & Segal 1964].

**AqVg: 4.1.1.** Alliance: *Lemnion minoris* O. Bolós & Masclans 1955

**AqVg: 4.1.2.** Alliance: *Hydrocharition morsus-ranae* Rübel ex Klika in Klika & Hadač 1944

**DISCUSSION**

In this study, the high-rank syntaxa of Turkey were reviewed. Accepted units were compiled into these systems of classes, orders, and alliances (AnatoliaVegCheckList-AVC) for communities dominated by vascular plants.
Arboreal, riparian woodland, steppic, halophytic and brackish, sand dune, chasmophytic, ruderal, and aquatic vegetation types include 30 classes, 1 subclass, 49 orders, 5 suborders, 107 alliances, and 9 sub alliances.

Turkey’s forest vegetation was examined in three parts. Mediterranean Woodlands (1 class, 2 orders, 7 alliances), Central Anatolian Woodlands (1 class, 3 orders, 11 alliances), and North Anatolian Woodlands (2 classes, 4 orders, 6 alliances) are included.

Steppes are one of the most ecologically important and vulnerable ecosystems in Turkey. The integrity of Turkey’s steppes faces three primary threats: habitat loss and degradation, overharvesting, and climate change. Expanding cultivation is likely the most evident driver of steppe habitat loss, though infrastructure development also contributes significantly. Overgrazing is the main cause of steppe habitat degradation.

Steppe vegetation is represented by 8 classes, 15 orders, 2 suborders, 49 alliances, and 6 suballiances. All syntaxa belonging to Turkey steppe vegetation are different from European grasslands. Phytogeographically, it represents the Central Anatolian, Eastern Anatolian-Iranian, and Mesopotamian sectors of the Irano-Turanian phytogeographic region.

Syntaxa belonging to other vegetation types are as follows: riparian woodlands: 2 classes, 4 orders, 2 alliances; halophytic and brackish swamp vegetation: 3 classes, 4 orders, 6 alliances; sand dune vegetation: 2 classes, 2 orders, 3 alliances, 3 suballiances; chasmophytic vegetation: 3 classes, 1 subclass, 4 orders, 3 suborders, 10 alliances; ruderal vegetation: 4 classes, 6 orders, 5 alliances; aquatic vegetation: 4 classes, 5 orders, 8 alliances.

CONCLUSIONS

This paper features the first comprehensive and critical account of syntaxa of Turkey and synthesizes more than 60 years of classification efforts by Turkish phytosociologists. It aims to document and stabilize the concepts and nomenclature of syntaxa for practical uses, such as the calibration of habitat classification used by Turkey, standardization of terminology for environmental assessment, management and conservation of nature areas, and landscape planning and education. The presented classification systems provide a baseline for future development and revision of syntaxonomy of Turkey.

ABBREVIATIONS

Art: Article of the ICPN; AVC: AnatoliaVegChecklist (the syntaxonomic system); EVS: European Vegetation Survey (Working Group of IAVS); IAVS: International Association for Vegetation Science; ICPN: International Code of Phytosociological Nomenclature (3rd edition); CAV: Central Anatolian Woodlands; MdW: Mediterranean Woodlands; NAW: North Anatolian Woodlands; RpW: Riparian Woodlands; StpVg: Steppic Vegetation; HlpVg: Halophytic and Brackish Swamp Vegetation; sdVg: Sand Dune Vegetation; ChVg: Chasmophytic Vegetation; RdVg: Ruderal Vegetation; AqVg: Aquatic Vegetation.

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TRANSFORMATION OF DECABROMODIPHENYL ETHER (BDE-209) IN A SOIL–SEDUM ALFREDDII SYSTEM AND THE EFFECT ON SOIL ENZYME AND ACYL-HOMOSERINE LACTONES

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ABSTRACT

The transformation behavior of decabromodiphenyl ether (BDE-209) and its influence on soil enzymatic activities and acyl-homoserine lactones in a soil–Sedum alfredii system were investigated by a 60-day culture with BDE-209 treatments (0, 2.5, 5, 10, 15, and 20 mg kg⁻¹). The dissipation of BDE-209 in rhizosphere soil (10.3%–33.4%) was higher than non-rhizosphere soil (4.4%–16.0%). The trend of polybrominated diphenyl ethers (PBDEs) was similar to the dissipation of BDE-209 (491.4–599.1 ng g⁻¹ in rhizosphere soil and 264.4–434.7 ng g⁻¹ in non-rhizosphere soil). Soil BDE-209 was taken up into the aboveground parts of S. alfredii. The BDE-209 concentration in plant tissues decreased in the order stem > root > leaf, except in the 20 mg kg⁻¹ treatment (root > stem > leaf). The lower-brominated PBDEs were preferentially accumulated in leaves (804.74, 789.76, and 576.24 ng g⁻¹ for leaves, roots, and stems, respectively) in the BDE-209 spiked groups. The activities of glucosidase, sulfatase, and nitrate reductase first decreased and then stabilized with BDE-209 increase. The dehydrogenase, urease, and alkaline phosphatase activities were significantly positively correlated with BDE-209 (p < 0.05). Three acyl-homoserine lactone signals (C4-HSL, 3-oxo-C6, and 3-oxo-C12) were detected in rhizosphere soil and non-rhizosphere soil, of which C4-HSL was dominant. This suggests that S. alfredii could accelerate the migration and transformation of BDE-209 and C4-HSL was positively correlated with soil enzymatic activities and BDE-209 treatments.

INTRODUCTION

Over the past few decades, polybrominated diphenyl ethers (PBDEs) have been extensively used as brominated flame retardants in many consumer products, such as plastics, textiles, electronics, and rubber [1-3]. Increasing amounts of PBDEs have been detected in many environmental matrices, including water, soil, sediment, and biota [4-7]. Due to their high octanol–water partition coefficient, PBDEs have the potential to bioaccumulate in fish and mammals throughout the food chain, which represents a risk to human health [8-10]. Soil has been considered as major sink for PBDEs [11-14]. Various aquatic and terrestrial plants have been used to study the accumulation ability of PBDEs from sewage sludge and soil, such as Phragmites australis, Oryza sativa, Alternanthera philoxeroides, pumpkin, radish, and alfalfa; however, few plants have both high accumulation and high biomass.

High PBDE contents have been found in electronic waste dismantling sites, such as Guiyu, Guangdong province, China [9], which is co-contaminated with heavy metal. Therefore, it is important to identify a plant that has the ability to remove these two kinds of pollutants. As the most active zone in soil, the rhizosphere contains the highest microbe concentration as they are attracted by the root secretions, animal and plant debris [15]. Many organic compounds are degraded in the rhizosphere or taken up by microbes, animals, and plants. The brominated PBDEs congeners (tri- through hexa-BDE) and HO-PBDE/MEO-PBDE are potential transformation products of BDE-209, which have been identified as having a higher toxicity than BDE-209[16-18]. Soil organic matter could provide energy for the growth of microorganisms, but persistent organic pollutants can inhibit the growth of microorganisms and even cause toxicity.

Quorum sensing (QS) is a regulatory mechanism that enables bacteria to make collective decisions with respect to the expression of a specific set of genes [19]. The diffusible signal molecules se-
creted by cells play an important role in the procedure of QS expression. When the signals reach a certain concentration threshold under a high bacterial cell density, cognate receptors begin to detect and bind signal molecules, then bacteria begin to regulate the expression of relevant genes. This mechanism could coordinate the behavior of all individuals in the population to better adapt to the environment. Acyl-homoserine lactone (AHL) is an important QS signal molecule, used by gram-negative bacteria as an autoinducer.

To date, the study of PBDEs in soils has mainly focused on their distribution, accumulation, and metabolism in soils and some edible plants and the potential risks to human health [20]. There have been few studies on microbial degradation of PBDEs in the soil–plant system, especially the correlation between microbial degradation and microbial QS. Rhizosphere QS is more intense than that in bulk soil because more microbes are present in this soil region. Previous studies have shown a phylogenetic overlap between aromatics degrading bacteria and AHL-based QS bacteria [21, 22]. Yong and Zhong [23] proposed that the rhl QS system could improve aromatic biodegradation by positively regulating the activity of catechol 2,3-dioxygenase, but the function stringency and scope of QS in PBDEs degradation remains little known. It is therefore of great importance to study the links among QS and PBDEs degradation in soil.

In this study, the typical Zn and Cd hyperaccumulator Sedum alfredii [24, 25] was planted in a BDE-209 contaminated soil (concentrations of 0, 2.5, 5, 10, 15, and 20 mg kg⁻¹) to investigate the transformation characteristics in the soil–S. alfredii system. The link between QS and the potential phytoremediation ability of BDE-209 was preliminary discussed based on research into AHL signals and soil enzyme activities in the rhizosphere.

MATERIALS AND METHODS

Chemicals and reagents. The following standards were purchased from AccuStandard (AccuStandard, New Haven, USA): BDE-28, -47, -99, -100, -153, -154, -183, -196, -207, and -209. The C4-HSL, 3-oxo-C6-HSL, C6-HSL, 3-oxo-C8-HSL, C8-HSL, and 3-oxo-C12-HSL Standards were purchased from Sigma (St. Louis, MO, USA). Acetonitrile for LC-MS/MS was chromatographic grade (Merk, Darmstadt, Germany). All other chemicals were analytical grade.

Soil properties and preparation. Soils were collected from 0–20 cm soil depth in a cropland in Zhejiang province, China. Selected characteristics are as follows: pH (H₂O), 6.52; organic matter, 25.28 mg kg⁻¹; cation exchange capacity, 19.87 cmol kg⁻¹; sand content, 25%; silt content, 44%; clay content, 31%; and no detectable PBDEs. After air-drying for 2 weeks, the soil samples were sieved through a 2-mm nylon sieve, then received N, P, and K fertilizer (N, 100 mg kg⁻¹; P₂O₅, 80 mg kg⁻¹; K₂O, 100 mg kg⁻¹). Soil was spiked with dichloromethane solution of BDE-209 at concentrations of 0, 2.5, 5, 10, 15, and 20 mg kg⁻¹ according to the method described by Lu and Zhang [26], and moisture was adjusted to 60% of the highest field water holding capacity. Root boxes were filled with 4 kg soil (dry weight) with three cavities (160 mm×140 mm×200 mm), rhizosphere zone (width 20 mm) in the middle, non-rhizosphere area separated by 600 mesh nylon net) in every treatment. The pots were used to plant the S. alfredii, after maintaining balance for 3 weeks.

The plant seedlings were grown in Hoagland’s nutrient solution until they germinated enough roots. Seedlings with similar size were selected and three seedlings were planted in each root box. The growth conditions were as follows: temperature 25°C, soil moisture 60% of the highest field water holding capacity, and an incandescent lamp was used as an auxiliary light source and the supplemental light source was turned on at 7:00–11:00 and 13:00–18:00 every day, with natural light only at 11:00–13:00.

Sample preparation. Plants were removed from the soil after 60 days. Two hundred grams of soil were randomly collected from each non-rhizosphere zone and rhizosphere zone. These soil samples were divided into two parts, the first of which was analyzed immediately for AHLs, soil microbial biomass, and soil enzymes activity, and the other was freeze-dried at low temperature, ground, and filtered through a 60-mm mesh, then stored at −20°C for PBDE determination.

Extracted and determination of PBDEs. Both soil sample and plant samples were extracted by Soxhlet apparatus, with a slight alteration from the method described by Covaci [27]. The samples were mixed with anhydrous sodium sulfate and then Soxhlet-extracted with dichloromethane:n-hexane (1:1, v/v) for 16 h. The extract was concentrated to approximately 2 mL, loaded onto a silica-gel column (10 cm × 6 mm ID), and then eluted with 13 mL mixture of n-hexane/dichloromethane (1:1, v/v). The sample was then blow dried under a gentle stream of nitrogen and re-dissolved with 1 mL n-hexane.

The instrument used for PBDEs quantitative analysis was Agilent 7980 gas chromatograph equipped with an micro electron capture detector (GC-μECD). The conditions were slightly changed from the method described by Augusto [28]. The capillary column was Agilent DB-5HT (15 m × 0.25 mm × 0.1 μm), carrier gas was high pure nitrogen (≥99.999%), with a stable flow speed of 1
mL min⁻¹. The injection port and detector temperatures were 280°C and 300°C, respectively. The injection volume was 1 mL, delivered through split injection, with a split ratio of 10:1. After sample injection at 100°C, this temperature was held for 2 min, followed by temperature increase at 30°C min⁻¹ to 325°C, and maintained for 5 min.

**AHL signal molecule extraction and detection.** The AHL extract used acidified ethyl acetate by acetic acid [29]. Two milliliter bacterial culture medium and acidified ethyl acetate (0.01% acetic acid, v/v) with 1:1 (v/v), then mixed for 30 s used the vortex mixture. The mixture was left to stabilize for 2 min to allow stratification and collect the upper organic layer. This process was repeated again and collected ethyl acetate was allowed to dry under a gentle nitrogen stream. Finally, the mixture was dissolved in 0.5 mL acidified ethyl acetate, and stored at -80°C until analysis.

The AHLs were analyzed with an Acquity UPLC® System (Waters, Milford, MA, USA) coupled to a mass spectrometer (electrospray ionization mode ESI+ tandem quadrupole), and 10 μL of each sample was injected into an Acquity UPLC RP-C18 column (2.1 × 100 mm; 1.7 μm; Waters, Ireland) at a flow rate of 0.4 mL min⁻¹ [30]. The column was maintained at 40°C. The UPLC system mobile phase A was 0.1% formic acid and mobile phase B was 0.1% formic acid in acetonitrile. The gradient profile was as follows: 0–0.5 min, isocratic elution at 10% B; 0.5–3.5 min, linear gradient to 60% B; 3.5–4 min, linear gradient to 90% B; 4–5 min, isocratic elution at 90%; 5–5.1 min, linear gradient to 10%; and finally, it was equilibrated to the initial conditions (10% B, v/v) for 0.9 min. The MS/MS conditions were as follows: source temperature, 120°C; desolvation temperature, 550°C; cone/desolvation gas flow, 70/650 L h⁻¹; capillary voltage, 4 kV. Quantification was performed using MassLynx™ software package (version 4.1, Waters, Milford, MA, USA).

**Soil microbial biomass carbon (SMBC) and enzyme activity.** The SMBC was determined by a modified chloroform fumigation extraction method [31]. Twenty grams of soil were fumigated in a desiccator with 50 mL NaOH solution (1 mol L⁻¹) and 50 mL chloroform (no ethanol) in a beaker. The chloroform was kept boiling for 3–5 min after vacuum pumping, then left for 24 h in the dark, at 25°C. After using the vacuum pump to completely remove chloroform, K₂SO₄ was used to extract and the total organic carbon was determined by a TOC-L carbon analyzer (Shimadzu Corporation, Kyoto, Japan) with ASI-L auto-sampler.

The colorimetric method was used to estimate activities of dehydrogenase (DH), glucosidase (GL), alkaline phosphatase (ALP), sulfatase (SP), nitrate reductase (NAR), urease (Ure), and fluorescein diacetate (FDA) hydrolase [32-34]. These enzymes activities were determined using 1 g of air-dried soil with their appropriate substrate and incubated for 1 h (37°C) at the suitable buffer solution. The absorbance of NaR(540nm), FDA(490nm), Ure(578nm), DH(492nm), GL(398nm), SP(398nm), and ALP(398nm) were determined with a UV/vis spectrophotometers.

![Graph](image)

**FIGURE 1**

The accumulation of BDE-209 in S. alfredii.
RESULTS

Accumulation and transformation of BDE-209 in S. alfredii. This study showed that BDE-209 can be absorbed by roots and transfer to aboveground parts. The concentration of BDE-209 decreased in the order of stem > root > leaf, except in the 20 mg kg\(^{-1}\) treatment, which decreased in the order root > stem > leaf (Fig. 1). There was little difference in the concentration of BDE-209 in S. alfredii from 2.5 to 15 mg kg\(^{-1}\). These findings indicate that S. alfredii has the potential to uptake BDE-209 from soil. The bioconcentration factor (BCF) is an important index to measure the capacity of plants to accumulate pollutants through the ratio of pollutant content in plants and in soil [35]. To further understand the potential of S. alfredii to accumulate and transport BDE-209 from soil to plants, the BCF and transfer factor for BDE-209 in S. alfredii were determined for all tissues of S. alfredii (Fig. 2). The results showed that S. alfredii had a good enrichment ability (BCF > 1) for BDE-209. The highest BCF values were found in the 2.5 mg kg\(^{-1}\) BDE-209 treatments at 10.05, 15.09, and 1.85 in root, stem, and leaf, respectively. When soil was spiked with 20 mg kg\(^{-1}\) BDE-209, the BCF values were 4.57, 4.15, and 0.79 in root, stem, and leaf, respectively, which was the lowest in all treatments. This suggests that S. alfredii has a good ability to bioconcentrate BDE-209, but high level of BDE-209 was harmful to the growth of S. alfredii (Table 1), and affected the uptake of BDE-209.

Nine brominated PBDE congeners were detected in plant tissues after plant harvest (Fig. 3). BDE-207 was the dominant PBDE in roots, stems, and leaves. For example, S. alfredii accumulations 529.37, 302.08, and 157.56 ng g\(^{-1}\) BDE-207 in roots, stems, and leaves, respectively, with the 20 mg kg\(^{-1}\) treatment. BDE-47, -100, -153, -154, -183, -196, and -207 were detected in S. alfredii root; BDE-28, -47, -99, -100, -153, -183, -196, and -207 were detected in S. alfredii stem; BDE-28, -99, -100, -153, -154, -196, and -207 were detected in S. alfredii leaf. The high-brominated PBDE congeners (hepta- through nona-) were present in higher proportions in plant roots and stems, whereas lower-brominated PBDEs (tri- through hexa-) were present in higher proportion in plant leaves. The concentration of lower-brominated PBDEs in plant roots and stems was 789.76 and 576.24 ng g\(^{-1}\), respectively, whereas it was 804.74 ng g\(^{-1}\) in leaves, for all BDE-209 spiked groups. This indicates that lower-brominated PBDEs preferentially accumulated in leaves.

![Figure 2](image)

**FIGURE 2**
Bioconcentration factor (BCF) of BDE-209 in S. alfredii (n=9).

| TABLE 1 |
The influence of BDE-209 on the growth of S. alfredii (fresh weight of root, stem and leaf, g plant\(^{-1}\), n=9).  
<table>
<thead>
<tr>
<th>BDE-209 treatments</th>
<th>Root (g)</th>
<th>Stem (g)</th>
<th>Leaf (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>1.86±0.16</td>
<td>2.27±0.14</td>
<td>12.31±0.50</td>
</tr>
<tr>
<td>2.5</td>
<td>2.79±0.38</td>
<td>2.88±0.13</td>
<td>15.17±1.46</td>
</tr>
<tr>
<td>5</td>
<td>7.70±0.34</td>
<td>3.78±0.32</td>
<td>17.93±1.12</td>
</tr>
<tr>
<td>10</td>
<td>3.86±0.86</td>
<td>3.60±0.56</td>
<td>17.96±1.22</td>
</tr>
<tr>
<td>15</td>
<td>4.08±0.57</td>
<td>3.67±0.73</td>
<td>18.35±1.38</td>
</tr>
<tr>
<td>20</td>
<td>3.19±0.11</td>
<td>2.78±0.56</td>
<td>12.09±1.02</td>
</tr>
</tbody>
</table>
The distribution and content of the debrominated products of BDE-209 in its root, stem and leave.

Dissipation and debromination of BDE-209 in soil. The dissipation of BDE-209 in the planted soil was significantly higher than that in the unplanted soil (Fig. 4), and BDE-209 content was lower in rhizosphere soil than that in non-rhizosphere soil. The reduction rates of BDE-209 were 10.3%–33.4% and 4.4%–16.0% in rhizosphere soil and non-rhizosphere soil, respectively, and the lowest rates were both found in the 20 mg kg⁻¹ treatments. This indicates that S. alfredii can
accelerate the dissipation of BDE-209 in soil and this effect is stronger in rhizosphere soil than non-rhizosphere soil. Nine main homologues of BDE-209 (tri- to nona-BDE) were all detected in rhizosphere soil, whereas only five homologues were detected in non-rhizosphere soil. The concentration of homologues of BDE-209 were 491.4–599.1 ng g⁻¹ in rhizosphere soil and 264.4–434.7 ng g⁻¹ in non-rhizosphere soil (Fig. 5). This showed that the homologues of PBDEs in rhizosphere soil were present at higher concentrations than in non-rhizosphere soil, which was different from BDE-209 (Fig. 4). The higher-brominated PBDE congeners (BDE-207, BDE-196, and BDE-183) were dominant in both rhizosphere soil and non-rhizosphere soil. The mean of the total percentages of the higher-brominated PBDEs congeners in the nine congeners ranged from 64.7% to 89.3% in rhizosphere soil and 86.1% to 100% in non-rhizosphere soil. This showed that more homologues of BDE-209 and lower-brominated PBDEs were generated in rhizosphere soil than non-rhizosphere soil. All nine brominated PBDEs were detected in rhizosphere soil, but BDE-28, -100, -153, and -154 were absent in the non-rhizosphere soil.

**Influence of BDE-209 on SMBC.** The SMBC is an important indicator reflecting microbial activities in soil. As shown in Fig. 6, the SMBC in rhizosphere soil was higher than that in non-rhizosphere soil in all treatments. The SMBC decreased as the soil BDE-209 content increased, whereas it was not significantly different to the control when BDE-209 treatments were less than 2.5–10 mg kg⁻¹. The SMBC in the 15 and 20 mg kg⁻¹ BDE-209 treatments were much lower than in the control group (p < 0.001), indicating that more than 15 mg kg⁻¹ BDE-209 significantly inhibits the growth of microbes in soil.

**Influence of BDE-209 on soil enzyme activity.** Soil enzyme activity appeared in the form of enzyme activity/SMBC to modify the change of soil enzyme activity caused by soil microbial biomass. As shown in Table 2, seven enzymes activities in rhizosphere soil were all higher than those in non-rhizosphere soil. The activities of GL, SP, and NaR first decreased and then stabilized with BDE-209 increase in soil. Conversely, the DH, Ure, and ALP activities were all significantly positively correlated to BDE-209 concentration (p < 0.05). The FDA hydrolyase activity slightly increased and then dropped and the highest value was 80.10 ± 7.67 and 75.49 ± 2.62 (mg FDA g microbial organic C⁻¹ h⁻¹) in rhizosphere soil and non-rhizosphere soil with 10 mg kg⁻¹ BDE-209 treatment.

![Image](image_url)

The debrominated products of BDE-209 in rhizosphere soil (R) and non-rhizosphere soil (NR).
### TABLE 2
The influence of BDE-209 on soil enzyme activity.

<table>
<thead>
<tr>
<th>BDE-209 Treatments (mg kg⁻¹)</th>
<th>GL/C (mg p-nitrophenol g⁻¹ microbial C h⁻¹)</th>
<th>PS/C (mg p-nitrophenol g⁻¹ microbial C h⁻¹)</th>
<th>SP/C (mg p-nitrophenol g⁻¹ microbial C h⁻¹)</th>
<th>NaRC (mg NO₂ g⁻¹ microbial C h⁻¹)</th>
<th>PDA/C (mg CO₂ g⁻¹ microbial C h⁻¹)</th>
<th>Ure/C (mg NH₃ g⁻¹ microbial C h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>13.06±1.09</td>
<td>108.73±5.25</td>
<td>40.21±2.01</td>
<td>58.8±3.88</td>
<td>15.53±1.55</td>
<td>64.99±2.94</td>
</tr>
<tr>
<td>NR</td>
<td>12.71±0.80</td>
<td>85.70±8.58</td>
<td>24.11±2.70</td>
<td>54.55±1.53</td>
<td>10.41±1.11</td>
<td>59.11±3.70</td>
</tr>
<tr>
<td>2.5 R</td>
<td>12.23±0.55</td>
<td>92.06±9.27</td>
<td>39.17±2.62</td>
<td>49.22±3.46</td>
<td>8.62±0.04</td>
<td>71.06±3.46</td>
</tr>
<tr>
<td>NR</td>
<td>10.64±1.59</td>
<td>84.4±1.00</td>
<td>36.86±1.35</td>
<td>48.23±3.03</td>
<td>7.49±0.86</td>
<td>71.85±6.44</td>
</tr>
<tr>
<td>5 R</td>
<td>12.55±0.76</td>
<td>47.53±3.18</td>
<td>57.52±4.94</td>
<td>41.52±0.94</td>
<td>6.51±0.48</td>
<td>79.8±5.25</td>
</tr>
<tr>
<td>NR</td>
<td>10.18±0.23</td>
<td>48.8±6.11</td>
<td>50.2±4.11</td>
<td>30.97±2.55</td>
<td>5.97±0.80</td>
<td>68.44±3.85</td>
</tr>
<tr>
<td>10 R</td>
<td>14.25±1.09</td>
<td>46.3±0.02</td>
<td>61.73±6.61</td>
<td>34.17±2.56</td>
<td>8.22±0.77</td>
<td>80.1±7.67</td>
</tr>
<tr>
<td>NR</td>
<td>12.87±0.70</td>
<td>48.00±2.00</td>
<td>65.48±1.49</td>
<td>33.32±1.08</td>
<td>5.66±0.37</td>
<td>75.49±2.62</td>
</tr>
<tr>
<td>15 R</td>
<td>15.60±1.46</td>
<td>47.39±4.27</td>
<td>71.7±7.51</td>
<td>36.74±4.93</td>
<td>8.42±0.59</td>
<td>68.87±2.66</td>
</tr>
<tr>
<td>NR</td>
<td>15.84±1.20</td>
<td>50.97±1.22</td>
<td>68.34±5.20</td>
<td>37.37±0.71</td>
<td>7.00±0.33</td>
<td>65.98±5.24</td>
</tr>
<tr>
<td>20 R</td>
<td>20.04±1.52</td>
<td>45.27±2.39</td>
<td>88.06±0.76</td>
<td>42.67±6.51</td>
<td>7.48±0.40</td>
<td>62.05±3.55</td>
</tr>
<tr>
<td>NR</td>
<td>17.13±2.58</td>
<td>41.55±1.25</td>
<td>81.79±9.47</td>
<td>38.51±2.28</td>
<td>6.32±0.52</td>
<td>58.99±1.79</td>
</tr>
</tbody>
</table>

(R: rhizosphere soil; NR: non-rhizosphere soil).

![FIGURE 6](image1)

**FIGURE 6**
The content of biomass C in rhizosphere soil (R) and non-rhizosphere soil (NR) (three stars symbol mean p<0.001).

![FIGURE 7](image2)

**FIGURE 7**
The rate of C4-HSL in rhizosphere soil (R) and non-rhizosphere soil (NR) effected by BDE-209.

9146
TABLE 3
Influence of BDE-209 on the concentration of AHLs in soil (n=5).

<table>
<thead>
<tr>
<th>BDE-209 Treatments (mg kg⁻¹)</th>
<th>AHLs (ng kg⁻¹)</th>
<th>C4-HSL</th>
<th>3-oxo-C6-HSL</th>
<th>C6-HSL</th>
<th>3-oxo-C8-HSL</th>
<th>C8-HSL</th>
<th>3-oxo-C12-HSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK R 2.84±0.32</td>
<td>0.37±0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.01±0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 0.86±0.40</td>
<td>0.35±0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.77±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 R 18.16±0.26</td>
<td>0.69±0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.57±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 1.56±0.08</td>
<td>0.48±0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.52±0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 R 11.87±0.22</td>
<td>0.66±0.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.74±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 0.50±0.12</td>
<td>0.36±0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.56±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 R 14.33±1.23</td>
<td>1.11±0.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.67±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 1.46±0.51</td>
<td>0.79±0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.61±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 R 1.54±0.20</td>
<td>0.35±0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.65±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 0.80±0.03</td>
<td>0.18±0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.69±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 R 1.08±0.24</td>
<td>0.38±0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.56±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR 1.18±0.23</td>
<td>0.22±0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.52±0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(R: rhizosphere soil; NR: non-rhizosphere soil; -: Not detected.)

TABLE 4
S. alfredii root parameters (n=9).

<table>
<thead>
<tr>
<th>BDE-209 treatments (mg kg⁻¹)</th>
<th>root diameter(mm)</th>
<th>root length(cm)</th>
<th>root surface(cm²)</th>
<th>root volume(cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>223.11±11</td>
<td>5.87±0.78</td>
<td>18.43±1.21</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>2.5</td>
<td>300.15±34</td>
<td>7.78±1.21</td>
<td>24.44±2.56</td>
<td>0.27±0.03</td>
</tr>
<tr>
<td>5</td>
<td>361.09±21</td>
<td>10.21±0.34</td>
<td>32.09±1.45</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>10</td>
<td>373.64±45</td>
<td>9.10±1.23</td>
<td>35.06±3.21</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>15</td>
<td>392.20±42</td>
<td>10.10±1.22</td>
<td>40.25±3.97</td>
<td>0.27±0.03</td>
</tr>
<tr>
<td>20</td>
<td>195.05±12</td>
<td>5.35±0.88</td>
<td>16.82±0.98</td>
<td>0.25±0.01</td>
</tr>
</tbody>
</table>

TABLE 5
Correlation coefficients between soil enzymes activities and BDE-209 treatments and AHLs.

<table>
<thead>
<tr>
<th>soil enzyme activity</th>
<th>BDE-209 treatments</th>
<th>ALP 0.972**</th>
<th>DH 0.926*</th>
<th>Ure 0.899*</th>
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<td></td>
<td>AHLS</td>
<td>C4-HSL</td>
<td>3-oxo-C6</td>
<td>3-oxo-C12</td>
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<td>ALP</td>
<td>0.972**</td>
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<td>DH</td>
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<td>Ure</td>
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<td>FDA</td>
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<td>0.837*</td>
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<td>SP</td>
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(one star symbol mean p<0.05, two stars symbol mean p<0.01)

Influence of BDE-209 on AHLs in soil. Six AHL signal molecules (C4-HSL, 3-oxo-C6-HSL, C6-HSL, 3-oxo-C8-HSL, C8-HSL, and 3-oxo-C12-HSL) were determined in rhizosphere soil and non-rhizosphere soil. As shown in Table 3, C6-HSL, 3-oxo-C8-HSL, and C8-HSL were not detected. C4-HSL was the dominant AHL signal molecular in rhizosphere soil. The highest concentrations of C4-HSL were 18.16 ng kg⁻¹ in rhizosphere soil and 1.56 ng kg⁻¹ in non-rhizosphere soil, respectively, with 2.5 mg kg⁻¹ BDE-209 treatment. The concentration of C4-HSL were 1.9–23.5 times higher under 0–15 mg kg⁻¹ BDE-209 treatments in rhizosphere soil than in non-rhizosphere soil (Fig. 7). This suggests that the microbes in rhizosphere soil could generate more AHLs than non-rhizosphere soil. The ratio of the average of C4-HSL in rhizosphere soil and non-rhizosphere soil was highest with the 5 mg kg⁻¹ BDE-209 treatment (Fig. 7). This indicates that 0–15 mg kg⁻¹ BDE-209 could induce C4-HSL, while 20 mg kg⁻¹ BDE-209 inhibited C4-HSL both in rhizosphere and non-rhizosphere soil.

DISCUSSION

Transformation of BDE-209 in the soil—S. alfredii system. Accumulation in S. alfredii tissue is an important mean to remove PBDEs from the contaminated soil. In this study, growth of S. alfredii was not inhibited, and was even slightly stimulated by BDE-209 spiking (Tables 1 and 4). This shows that S. alfredii has a good tolerance to BDE-209. BDE-209 and nine de brominated products were detected in different parts of S. alfredii (Figs 1 and 3), whereas BDE-28 and BDE-99 were absent in roots and rhizosphere soil (Figs 3 and 5). This indicates that PBDEs could not only be taken up, transported, and transformed in S. alfredii from soil, but also degraded in S. alfredii by plant en-
zymes and endophytes. It has been reported that plant root exudates could provide nutrition and stimulate the activities of soil bacteria, and change the soil physical and chemical properties (e.g. pH and viscosity), directly enhancing the bioavailability of PBDEs and encouraging microbial degradation and plant uptake [36]. BDE-28, -100, -153, and -154 were absent in non-rhizosphere soil, although the nine debrominated products were all detected in rhizosphere soil (Fig. 3). This could be due to SMBC and enzyme activities were lower in the non-rhizosphere soil than in the rhizosphere soil. The previous studies also showed that plant uptake of PBDEs only represented a small proportion of the total PBDE content in soil [26, 37]. These findings suggest that the concentration differences of BDE-209 and PBDEs in rhizosphere soil and non-rhizosphere soil were mainly due to the activities of soil microbe.

Microbial degradation plays one of the most important roles in the removal process of PBDEs from contaminated soil. Wang et al. [6] found that BDE-209 removal was positively related to the microbial biomass in soil. Chen et al. [38] found that rhizosphere soil with the higher bacterial abundance and activity also had high removal of BDE-47. Similarly, this study showed that rhizosphere soil with higher SMBCs, soil enzymes activities and BDE-209 removals (Figs 4 and 6, Table 5). These results support the suggestion that the SMBC and enzyme activities can be used to characterize the PBDE degradation potential of soil microbes. All the results showed that there was a high efficiency removal of BDE-209 in soil planted with S. alfredii.

**Effect of BDE-209 on soil enzymes activities and AHLs.** In the present study, the soil enzymes activities of ALP, DH, and Ure were significantly positively correlated with BDE-209 treatments (Table 5). These results indicate that BDE-209 increased the activities of these three enzymes in all treatments. Although the result was different from Zhu et al. [39], who found that the activity of Ure was inhibited with 1, 10, and 100 mg kg⁻¹ BDE-209 applied, it was consistent with the results of Huang et al. [34]. This could be due to BDE-209 is used as the carbon source by microorganisms at a low level of PBDEs [33, 40-42]. The enhancement of the microbial biomass also confirmed this explanation under 15 mg kg⁻¹ BDE-209 (Fig. 6). In addition, the FDA activity was significantly positively correlated with C4-HSL and 3-oxo-C6-HSL, and NaR activity was also significantly positively correlated with 3-oxo-C12. These results indicate that the FDA and NaR enzyme activities might be controlled by the QS of soil microbes. Yong et al. [23] found that the rhl QS system could improve biodegradation of aromatics by positively regulating the activity of catechol 2,3-dioxynogenase. Huang et al. [43] found that the QS activity of the AHL-producing marine bacterium _Crociceccus naphthovorans_ had a positive influence on polycyclic aromatic hydrocarbon degradation. Kumari et al. [44] found that _Pseudomonas aeruginosa_ N6P6 could improve the degradation rate of phenanthrene and pyrene with enhance biofilm regulated by las and rhl QS system. These previous studies also confirmed our hypothesis that QS has a positive role in the degradation of BDE-209 by soil microbes.

The activities of GL, SP, and NaR were first decreased and then stabilized with increasing BDE-209 concentrations which may be because the activity of some microbes had been inhibited by the small BDE-209 application (0–5 mg kg⁻¹), but they were not killed. With the increase of the BDE-209 concentration (5–20 mg kg⁻¹), the microbes began to die which could not tolerate BDE-209 and the tolerant species then had more space and substrate for development. As a result, all enzyme activities stabilized or increased, for example, the activities of GL, SP, and NaR were stable or slightly increased when more than 5 mg kg⁻¹ BDE-209 was added (Table 2). Finally, the FDA activity, which represents the whole microbial activity [45, 46], was in a dynamic equilibrium (Table 2). The decreased and stabilized SMBC might be a response to our analysis.

**CONCLUSION**

More dissipation and homologues of BDE-209 and lower-brominated PBDEs were generated in rhizosphere soil than in non-rhizosphere soil, indicating that _S. alfredii_ can accelerate the transformation of BDE-209 in soil. The high BCF of _S. alfredii_ for BDE-209, suggested that _S. alfredii_ has a good ability to bioaccumulate BDE-209. Higher amounts of lower-brominated PBDEs were detected in leaves, indicating that lower-brominated PBDEs preferentially accumulate in leaves. BDE-209 increased the activities of ALP, DH, and Ure, although the trends of GL, SP, and NaR first declined then stabilized. Three AHLs signals (C4-HSL, 3-oxo-C6, and 3-oxo-C12) were detected in rhizosphere soil and non-rhizosphere soil, of which C4-HSL was dominant. C4-HSL could be induced by BDE-209 concentrations under 15 mg kg⁻¹ both in rhizosphere and non-rhizosphere soil, whereas 20 mg kg⁻¹ of BDE-209 inhibited C4-HSL. The positive correlation of both FDA activity with C4-HSL and 3-oxo-C6-HSL and NaR activity with 3-oxo-C12, showed that AHLs have a positive role in the degradation of BDE-209 by soil microbes, especially C4-HSL. This study linked QS, soil enzyme and the phytoremediation of BDE-209 in soil, but the precise role of QS in phytoremediation still need further investigation.
ACKNOWLEDGEMENTS

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SEDIMENTARY CHARACTERISTICS OF 1ST AND 2ND MEMBERS OF CRETACEOUS QUANTOU FORMATION IN NORTHERN SONGLIAO BASIN

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ABSTRACT

Recently, an important development trend of oil and gas exploration extends to the deep part of the basin. At present, the oil and gas reservoir in the Songliao Basin is still located in the mid-deep layer, and the deep reservoir of the first and second members of the Quantou Formation (Ku1q), which is the focus of oil and gas exploration. In particular, there is no clear understanding of the deposition system, which also limits the further exploration and development of the deep reservoir. Based on the analysis of heavy mineral assemblage and formation sand ratio, provenance of five tectonic zones in the first and second member of Quantou Formation are identified. Sedimentary system of the first and second members of the Quantou Formation in the northern part of the Songliao Basin is discussed based on drilling data and logging curve. Results show that the first and second members of Quantou Formation are mainly composed of five significant sedimentary facies including meandering river facies, braided river facies, alluvial fan facies, delta facies and shallow lake with characteristics of ‘steep slopes into a fan, gentle slope into the river’. Sedimentary facies are further subdivided into eleven sub-facies and characteristics of the sedimentary system, and the spatial arrangement is identified combined with the current exploration practice. On this basis, the distribution characteristics of the sedimentary system and the spatial configuration of the "steep slope type-gentle slope type-straight type " are figured out.

KEYWORDS:
Northern Songliao Basin, Quantou Formation, Sedimentary system, Spatial Configuration

INTRODUCTION

Main oil layers of some mature blocks such as Daqing placentcline of the Songliao Basin has entered the production later stage, showing a highly uneven situation with a high water cut after more than fifty years of exploration and development while some of the new reservoir and the new blocks to be implemented is facing a series of problems such as low degree of exploration, lack of resources, low degree of the overall understanding of the exploration and other issues [1-4]. In view of this problem, the focus of the future production of oil and gas Songliao Basin are addressed in two aspects: one is to improve the reservoir development rate of old oilfield, and the other is to speed up the exploration process of the new blocks. The former is not the focus of this article. Most of the geological scholars are more concentrated in the structure, deposition, reservoirs and plays of the main reservoir (shallow to mid-deep layer), and other non-main reservoirs report less from the current survey. At present, the exploration and development activities of the Quantou Formation in Songliao Basin are still in the mid-deep sections of the Quantou Formation in the third and fourth members, low-yielding gas flow and oil and gas show are found in the Suileng anticline zone and the central depression area in the northeastern uplift area [5-7]. However the first and second members of Quantou Formation is still in the stage of low exploration and less well locations. So there are a lot of gaps in the reservoir research, especially the depositional characteristics of the reservoir have not been systematically studied. In view of this deficiency, it is necessary to carry out in-depth study on non-core reservoirs. Based on the previous studies of the Songliao Basin and the analysis of the drilling and logging data, the sedimentary system with fluvial facies as the main sedimentary facies in this group is revealed, and the sedimentary characteristics and spatial distribution of the first and second members of Quantou Formation in the northern Songliao basin are discussed with a view to provide the basis in the future deep oil and gas exploration and development of Songliao Basin.
**MATERIALS AND METHODS**

**Geologic setting.** The Songliao Basin is a Mesozoic large-scale fault-off basin between Siberean craton and northern China with a length of 750 km and a width of 330 to 370 km and an area of about $26 \times 10^4$ km$^2$. There are six primary tectonic units and Cretaceous as the main oil and gas layers [8-11]. The northern part of the Songliao Basin bounded by the Heilongjiang Jilin boundary mainly includes five primary tectonic units, the western slope area, the northern dumping area, the central depression area, the northeastern uplift zone and the southeast uplift area (Fig. 1a). The total proven oil reserve in the northern part of the Songliao Basin is about $60 \times 10^8$t and the natural gas geological reserve is about $2700 \times 10^8$ m$^3$, among which the potential of deep natural gas resources is $1200 \times 10^8$ m$^3$ and the potential for oil and gas resources is huge [12].

The tectonic evolution of the Songliao Basin includes three stages: fault depression, depression and inversion. From bottom to top it can be divided into: faulted fault layer composing of lower Cretaceous Huoshiling Formation (K$_1$h), Shahezi Formation (K$_1$sh), Yingcheng Formation (K$_1$y), Quantou Formation (K$_1$q) and Upper Cretaceous Qingshankou Formation (K$_2$qn), Yaojia Formation (K$_2$y), Nenjiang Formation (K$_2$n), Sifangtai Formation (K$_2$s), Mingshui Formation (K$_2$m) and the Cenozoic [13] (Fig. 1b, Fig. 2). In the early stage of the Lower Cretaceous depression in the Songliao basin, the large fluvial facies of the Quantou Formation are mainly developed. The sedimentary strata is interactive overlaid with the large river, delta sandstone, the large lacustrine mudstone, the oil shale with a thickness of 1084 ~ 1800 m [14-16]. We mark the lithology as the dark purple, purple brown mudstone gray, purple gray sandstone and gray sandstone.

![FIGURE 1](image)

Division of tectonic units (a) and profile (b) of northern Songliao Basin
(a: Cretaceous water system direction reference Feng et al., 2010 [13] b: Position shown in Fig. a, A-A ’)
Materials and analytical methods. Study data such as seismic, drilling, logging& core, cuttings and other data are provided by Daqing Petroleum Exploration and Development Research Institute. The logging data of 107 wells were analyzed, mainly based on natural gamma curve and resistivity curve, among which 26 wells were obtained core and cuttings data, and the heavy mineral and lithologic grain size analyses were carried out.

Heavy mineral study is based on the traditional analytical method. 1335 heavy mineral samples of the first and second members of Quantou Formation of the northern Songliao Basin are separated and identified by the thin film identification and oil immersion method [17, 18]. The main processing workflow of the sample is as follows: select the fine sand with a particle size between 0.125 and 0.063 mm, remove the impurity at a concentration of 5 g/L sodium metaphosphate solution [19], separate heavy mineral in tribromomethane by stereomicroscope and polarizing microscope at a specific gravity of 2.86 g/cm³ [20], make clear the relative content of the various particles in the mineral. We also performed particle statistical analysis, and each sample counts 500 to 600 mineral particles [21].

The lithology particle size was completed by sieving. The samples were treated with the addition of 30% hydrogen peroxide and 10% dilute hydro-
chloric acid to remove the organic matter and carbonate and repeatedly washed with water to dry and dried [22, 23], grinding to the particles completely spread, sieve in the standard about 10 minutes, and then graded weighing, calculate the relative content of each grain level.

Among them, the source of sediments can be effectively identified by heavy mineral assemblage and formation sand ratio data, and clear the main source direction and distribution characteristics of the whole Songliao Basin. The logging curve response feature can accurately identify the type of sediments, which is combined with the lithologic grain probability curve to ensure the accuracy of the sedimentary system of the first and second members of Quantou Formation in the overall understanding and specific division.

RESULTS AND DISCUSSION

Sediment source analysis. Six major river systems and provenances are developed in the Cretaceous of Songliao Basin, according to the previous research, Nehe River system, Qiqihaer River system and the Baiquan River system is the northern provenance, Baicheng water system and Changchun river system are the southern provenance, and the three large northern water systems control the sedimentary effects of the northern Songliao Basin (Fig. 1).

Heavy mineral assemblage. Heavy minerals can be used to efficiently record the provenance orientation and the geological setting of the provenance area, which are thus widely used for sediment provenance analysis [24, 25].

FIGURE 3
Heavy mineral distribution map of the first member (a) and the second member (b) of the Quantou formation of the northern Songliao Basin
(The heavy mineral boundary is shown in Fig.1, a)
The main types of heavy minerals in the first and second members of Quantou Formation are zircon, crest, garnet, celite, chlorite, titanumite and magnetite. The whole area is mainly unstable minerals except for the Baiquan water system and the Baicheng water system. (Fig. 3). Heavy mineral assemblage is inherited in the vertical direction, especially in the western and northern provenance; contents are basically maintained consistent except for the relatively low content of titanium dioxide in the north. At the same time, the plane distribution shows a distinct zoning, and it has a good match with the distribution of water system in the basin, which proves the representative provenance orientation of the period indicating five major provenances and the six provenance branch.

During the depositional period of the first member of Quantou Formation, the provenance characteristics of the western Dewartite-Zircon-titaniumite magnetite heavy mineral assemblage, which is matched with the Qiquaer water system, is characterized by the combination of the bushite-zirconite-titaniumite and garnet compound; The provenance of the northeastern zircon is the zirconite-titaniumite and garnet compound; The provenance range and distribution characteristics of the sand ratio in the first and second members of Quantou Formation have a good agreement with the provenance direction determined by the assemblage of heavy minerals.

During the sedimentary period of the second member of Quantou formation, the heavy mineral assemblage in the west and the north is the same as that in the period of the first member of Quantou Formation. Baiquan water system in the northeast refers to Zirconite-white titanium assemblage; Changchun water system in the south refers to boulder - zircon - garnet / magnetite heavy mineral assemblage (Figure 3, b).

**Formation sand rate.** The distribution of the high sand content area and its separation effect on the low value can effectively determine the provenance range and distribution characteristics [26]. The distribution of sand content in the first and second members of the Quantou Formation of the northern Songliao Basin is characterized by a decreasing tendency from the edge of the basin to the central basin, and the central depression is a low area. At the same time, the plane distribution characteristics of the sand ratio in the first and second members of Quantou Formation have a good agreement with the provenance direction determined by the assemblage of heavy minerals.

Among them, the sandstone distribution in the first member of the Quantou formation is characterized by strip shape and sheet shape with a decline from the inner to outer of the layers (Fig. 4, a). Sand ratios of the northern, eastern and southeastern areas have a higher range of about 40-60%, respectively up to 75%, corresponding to Nehe, Baiquan and Changchun water system provenance direction while the western and southwest have a relatively low range of about 20 to 40%. The performance of the film-like range of decline can identify two provenance direction respectively, corresponding to Qiquaer and Baicheng water system (Fig. 4, b), the distribution of the high values is
between 20 and 40%, and the highest in the southeastern region can reach 50%, but its zonation characteristics are similar to that of the first member of the Quantou Formation, and there is a clear boundary of the provenance.

Depositional environment and its type. The sedimentary facies of the first and second member of the Quantou Formation in the northern Songliao Basin include fluvial facies, alluvial facies, delta facies and lacustrine facies (Fig. 6, Fig. 7). Among them, the fluvial facies are the main sedimentary facies of the first and second members of the Quantou Formation (Fig. 6), the fluvial facies of the first member of the Quantou Formation is mainly braided river facies, and the second member of the Quantou Formation is mainly composed of meandering river facies. The two fluvial facies can be divided into three sedimentary sub-facies: channel, dike and river, and the effective identification of them is mainly based on the following three sedimentary features:

Grain size probability curve. The grain size probability curve of the meandering river is a two-stage curve, and the braided river is a three-stage probability curve. The overall probability of the rolling in the two-stage probability curve is basically not seen in well SuiShen4, well Dashen10 and well Linshen3, showing the combination of the two-stage jump and suspension features. The two overall cut-off points are between 3 and 3.5. The overall jump content of about 30% to 50%, the slope is higher between 55 ° ~ 60 ° (Figure 5, a). The rolling degree of the grain size probability curve is smaller than that of the jump and suspension, the overall jump is 50% and the suspension is about 35% in well Linshen4, well Linshen1 and well Linshen3 in the first member of Quantou Formation (Figure 5, b), representing the sedimentary characteristics of the small diversion channel or the upper part of the diversion channel.

(b) Lithology and grain size. The slope of the meandering river is small, so the transport form is dominated by suspended load and mixed load. Therefore, the sediments are fine and usually deposited by mud and sand. Meandering river contains more mudstones; its physical properties is poor, which exhibits the "sand in mud" lithological combination and develops staggered bedding. The braided river owns large content of sandstone, relatively large particle size, good physical properties, and more common horizontal bedding.

(c) Logging curve response. The meandering river GR logging curve is mainly box-shaped, local represent the finger-like features and mid-high resistivity curve amplitude shaped like smooth tongue. Braided river GR logging curve is more smooth in general, shaped like box. Resistivity curve is like tongue; tooth characteristics are obvious (Figure 6). At the same time, the meandering river perform more typical positive rhythm relative to braided river, rhythm thickness is small than braided river.

Well Suish 4 (2430.1–2430.2 m)    Well Dash 10 (2281.4–2281.5 m)    Well Linsh 3 (2313.0–2313.1 m)
(a) Grain size probability curve of meandering river

Well Linsh 4 (2580.7–2580.8 m)    Well Linsh 1 (2513.5–2513.6 m)    Well Linsh 3 (2657.4–2657.5 m)
(a) Grain size probability curve of braided river channel

FIGURE 5
Characteristics of probability curve of grain size of fluvial facies in the Quantou Formation of northern Songliao Basin
**Fluvial sedimentary profile of the first(a) and second (b)member of Quantou Formation of the northern Songliao Basin**

(Logging lithology and curve are chosen from well Linsh3)

**Meandering river sedimentary system. River sub-facies.** The river sedimentation is mainly composed of thick sandstones, occasionally existing grain sandstone sandwiches, the bottom has a scouring surface, the color is gray, and the silty siltstone is purple with visible interlaced and parallel bedding. Gamma logs are box-shaped or bell-shaped, and the natural potential curve is shown as a tooth-box pattern.

**Embankment sub-facies.** Embankment sub-facies often occur in the upper part of the river bed deposition, belonging to the top layer deposition relative to the river sub-facies. Due to the rising of the water level, the river in the flood period exceed the river bed, fine particles suspended in the river overflow river bed on both sides and deposit. Floods due to sudden increase in the amount of water, with a large area of water diffusion, rapid decline in hydrodynamic produce rapid sedimentary characteristics. Purple silty mudstone, sandstone development, sandstone generally thick 0.3 m ~ 0.6 m and calcareous tuberculosis phenomenon are easy to find in this area, in which wave-like bedding, horizontal layers were often developed and the natural potential curve was low tooth-shaped negative protrusion.
River diffuse sub-facies. This type of sedimentary facies is relatively simple, mainly the lithologic type of siltstone and mudstone. Mudstone sediments were purple-red or purple, visible significant calcareous mass, mudstone broken degree is high, common cracks. Gamma and resistivity logging curves are close to the base value, with silt position curve showed micro-dentate processes.

Braided river deposition system. Braided river sediments can be divided into gravel braided river and sandy braided river, the braided river sandstone is coarse grained, and from coarse to fine, it transitions from gravel sandstone to sandstone and siltstone.

River sub-facies. Mainly are (Sandy) conglomerate, (including gravel) sandstone, siltstone. Low maturity, with large plate-like, large groove-like interlaced bedding, the curve is more to box, Type-based, higher range.

Embarkment sub-facies. Braided river bank sub-facies is not developed well, purple muddy silt sandwich appear, the general thickness of 0.3 m ~ 0.6 m, the generally develop flat bedding and wave marks, gamma and resistivity logging curve was low value raised.

River diffuse sub-facies. Floods with large areas of the river, mostly purple, purple and red muddy siltstone and mudstone interbeds, gray, light gray and green siltstone, developed horizontal bedding and microwave horizontal bedding. Logging characteristics for the resistivity curve is low, but visible micro-tooth-like bulge.

Alluvial fan deposition system. The alluvial fan has the characteristics of near-provenance deposition, alluvial fan concentrated in the northern basin. From core observation and logging data of the first and second member of Quantou Formation it can be seen that alluvial fan lithology is mainly gray sandy conglomerate, the largest gravel diameter is about 6 cm, gravel messy arrangement, complex composition, low maturity, more gravel sand, no obvious bedding, logging curve for the box type, bell type (Figure 7). In the seismic section, the main features of the subfacies are mainly root-fan, mid-fan and marginal-fan sub-facies.
Root-fan sub-facies. The top of the alluvial fan is characterized by the high slope of the sedimentary slope; the rock is gravel; the particle size is the largest in the alluvial fan deposition; the inclusions are low in maturity; the singularity is rounded, and the logging curve is continuous type, tooth box type, where the resistivity curve range is large (Figure 7, a).

Mid-fan sub-facies. The deposition slope angle of mid-fan sub-facies is small. Lithology is mainly composed of gravel deposits, sandy content is high, logging curve is high (tooth) box type, bell type, rhythm obvious (Figure 7, b).

Marginal-fan sub-facies. The deposition slope angle is low; the type of deposition to diffuse-based; the lithology refers to the fine gravel and the coarse sandstone, and the sorting degree and grinding round are poor; logging curve is like the high box, (Fig. 7, c).

Delta deposition system. Delta can be divided into three sub-facies, delta plain, delta front, pre-delta.

Delta plain sub-facies. The delta plain is part of the delta above the lake, including a large number of riverbank between the large bifurcation position of the river and the lake. Areas from the river crossing point to the average high water level above the region belong to the plains on the delta. Coastline frequent swing area belong to the lower plains category while the lake basin is in the dry seasons between the flood period.

The lithology of the upper plain is dominated by sandstone, fine sandstone and siltstone. It is common to find large plate-like interlaced bedding, large-scale trough-like interlayer, parallel stratification, logging curve to box type and bell type. The sedimentary characteristics of the lower plain are similar to those of the upper plain, but the width of the river is narrower than that of the upper plain. The lithology refers to siltstone, argillaceous siltstone and silty mudstone. The logging curves are shown as long bells (Fig. 7, d).

Delta front sub-facies. The delta front is the part of the delta between the average low water level and the normal low water level. The leading edge of the delta is dominated by the underwater distributary channel. The lithology is dominated by sandy conglomerate, coarse sandstone, dark silt and muddy sandwich. The logging curve shows high amplitude box type, relatively bell type, but also dentate, straight (Figure 7, e).

The pre-delta sub-facies. The pre-delta is located outside the leading edge of the delta. The lithology is characterized by a large set of thick purple and gray shale deposits. Horizontal bedding and massive bedding can be seen, and electronically
measured curve is Smooth for baseline display (Fig. 7, f).

Lake sediments. The sedimentary period of the first and second members of Quantou Formation of the Songliao Basin is affected by the basement fault, and the depression basin is gradually formed. Only the central depression area has a settlement center and the limnetic facies is not developed. In this study, two sub-facies including lacustrine and flood plain are identified in the first and second member of Quantou Formation, but shallow lacustrine sub-facies only has lithologic and log response in the second member of Quantou Formation.

Shallow lake sub-facies. Thick layer of gray mudstone with thin layer of silty sandstone interbedded; high structural maturity; resistivity and gamma-ray logging curve form is low. The argillaceous siltstone development location curve appears to be dactyline. The gamma curve is relatively low (Figure 7, g); The core distribution is relatively uniform and horizontal bedding is developed.

Flood plain sub-facies. Large area of flood plain appears in the central depression area, because the slope of the depression is relatively shallow and the water level is shallow during the period of the first and second members of Quantou Formation; sediments of the coarse debris from the lake shore is close to the surface and the oxidation is strong. Purple mudstone, purple-red mudstone and gray muddy siltstone is developed, mudstone thickness is larger, logging curve is dactylic (Fig. 7, h). The core distribution is uniform, the lithology is sorted, and the grinding is round; there is no obvious laminae in the core observation, and occasional wavy bedding is found.

Depositional System and Its Spatial Distribution. Plane distribution of sedimentary systems. Affected by the distribution of the source plane, the first and second members of Quantou Formation are characterized by cyclic sediment (Figure 8). There are five main provenances during the deposition of the first and second member of Quantou Formation, in which the first member of Quantou Formation shows six provenance branches (Figure 8, a). The western slope area, the northeastern uplift area and the southern area are short provenance sediment, in which the western slope area, the northeastern uplift area is covered by basic depositional characteristics of most area of the structural area. The northern dumping area is wide strip long provenance with eight branches in the second member of Quantou Formation (Figure 8, b), the deposition capacity is relative poor and main provenance is in the north dumping area. Both of the two sedimentary periods have a sedimentary system of the alluvial fan-braided river-meandering river-delta-flood plain. Braided fluvial facies are developed in the first member of Quantou Formation and meandering river facies are developed in the second member of Quantou Formation. Delta deposits are widely distributed and are the main reservoirs of oil and gas in this period.

During the depositional period of the first member of Quantou Formation, alluvial fan deposition is located near the edge of the basin. The floodplain deposits are mainly located in the central depression, and the river facies are parallel to the five water systems (north and south) and fans (west and northeast). The meandering river facies of provenance direction of northern dumping area and the northeastern uplift area is lake-inlet sediments, and the northern meandering fluvial facies deposit extends from north to south in all lake basin; lacustrine-inlet delta facies are developed with the meandering river facies of southeast uplift provenance area.

The sedimentary system of the second member of the Quantou Formation is characterized by a stripe-like distribution with the five water systems, and the northern dumping area is the main provenance of this period. In this period, the alluvial fan is limited in the northern part of the basin, and the sedimentary area of the basin is shrinking. The shallow lake facies are found in the southwest direction. The braided river is developed poorly, representing a short distance edge deposition. The meandering river facies are developed well in general, representing a remote provenance sedimentary features. The river channel is banded, and the local deposition curvature is bigger. Delta facies are significantly developed in this sedimentary system, which is mainly shown as the lake-inlet delta.

Depositional system and its spatial configuration. The basin extension is weakened during the period of Quantou Formation, and the regional subsidence is started. The center of the settlement is located in the central depression and is shown as the “high north-low south” tectonic characteristics. The first and second members of Quantou Formation controlled by the tectonic movement represent a combination sedimentary mode of the "steep slope type - gentle slope type - straight type" with the inheritance and regularity. The steep slope type is the alluvial fan deposition at the edge of the basin, and the gentle slope type is the river facies sedimentary assemblage of the slope area and the uplift area. The straight type is the characteristics of the sedimentary system in the north dumping area.

During the depositional period of the first member of Quantou Formation, the sedimentary range of the steep slope type alluvial fan is large, and it is located in the northern boundary of the study area, which is short-distance deposition, but the distribution range is wide. The western slopes
are similar to those in the southeastern uplift, and are in the long-term regional monoclinic form in the process of basin development [27]. The gentle slope deposition is the major sedimentary type, and sedimentary facies refer to braided river facies and meandering river facies. The main features are that a plurality of braided and meandering river frequently swing and cross convergence, forming a large area of river sand with conditions of broad terrain and sufficient material supply and river sand superimposition features are obvious, sandstone thickness is big and lateral continuity is strong.

The sedimentary development of the steep slopes of the second member of Quantou Formation is limited, only in the northern part of the basin, and the sedimentary model is mainly gentle slope type. Although the inheritance of the first member of Quantou Formation is obvious, the river sedimentation is weakened. Degree of development of the sand body is deteriorated. Deposition is short-distance provenance in general. The straight type of the first and second members of Quantou Formation is strong intergrade in the vertical direction, and the characteristic change is not significant, which shows that the river channel is stretched to the central depression in the form of wide stripes, extending from south to north through the entire northern Songliao Basin.

CONCLUSION

The method of comprehensive analysis of heavy mineral and Formation sand ratio yields to five main provenances of the first and second member of the Lower Cretaceous Quantou Formation in northern Songliao Basin including six provenance branches of the first member of Quantou Formation and eight provenance branches of the second member of Quantou Formation distributed around the basin. The distribution of the source in the plane shows a clear inheritance. The northern part is the main provenance of the study area in this period and its sand ratio is relatively high to the whole with a large extension length from north to south.

Five sedimentary facies and eleven sedimentary sub-facies are identified in this paper. Sedimentary facies of the first and second member of Quantou Formation is mainly river facies, in which the first member of Quantou Formation is braided river facies and the second member of Quantou Formation is meandering river facies. Alluvial fan facies are mainly developed at the edge of the basin. The central depression is the main subsidence area, with a large area of lacustrine deposits and delta facies and the lacustrine sedimentary area in the second member of Quantou Formation is smaller.

The combination sedimentary mode of the first and second member of the Quantou Formation is "steep slope type - gentle slope type - straight type" with the inheritance and regularity. The alluvial fan is extensively developed in the first member of the Quantou Formation, and the gentle slope type is a large-scale braided river and meandering river channel transiting to a short-distance deposition of a single channel during the period of the second member of the Quantou Formation. The vertical sedimentary model of the northern provenance yields to the characteristics of strong vertical transition, which are wide stripe-like extending to the central depression.

ACKNOWLEDGEMENTS

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OCCURRENCES AND SORPTION OF FLUOROQUINOLONE ANTIBIOTICS IN VEGETABLE FARM SOIL FROM SICHUAN BASIN, CHINA

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ABSTRACT

The occurrence of four fluoroquinolone antibiotics (ciprofloxacin, enrofloxacin, norfloxacin, and ofloxacin) and sorption process of enrofloxacin in soils from the vegetable farms in Sichuan Basin, southwestern China, were investigated in this study. Concentrations of target antibiotics in the soil samples ranged from not detectable to 29.8 µg/kg dry weight. Enrofloxacin was the dominate fluoroquinolone antibiotics in soil samples from vegetable farms with the highest average concentration (1.73 µg/kg) and detection frequency (81.7%). Enrofloxacin was rapidly adsorbed by purple soil with high sorption coefficients. Sorption isotherms were well fitted by Freundlich model. Solution pH strongly affected the sorption process. When solution pH was increased from 5 to 10, the sorption capacity and coefficients of enrofloxacin in the purple soil declined by 19.6% and 95.8%, respectively. The cation-exchange was the dominant sorption mechanism of enrofloxacin sorption in purple soil according to the sorption energy and ionic strength effects. The sorption process showed the high sorption affinity of fluoroquinolone antibiotics in purple soil, which may cause the accumulation of fluoroquinolone antibiotics in soil.

KEYWORDS:
Fluoroquinolone antibiotics, Enrofloxacin, Soil, Concentration, Sorption

INTRODUCTION

Fluoroquinolone antibiotics (FQs) are extensively used to treat various bacteria-related diseases in humans and animals and elevate livestock productivity [1, 2]. Ciprofloxacin (CIP), Enrofloxacin (ENR), Norfloxacin (NOR), and Ofloxacin (OFL) are the commonly used categories of FQs. Except ENR is only used in veterinary antibiotic, CIP, NOR, and OFL are the medicine for human and animal. In 2013, CIP, ENR, NOR, and OFL consumption in China was 5340 tons, 5180 tons, 5440 tons, and 5510 tons, respectively, approximately 4/5 of the total FQs usage in China [3]. The occurrence of FQs in the environment may contribute to the propagation of antibiotic-resistant genes (ARGs) and may thus present long-term risks to human and ecological health [4, 5].

FQs is discharged into the soil environment mainly through the excretions of animals. FQs in body cannot be totally metabolized by animals. Approximately 40%-80% of FQs was eliminated from the body through excretions [1]. The residual FQs concentration in manure was several mg/kg [4, 6]. Presently, no regulations address the presence of antibiotics in the manure applied to agricultural production as well as in soil. Soil fertilized with manure and irrigated with wastewater may have a potential continuous accumulation of FQs in soil [2, 7]. Previous studies reported that FQs was frequently detected in agricultural soils with high concentrations and detection frequency in China. Wu et al. reported the presence of FQs in soils from organic vegetable farm in southern China with a mean concentration of 14 µg/kg and a detection frequency of 100% [8]. Li et al. found that the maximum concentrations of CIP and ENR in soils from greenhouse vegetable production bases in northern China were 253 µg/kg and 348 µg/kg, respectively [9], much higher than the ecotoxic effect trigger value (100 µg/kg) of veterinary medicine from the Food and Drug Administration [10].

FQs are slowly degraded in soil environment with a half-life between several weeks and several months [11, 12], and can exist as cationic, zwitterionic, and anionic species under various environmental conditions [13, 14]. FQs were adsorbed in soil with high sorption coefficients and sorption process was strongly influenced by the properties of soil, such as clay content, pH, organic matter content, and cation exchange capacity. Sorption mechanisms, such as physical sorption, cation exchange and surface complexation, may vary with soil type [13, 15-17]. Sorption behavior can significantly affect the transportation process of FQs in soil, and influence the distribution of FQs in soil and water environment [16, 18]. The sorption studies of FQs in literature inferred that FQs showed low leaching and highly immobile in soil [19-23]. However, the experiments in lab and field has stated that FQs can be migrated to deep soil and groundwater through seepage water or
runoff [24, 25]. In Beijing and Changzhou, China, ENR, NOR, and OFL were detected in all groundwater samples with a maximum concentration of 36.2–96.8 ng/L during long-term irrigation with reclaimed water [26]. Residual FQs could not only increase the risk of antibiotic contamination in soil and water environment, but also have adverse effects to human health through the consumption of agricultural productions [5, 27]. Therefore, the distribution and environmental behavior of FQs in soil environment should be given more attention.

Housing a 90 million population, the Sichuan Basin is located in southwest of China with area approach 170 thousand km² and provides a large number of agricultural products for people in Sichuan province and Chongqing. The major soil type in this area is purple soil, which is formed from a series of red or purple rocks from the Trias-Cretaceous system and classified as regosol taxonomies in Chinese soil classification system (GB/T 17296-2009, Chinese National Standards). Population growth and market demands of vegetable production have resulted in the expanding of vegetable farms (traditional farms, green- food, and organic vegetable farms) with different scales have expanded rapidly in Sichuan Basin. Large and repeated amounts of animal manure is always adopted under cultivation conditions to fertilize soil and achieve high vegetable production, which may cause the residues of antibiotics in the soils of vegetable farms and present in high levels [4,28]. Many studies in recent years has reported the occurrence of antibiotics in vegetable farm soil from the Pearl River Delta region and the Yangtze River Delta region [6, 8, 9, 29, 30]. However, few studies have been done on the general status of antibiotic pollution in agriculture soil of Sichuan Basin, one of the most important agricultural areas in China. The purpose of this study is to investigate the occurrence of four FQs compounds in vegetable farm from Sichuan Basin and explore sorption behavior of FQs in purple soil, one of the representative soil types and widely distributed in Sichuan Basin.

MATERIALS AND METHODS

Reagents and standards. Four FQs compounds (CIP, ENR, NOR, and OFL) of HPLC-grade purity was obtained from Aladdin biochemical technologies Co., Ltd. (Shanghai, China). Acetonitrile (HPLC grade) was purchased from Honeywell Burdick & Jackson (Cheoyong, Korea). Ultrapure water (18.25 MΩ/cm) was obtained from Ulupure UPH system (Chengdu, China). Other chemicals were of analytical grade and purchased from Kelong Chemical Co., Ltd. (Chengdu, China). A stock solution of FQs was prepared by dissolving accurate quantities of powdered standards in a water solution of 0.01 M CaCl₂, and stored in dark at 4 °C. The mass concentration of FQs in the stock solution was 500 mg/L, and used within 90 days. Working standard solutions were prepared from this stock by serial dilution.

Soil sample collection and characterization. In this study, vegetable farms in Pengzhou, Sichuan provinces (31°15′54.63″ N and 103°58′42.72″ E, altitude 620 m) and Tongnan, Chongqing (30°15′46.64″ N and 105°48′3.11″ E, altitude 380 m) were selected for soil sampling. Pengzhou and Tongnan are the major vegetable cultivation bases in Sichuan Basin and the vegetable output in 2016 was more than 3.2 million tons. A total of 82 soil samples from vegetable farms were collected in March 2017. Nine soil subsamples (depth 5-20 cm) were collected randomly from the field and then fully mixed to get one composite sample. Soil samples were picked into brown glass bottles and then transported to the laboratory immediately. The samples were freeze-dried under vacuum at -50°C using a freeze dryer (MudolYOD-230, Thermo Fisher, American) for 48 h, and then sieved with a 1-mm standard mesh before analysis.

The main physical and chemical properties of soil samples for sorption experiments were measured. Soil pH was measured in deionized water at a soil/solution ratio of 1: 2.5 using a pH meter (SL1000, HACH, USA). Cation exchange capacity of soils was determined by the EPA Method-9081 (EPA, 1998). Soil organic matter (SOM) was measured by a potassium dichromate oxidation spectrophotometric method (HJ 615 -2011, Chinese National Standards). The Fe, Al, Ca and K in soil were determined by X-ray fluorescence (XRF) spectrometry (Alpha4000, Innov-X System, USA). The particle size composition of soil samples was analyzed by method of Tang et al. [31]. The results based on a dry weight were as follows: 6.29 ± 0.24 % silt and 26.2 ± 6.1 % clay. Soil samples were silty loam with 22.7 ± 3.8 % sand, 51.1 ± 2.3 % silt and 26.2 ± 6.1 % clay.

Soil sample extraction and cleanup. The procedures of sample extraction and cleanup were conducted following the methods by Wu et al. [8], with small modification. One gram of soil sample was placed in a 10 mL glass centrifuge tube and extracted with 5 mL of 5% Mg (NO₃)₂ aqueous solution containing 4% aqueous ammonia. The tubes were vortexed (XW-80A, Haimen, China) for 5 min and extracted triplicate in an ultrasonic bath (KQ-200KDE, Kunshan Ultrasonic Instrument, China) for 10 min. Then, the extracts were centrifuged (TG20WS, Changsha Xiangzhi, China) at 5000 rpm for 10 min. The supernatants were concentrated to near dryness using a rotary evaporator (RE-2000, Shanghai,
China), and then was purified by solid-phase extraction device (SEP100, Hanon, China) with Oasis HLB extraction cartridges (3 mL/60 mg, Waters, USA). The cartridge was conditioned with 3.0 mL of methanol, followed by 3.0 mL of ultrapure water. The flow speed was set at 1.0 mL/min. The extracts were loaded onto the cartridges and then rinsed with 3 mL of 10% methanol solution and dried for 5 min. The cartridges were eluted with 3 mL 1% methanol at a flow rate of 1.0 mL/min. The eluate was collected and concentrated under a gentle stream of nitrogen gas to about 0.1 mL, and then dissolved in acetonitrile and 0.05 M phosphoric acid solution (10/90, V/V) to a final volume of 1.0 mL. The solution finally was filtered through 0.22 μm syringe filters (Tianjin Jinteng, China) for instrument analysis.

**Sorption experiments of ENR in soil.** The molecular structure of FQs are similar and contain carboxyl group and piperazinyl amine group. Therefore, the sorption process and mechanism of FQs in one soil type are usually consistent [15,17,18]. In this study, the mean concentration of ENR in soil samples was higher than CIP, NOR, and OFL (Table 1). Batch sorption experiments of ENR in purple soil were conducted to evaluate sorption process of FQs in purple soil. The soil samples for sorption experiment were collected from the above vegetable farms and initially free of ENR. Briefly, 100 mL of 0.01M CaCl$_2$ solution was added to 10 g of purple soil in 500 mL conical glass flasks. After an equilibration period of 12 h under stirring, 100 mL of solution containing ENR prepared in 0.01 M CaCl$_2$ was added. All the flasks were shaken in a constant-temperature horizontal shaker (ZHWY-2102C, Shanghai Zhicheng, China) at 200 rpm and kept in dark. The pH automatic regulator (GS-pH02, Shanghai Goodshine, China) was applied to control the solution pH at predetermined pH value in sorption procedure by adding acid 0.01M NaOH and 0.01M HCl. The temperature was kept at 25 °C. After sorption experiments were completed, the mixtures in tubes were centrifuged (TG20WS, Changsha Xiangzhi, China) at 5000 rpm for 10 min. The supernatants were collected and filtered through 0.22 μm syringe filters. ENR in aqueous phase was analysed by HPLC. All experiments were conducted in duplicate.

In kinetics experiments of ENR sorption in purple soil, the initial concentration of ENR in the solution were 5 mg/L and 20 mg/L with solution pH value of 6. The analyses were performed after 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 h of contact time. Eight different concentrations of ENR solutions (0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, and 20.0 mg/L) were used to determine the sorption isotherms with different CaCl$_2$ concentration (0.001 M, 0.01 M, and 0.05 M) in an acid solution (pH=6). The mixture was subjected to continuous shaking for 8 h, respectively. The influence of solution pH and SOM on ENR sorption in purple soil were also investigated. A mixture containing 10 g of purple soil and 200 mL of 10 mg/L ENR solution were subjected to shaking for 8 h. For solution pH effect, the pH values of ENR solution were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. For SOM effect, the purple soil with different SOM content were firstly prepared following the method of Nam et al. [32], with small modification. Briefly, SOM was removed using H$_2$O$_2$ with solution to solid ratio of 10:1 (mL:g) at 25°C in 1 h ~ 5 h to form purple soil with different SOM content. Afterwards the residual soils were washed using distilled water and air-dried for batch sorption experiments.

**HPLC analyses.** CIP, ENR, NOR, and OFL was determined by HPLC equipped with a UV detector (Chromaster, Hitachi, Japan). The detection wavelength was 294 nm of OFL and 280 nm of CIP, ENR, and NOR. The oven temperature was set to 30 °C. The HPLC column was Hitachi Eclipse Plus C18 column (5um, 2.1 × 150 mm). Analyses were performed at a flow rate of 1 mL/min, and a sample injection volume of 0.02 mL. Pure acetonitrile (A) and 0.05 M phosphoric acid solution (pH adjusted to 2.4 by triethylamine) (B) were used as the mobile phase. The proportion of A:B for the determination of target antibiotics was 10:90 (v/v). The retention time of CIP, ENR, NOR, and OFL was 4.8 min, 6.1 min, 10.2 min, and 6.8 min, respectively.

**Data analysis.** The sorption capacity of ENR in soil was calculated on the basis of mass balance and by using Eq. (1).

\[
Q_t = (C_0 - C_t) \cdot V/M
\]

where $Q_t$ is the sorption capacity (mg/kg) at time $t$, $C_0$ and $C_t$ (mg/L) are initial and aqueous phase concentrations at time $t$, respectively, and $V/M$ (mL:g) is the solution to solid ratio.

The sorption coefficients $K_d$ (L/kg) values were calculated as follows:

\[
K_d = Q_e/C_e
\]

where $Q_e$ is the equilibrium sorption capacity (mg/kg), and $C_e$ is the equilibrium concentrations in the aqueous solution (mg/L).

Model fitting and statistical analysis were conducted using SigmaPlot 12.5 and SPSS 13.0, respectively.

**RESULTS AND DISCUSSION**

**Concentration of FQs in soils.** Four FQs compounds (CIP, ENR, NOR, and OFL) in vegetable farms soil samples from the Sichuan Basin were analyzed and shown in Table 1. ENR was detected in more than 80% of soil samples. The detection frequency of OFL was lowest (47.6%) among for the four FQs compounds. The maximum concentration for ENR was 29.8 μg/kg, higher than that for CIP, NOR, and OFL. The average concentrations of individual compounds ranged from 0.62 to 1.73 μg/kg,
However, the FQs concentrations in soils from vegetable farm in research area were not in high levels. The low FQs concentrations in soils could be attributed to local climate, fertilization practices, and planting age. The long-term rainy weather in Sichuan Basin caused high moisture in soil. The temperature in research area ranged from 10°C to 40°C over the years. The high moisture and temperature may accelerate biodegradation rate of antibiotics in the soils [34]. Furthermore, fertilizer types and dosage would affect the behavior and fate of FQs in the soil [35, 36]. The dominant organic fertilizers used in research area were composted cow and pig manure, which usually contained a lower level of residual FQs [4-6, 33]. The application of chemical fertilizers in cultivation could decrease the FQs accumulation in vegetable farms soil. In addition, the planting ages also affected the FQs residues in soil [8, 37]. The research vegetable farms were established in 2009 with a short history for continuous vegetable cultivation.

ENR sorption in soil. Kinetics of sorption. The effect of contact time on the sorption of ENR in purple soil were studied with the initial ENR concentrations of 5 mg/L and 20 mg/L (Fig. 1). The data obtained from the sorption of ENR in purple soil showed that 90% of the amount of ENR in solution was absorbed by purple soil in 3 h. Furthermore, a contact time of 6 h was sufficient to achieve equilibrium, and the sorption capacity did not change significantly at increased contact time. A fast sorption process of FQs in soil and sediment was observed within the first 10 h [13, 20, 38]. The equilibrium sorption capacity of ENR in purple soil was 99.1 mg/kg at initial ENR concentrations of 5 mg/L. When the initial ENR concentration was increased to 20 mg/L, the equilibrium sorption capacity of ENR in purple soil was 393 mg/kg. When the sorption reached equilibrium, the \( K_d \) values of ENR in purple soil were 2305 L/kg and 1179 L/kg at initial ENR concentrations of 5 mg/L and 20 mg/L, respectively. The \( K_d \) values of ENR in purple soil were similar with literature data, ranging between 260 L/kg and 5160 L/kg according to different soil types [18, 39, 40]. Leal et al. reported that the \( K_d \) value of ENR in Brazilian soil was 40 656 L/kg at initial ENR concentration of 2.72 mg/L, which was much higher than that obtained from this study [20].

### TABLE 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>NOR</th>
<th>CIP</th>
<th>ENR</th>
<th>OFL</th>
<th>Total FQs</th>
</tr>
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<tbody>
<tr>
<td>Detection frequency (%)</td>
<td>58.5</td>
<td>74.4</td>
<td>81.7</td>
<td>47.6</td>
<td>91.5</td>
</tr>
<tr>
<td>Minimum</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Maximum</td>
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<td>18.4</td>
<td>29.8</td>
<td>17.0</td>
<td>42.6</td>
</tr>
<tr>
<td>Average</td>
<td>0.98</td>
<td>1.41</td>
<td>1.73</td>
<td>0.62</td>
<td>4.75</td>
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Percentage distribution (%)

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<tr>
<th>Concentration (μg/kg, dry weight) of FQs in soil from vegetable farms</th>
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**Concentrations (μg/kg, dry weight) of FQs in soil from vegetable farms**

- **NOR**: Norfloxacin
- **CIP**: Ciprofloxacin
- **ENR**: Enrofloxacin
- **OFL**: Ofloxacin

**Detection frequency (%)**

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  - NOR: 58.5%
  - CIP: 74.4%
  - ENR: 81.7%
  - OFL: 47.6%

**Minimum**

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- CIP: ND
- ENR: ND
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**Maximum**

- NOR: 23.5 μg/kg
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For the evaluation of the kinetic sorption mechanism, the pseudo-first-order model, pseudo-second-order model and two-compartment-first-order model were applied to fit the sorption kinetic data [41, 42]. These models have the following expressions:

Pseudo-first-order model:
\[
\ln(Q_e - Q_t) = \ln Q_e - k_{1F}t/2.303
\] (3)

Pseudo-second-order model:
\[
t/Q_t = 1/(k_{2S} Q_e^2) + t/Q_e
\] (4)

Two-compartment-first-order model:
\[
Q_t/Q_e = f_1 \left(1 - e^{-k_1 t}\right) + f_2 \left(1 - e^{-k_2 t}\right)
\] (5)

where \( k_{1F} \) and \( k_{2S} \) are the rate constants of the pseudo-first-order model and pseudo-second-order model (1/h), respectively, \( k_1 \) and \( k_2 \) are the rate constants of the two-compartment-first-order model (1/h), \( f_1 \) and \( f_2 \) represent the fractions of the two-compartment-first-order model and \( f_1 + f_2 = 1 \).

The fitted parameters of these kinetic models are presented in Table 2. Based on the \( R^2 \), the pseudo-second-order model was a better fit (\( R^2 >0.998 \) ) compared with the pseudo-first-order model two-compartment-first-order model with respect to the kinetics of ENR sorption, suggesting that sorption was governed by the availability of sorption sites on the surfaces of purple soil particles instead of the ENR concentrations in solution. The pseudo-second-order model also exhibited better performance in the sorption kinetics of other FQs, such as NOR and OFL adsorbed by soils [22, 42]. The two-compartments-first-order model also had a good fit on ENR sorption in purple soil. The ratios of \( k_1/k_2 \) varied from 26 to 77, suggesting distinct sorption characteristics of the fast and slow compartment. The fast compartment can be attributed to the exposed surface functional groups of the soil particles. The slow compartment may be the inner pores of inorganic fractions and the matrix of organic matters [42].
ENR concentrations with different CaCl₂ concentrations (L-shape) were observed for the investigated results were shown in Fig. 2. Non-linear isotherms of ENR in purple soil at 25 °C, and mg/L were selected for the acquisition of the sorption processes [43]. The above-mentioned equation was provided by the following relationship:

\[ Q_e = Q_m \exp \left(-\beta \varepsilon^2 \right) \]  

where \( Q_e \) is the equilibrium solid phase concentration (mg/kg), \( Q_m \) is the theoretical saturation capacity (mg/kg), \( \beta \) is the constant of the sorption energy (mol²/kJ²), and \( \varepsilon \) is the Polanyi potential, which is described as [43]:

\[ \varepsilon = RT \ln(1 + 1/C_p) \]  

The parameters of the two sorption isotherm models are listed in Table 3. The \( R^2 \) values of Freundlich isotherm model showed a higher value than that obtained in the Dubinin-Radushkevich isotherm model, suggesting that the sorption of ENR in purple soil was not the monolayer molecular sorption. The magnitude of the exponent \( n \) reflected the favorability of sorption. The \( n \) values (0.689-0.728) indicated that the sorption of ENR in purple soil was favorable and the high energy sites on soil particles were occupied first followed by sorption at lower energy sites [44]. The purple soil exhibited a good potential to adsorb ENR. The Freundlich affinity coefficient, \( K_F \), ranged from 312 mg⁻¹·L¹·kg⁻¹ to 567 mg⁻¹·L¹·kg⁻¹. The high \( K_F \) values indicated that ENR was highly adsorbed by the purple soil [22].

### Table 3

The sorption isotherms of ENR in purple soil at different CaCl₂ concentration

<table>
<thead>
<tr>
<th>CaCl₂ concentration</th>
<th>Freundlich</th>
<th>Dubinin-Radushkevich</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001M</td>
<td>0.704</td>
<td>567</td>
</tr>
<tr>
<td>0.01M</td>
<td>0.689</td>
<td>558</td>
</tr>
<tr>
<td>0.05M</td>
<td>0.728</td>
<td>312</td>
</tr>
</tbody>
</table>

Sorption isotherms. Sorption isotherm is crucial in understanding the nature of the interaction between a sorbate (ENR) and sorbent (purple soil). ENR concentrations ranging from 0.5 mg/L to 20 mg/L were selected for the acquisition of the sorption isotherms of ENR in purple soil at 25 °C, and the results were shown in Fig. 2. Non-linear isotherms (L-shape) were observed for the investigated ENR concentrations with different CaCl₂ concentration. The data obtained from the sorption experiments were fitted to the Freundlich and Dubinin-Radushkevich isotherm models to elucidate the sorption process of ENR in purple soil [22, 43, 44]. Based on sorption occurring on a heterogeneous surface with non-uniform distribution of sorption heat and affinities through a multilayer sorption, the widely used empirical Freundlich isotherm can be expressed as:

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

where \( K_F \) is the Freundlich affinity coefficient for indicating relative sorption capacity of the sorbent (mg⁻¹·L¹·kg⁻¹), and \( 1/n \) is the heterogeneity factor.

The Dubinin-Radushkevich isotherm, a two-parameter model, was used to estimate the apparent free energy of sorption and to determine the difference between physical and chemical sorption processes [43].
To further study the nature of the sorption process, the sorption energy ($E$) of ENR sorption in purple soil were evaluated by the experimental data in Table 3. The value of sorption energy $E$ can be correlated to $β$, which is a parameter of the Dubinin-Radushkevich isotherm model and was calculated using the following equation [43]:

$$E = 1/\sqrt{2β} \quad (9)$$

The sorption energy $E$ provides information on whether the sorption mechanism is physical sorption ($E < 8$ kJ/mol) or cation-exchange (8 kJ/mol $< E < 16$ kJ/mol) [43, 44]. In the purple soil subjected to different CaCl$_2$ concentrations, the values of $E$ evaluated from Eq. (9) ranged from 8.43 kJ/mol to 9.22 kJ/mol, larger than 8 kJ/mol. It indicated that the sorption mechanism of ENR in purple soil might be dominated by cation-exchange.

**Solution ionic strength effects.** The influence of solution ionic strength on ENR sorption in purple soil was investigated at 0.001M, 0.01M, and 0.05 M CaCl$_2$. As shown in Fig. 2, the ENR sorption in purple soil was strongly affected by CaCl$_2$ concentration. The sorption capacity and sorption coefficients of ENR in purple soil was decreased as the CaCl$_2$ concentration increases. The $Q_e$ and $K_d$ were 396 mg/kg and 2142 L/kg at initial ENR concentrations of 20 mg/L with 0.001M CaCl$_2$, respectively. As the background CaCl$_2$ concentration increased from 0.01M to 0.05 M, the $K_d$ decreased from 1180 L/kg to 401 L/kg at initial ENR concentrations of 20 mg/L. The results obtained from this study and literatures demonstrated that the sorption of FQs compounds in soil depended on the solution ionic strength [13, 22, 38]. As strongly hydrated exchangeable cations, Ca$^{2+}$ retain a relatively large hydrated radius and thereby occupy more unobscured hydrophobic regions of the soil particle surface. This hydrophobic region is considered to be the primary domain for the sorption of weakly polar organic contaminants [45]. High concentration of CaCl$_2$ in solution caused strong competitive sorption between ENR and Ca$^{2+}$. The proposed cation exchange mechanism for ENR sorption was also supported by the strong ionic strength dependence. The sorption of FQs in soil and clay mineral was not only affected by solution ionic strength, but also significantly influenced by the nature of exchangeable cation. Yan et al. found that the sorption capacity of ENR in montmorillonites with 0.01M KCl was larger than that with 0.005 M CaCl$_2$ due to the different estimated hydrated radii of exchangeable cation [13].

**Effect of solution pH.** The sorption of FQs was strongly affected by the pH of aqueous solution. The $Q_e$ and $K_d$ values of ENR in purple soil were determined under different solution pH (Fig. 3). High sorption capacity of ENR was obtained at initial solution pH ranging from 4 to 6, and more than 96% of ENR was adsorbed by the purple soil. The highest $K_d$ value of ENR was 1905 L/kg in acid purple soil at an initial solution pH of 5. When solution pH was increased from 6 to 10, the $Q_e$ of ENR in the purple soil declined by 18.9%, and the $K_d$ value of ENR apparently decreased from 989 L/kg to 78 L/kg. Although the $K_d$ values of ENR in purple soil were low at initial solution pH of 10 in this study, more than 76% of ENR was adsorbed by purple soil. Yan et al. also found that the solution initial pH significantly affected ENR sorption in clay minerals (containing montmorillonite and kaolinite). The highest sorption capacity of ENR in clay minerals was observed at initial solution pH of 5.5 [13].

ENR is an amphoteric molecule containing a carboxyl group and piperazinyl amine group with two different $pK_a$ values: carboxylic ($pK_{a1} = 6.27$) and amino ($pK_{a2} = 8.30$) [21, 46]. A great fraction of dissolved ENR is cationic form at pH of $< 6.27$ because of the protonation of the piperazinyl amine group, meanwhile, over 60% of the anionic species of ENR are observed at pH of $> 8.3$ because of the deprotonation of the carboxyl group [13]. The protonation of the piperazinyl amine of ENR generates an appreciable amount of cationic ENR species in the acid pH range, which is subsequently adsorbed at the soil-water interface through the cation-exchange [16]. Meanwhile, the surface complexation and cation bridging may also contribute to the ENR sorption in soil, especially in the neutral and alkaline conditions. Vasudevan et al. investigated the sorption of ciprofloxacin in 30 soils from the United States and stressed out that even cation bridging and surface complexation contribute to the sorption, cation-exchange appeared to be the soil factor with the greatest influence on the magnitude of the sorption coefficient at all pH-values evaluated (pH 3-8) [14].

**Effect of SOM.** As the main active component in the nature soil, organic matter contains large numbers of the functional group and provide sorption sites. The quantity and the composition of SOM may impact the sorption of antibiotics in soil. Figueroa-Diva et al. reported that organic matter in soil had little contribution to FQs (NOR, CIP and ENR) sorption in soils [19]. However, Teixido et al. demonstrated that the addition of humic acid, a typical SOM, to agriculture soil samples apparently increased the magnitude of ENR sorption [23].

The effect of SOM on ENR sorption in purple soil was shown in Table 4. The $Q_e$ and $K_d$ value of ENR in purple soil with original SOM content were 194.1 mg/kg and 657 L/kg at initial ENR concentration of 10 mg/L, respectively. After a part of SOM in purple soil was removed, the $Q_e$ of ENR in purple soil fluctuated between 194 mg/kg and 197mg/kg. And the $K_d$ were ranging from 600 L/kg to 1000 L/kg. The relationship between ENR sorption coefficients and SOM content was also analyzed. The correlation analysis between $K_d$ and SOM content provided a correlation coefficient of 0.727,
suggesting that SOM may have limited effect on ENR sorption in purple soil.

Although the FQs can be adsorbed by SOM through cation-bridging, electrostatic interaction and H-bond [46], SOM may compete with FQs for sorption sites on clay minerals and SOM removal may expose more sorption sites for FQs sorption [18,47]. Moreover, the content of SOM is generally low in the nature soil. Therefore, SOM is not always the main contributor for antibiotics sorption in soil. The mineral fractions in soil also showed comparable or even higher sorption than organic matter. The metal oxides exposed on mineral particles, particularly Fe and Al, also play an important role in FQs sorption through interaction with the carboxyl group of the FQs on metal oxide surfaces [14, 17].

![Graph A](image)

**FIGURE 3**
ENR sorption in purple soil (A) and ENR fraction (B) under different solution pH

<table>
<thead>
<tr>
<th>Table 4</th>
<th>The ENR sorption in purple soil with different SOM content</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOM Content (g/kg)</td>
<td>47.5</td>
</tr>
<tr>
<td>$Q_e$ (mg/kg)</td>
<td>194.1±2.8</td>
</tr>
<tr>
<td>$K_d$ (L/kg)</td>
<td>744±362</td>
</tr>
</tbody>
</table>
CONCLUSION

Four FQs compounds were detected in soils from organic vegetable farms in Sichuan Basin, China. The concentrations of individual compounds ranged from ND to 29.8 μg/kg, less than the ecotoxic effect trigger value (100 μg/kg) set by the Food and Drug Administration. ENR was the dominate compound of FQs in soils. The climate and short planting age caused the relatively low level of FQs in the soils from vegetable farms. ENR was quickly adsorbed by the purple soil and found to display high sorption affinity with high \( K_f \) values. The sorption isotherm experimental data were well-fit by the Freundlich isotherms model. The sorption process was apparently affected by solution pH and ionic strength. High sorption capacity and \( K_f \) value of ENR sorption in purple soil were observed at initial solution pH of < 7 and low ionic strength. The cation exchange was the major sorption mechanism of ENR sorption in purple soil. The application of animal manure in vegetable farm and high sorption affinity of FQs in soils may cause accumulation of FQs in soil environment, and also increase the potential risks of ARGs propagation and contamination in water environment. Further studies on the eco-toxicity and transport behavior of antibiotics in soil are necessary.

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BUFFONIA ALI-NIHATII (CARYOPHYLLACEAE),
A NEW SPECIES FROM TURKEY

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ABSTRACT
A new annual species, Buffonia ali-nihatii Özdeniz (Caryophyllaceae) is described and illustrated from Central Anatolia. The specimens were collected from Sivas (Turkey). The new species is related Buffonia temuifolia L. The differences between the species are discussed. A morphological comparison was made with the morphologically similar species, and its distribution, description, is given as well as the ultrastructure of the seed.

KEYWORDS:
Buffonia, Central Anatolia, taxonomy.

INTRODUCTION
The Caryophyllaceae family has approximately 90 genera and approximately 2500 species, huge part of which is found in the northern temperate areas. The genus Buffonia is located within the tribe Alisinae of the family Caryophyllaceae [1]. It is one of the smallest genus of Caryophyllaceae in terms of numbers of taxa. The genus has approximately 23 species that are mostly grown in Asia and Europe [2].

The genus Buffonia comprises ca. 20 species having a distribution mostly in mountainous areas; Canary Islands, the Mediterranean and Irano-Turanian region [1]. The genus has been divided into 6 sections (Loliopsis, Leptocladia, Enervis, Calyculatae, Micrantha, and Longipedicellata) in recent years [3]. Based on the Flora of Turkey and the East Aegean Islands, Buffonia genus has 4 species in Turkey [4]. In the recent studies, 1 taxon (Buffonia virgata Boiss.) was determined as synonym in this genus. Also, 4 taxa (Buffonia anatolica Chrtek & Kriša, B. anatolica var. elatior Chrtek & Kriša, B. paniculata F.Dubois subsp. orientalis Chrtek & Kriša and B. yildirimhansi İlcım & Behçet) were added [3, 5]. Consequently; the number of the taxa of Buffonia increased to 7 in Turkey.

MATERIALS AND METHODS
A strange of Buffonia was noticed during examinations made on the ANK herbarium by author. Then, the specimens were compared with other herbaria species (GAZI, ANK, HUB, Bozok University Herbarium, VANF) and literature [4, 6, 7, 8, 9, 10, 11, 12] and therefore was described as a species new to science. The digital images of the new species were prepared using a CANON EOS 60D digital camera. The vegetative characteristics were measured with a ruler with 0.5 mm accuracy and the floral characteristics were determined using an ocular micrometer. Seed micromorphology were examined by LEO 440 scanning electron microscope (SEM).

RESULTS
Buffonia ali-nihatii Özdeniz, sp. nov. (Figures 1-3).
Section: Loliopsis Chrtek & Krisa
Type: Turkey B6 Sivas: 8 km from Beypinari to Zara, steep slopes, 07.08.1982, P.H.Davis & Ekim 68799 (holotype: ANK, isotypes: Bozok Univ. Herb., GAZI).

Diagnose. Buffonia ali-nihatii is involved in Buffonia temuifolia. It primarily differs from B. temuifolia since it has pedicels densely pilose (not scabrid), inner sepalis 3-4 mm long, ovate-lanceolate, 5-nerved (not 2-3 mm, lanceolate, 3-nerved), petals oblancoate (not linear), seeds 1.5-2.2 mm long, ventral surface papillate (not 1-1.5 mm long, ventral surface acute tuberculate).

Description. Annual herb. Stems erect or ascending, 35–45 cm tall, 5-9-noded, laxly branched at base, sparsely crispy hairy below, glabrous above. Leaves linear-subulate, adpressed stem, 1.9–2.5 × 0.3–0.4 cm, scarios and ciliate margins, vagina 1.5–2.5 mm long. Inflorescence in monochasia at the axiles of bracts, terminal and lateral flowers, usually a dense terminal pedicelled cyme of 5–12 flowers, a dense lateral pedicelled cyme of 3–9-flowers. Bracts subulate, 2–3 × 0.6–0.9 mm, scarios and ciliate margins. Pedicels 2–3 mm tall, densely pilose. Sepals glabrous, unequal, divided.
inside and outside, outer sepals 2.5–3.5 × 1.3–1.6 mm, ovate-lanceolate, 5-nerved, margin 0.2–0.3 mm scarious, inner sepals 3–4 × 1.3–1.6 mm, ovate-lanceolate, 5-nerved, margin 0.2–0.3 mm scarious, apex acuminate; petals white, oblanceolate, 2.3–2.5 × 0.6–0.8 mm, short from sepals; filaments shorter than petals, 1.2–1.5 mm long. Styles shorter than ovary, 0.3–0.5 mm long; ovary 2–2.3 mm long, stipitate. Capsule elliptic 2–3 × 1.8–2.0 mm, shorter than calyx; seed 2. Flowering in July and fruting in August.

FIGURE 1
Bufonia ali-nhatii (P.H.Davis 68799 & Ekim) A- habit, B- inflorescence, C- sepal, D- petal, E- capsule
**Seed micromorphology.** Seeds horse shoe shaped, 1.5–2 × 0.8–1 mm, brown, granulate, ventral surface short papillate, cells shaped linear-oblong, cells edge teeth V undulated; dorsal surface long papillate, cells shaped stellate, cells edge teeth V undulated, not a channel in the back.

**Specimens examined.** — *Bufonia tenuifolia*: **A4** Kırıkkale: Koçubaba Kasabası, Elmalı yöresi, 1200 m, 28.07.1990, A.A.Dönmez 2722 (HUB); **B4** Ankara: Kırıkkale, İrmağ ist. civarı, ca. 750 m, 09.07.1985, E.Yurdakulol 2353 (ANK); **B6** Kayseri: Sarz, Yalak mevkii, Binboğa Dağı, 1500-1700 m, 04.08.1991, Z.Aytac 4330 & H.Duman (GAZİ); **B9** Bıtıs: Hızan, Karbastı Köyü,1550 m, 01.08.1989, Y.Altan 2425 & L.Behçet (VANF); Bıtıs: Alacabük Dağı, Zerzemin sırtları, 22.05.2004, F.Özgökçe 12478 (VANF); Van: Gürpinar, Zernek Barajı ile Çörekli Köyü aras, 1980 m, 26.07.2007, İ.Demir 821 (VANF); Muş: Malazgirt, Aktuzla doğusu- kuzeýdoýusu, 1500-1600 m, 21.07.2014, L.Behçet 7087(VANF); **C2** Muğla: Girdev Dağı, ca. 170 m, P.H.Davis 13881 (ANK); Antalya: Avlan Gölü yakınınndaki kayalıklar, 1968, P.Quezel s.n. (ANK); **C3** Konya: Beyşehir, Kurucova, Beyşehir Gölü kıyısı, 1200 m, 25.07.1975, H.Peymen & A.Güner 2312 (HUB); Antalya: Perge, 10.07.1970, A.Pamukçuoglu s.n. (HUB); **C4** Karaman: Bucakkışla-Bayır, 750 m, 23.08.1994, M.Yural 7184, N.Adıgüz & A.Dönmez (GAZİ); Karaman: Dinek-Kılbasan, 1040 m,31.07.1997, M.Yural 7980, N.Adıgüz & F.Ertuğ (GAZİ); **C5** Içel: Mersin, fındıkpınarı, Çopurgediği çevresi,1200-1400 m,14.09.1987, Ş.Yıldırım 9912 (HUB); Niğde: Ulukışla, 10.07.1979, T.Ekim 1302 & T.Tükel (ANK); **C6** Kahramanmaraş: Engizık Dağı, Engizık Köyü kuzeyi, 2000 m, 28.08.1986, H.Duman 2378 (GAZİ).
**Conservation Status.** According to the current research *Bufonia ali-nihatii* is known only in one address. The number of mature individuals in each subpopulation is less than 1000 (Criterion C2). Therefore, it should be considered vulnerable (CR) according to the IUCN Red List Criteria [13].

**Etymology.** The species is named in nature loving businessman Ali Nihat GÖKYİĞİT.

**DISCUSSION AND CONCLUSIONS**

*Bufonia ali-nihatii* is included in section *Loliopsis* as it is annual, its stems are usually simple, erect, branched from the base, its leaves are linear-subsulate, glabrous, its sepals are ovate-lanceolate, its petals shorter than sepals, its capsules shorter than petals. This species is similar to the *Bufonia tenuifolia* species in terms of annual, hairs, pedicels length, unequal sepals shape, petal/sepal rate, stamens number and capsule/sepal rate. However, the differences in pedicel hairs, sepall length, shape and nerve number, petals shape, and seed surface were detected from the above-mentioned taxon (Table).

**Table**

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>B. ali-nihatii</em></th>
<th><em>B. tenuifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedicels</td>
<td>densely pilose</td>
<td>scabrid</td>
</tr>
<tr>
<td>Inner sepals</td>
<td>3-4 mm long, ovate-lanceolate</td>
<td>2-3 mm, lanceolate, 3-nerved</td>
</tr>
<tr>
<td>Petals</td>
<td>oblongulate</td>
<td>linear</td>
</tr>
<tr>
<td>Seeds</td>
<td>papillate, not a channel in the back</td>
<td>acute tuberculate, a channel in the back</td>
</tr>
</tbody>
</table>

**Key to *Bufonia ali-nihatii* and its related species in Turkey**

1. Stamens 8, about 1/2 as long as inner sepals ........................................... *B. oliveriana*  
   – Stamens 2-4, at most 1/3 as long as inner sepals ........................................... 2
2. Pedicels densely pilose, inner sepals 3-4 mm long, ovate-lanceolate, 5-nerved, petals oblongulate, seeds 1.5-2.2 mm long, ventral surface short papillate, not a channel in the back.................. *B. ali-nihatii*
   – Pedicels scabrid, inner sepals 2-3 mm long, lanceolate, 3-nerved, petals linear, seeds 1-1.5 mm long, ventral surface acute tuberculate, a channel in the back ................. 3
3. Stamens 4, about 1/3 as long as inner sepals ............................................... *B. virgata*
   – Stamens 3(-2), less than 1/3 as long as inner sepals ..................................... *B. tenuifolia*

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REMOTE SENSING OF CUCUMBER POWDERY MILDEW USING ADVANCED UNMANNED VEHICLE AND IMAGE PROCESSING TECHNIQUES

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ABSTRACT

Pesticides is considered one of the most hazardous compounds that caused serious health implications to man and environment. However, their use is perquisite to guarantee a high yield especially, for greenhouse cucumber production. In fact, the process success depend on spraying coverage, rate and early detection of infection symptoms. In this study, a novel remote sensing approach for detecting Powdery Mildew symptoms in cucumber (Cucumis sativus) leaves has been developed. The experiment was conducted under greenhouse conditions. An unmanned vehicle platform used to carry the remote sensing sensors. Infected leaves were then analyzed using a pioneer image processing model. In this model, leaves were classified according to different feature especially, the reflect color. Image processing results were then assessed using ground reference data (lab analysis). The accuracy assessment of remotely-sensed data was ground reference data were comparable. Because changes in infected leaves associated with the imagery model data, we conclude that remotely-sensed data derived from the unmanned vehicle sensor data hold promise for detecting Powdery Mildew symptoms in cucumber leaves.

KEYWORDS:
Pesticides, Environment, Cucumis sativus, Leaf Disease, Erysiphe cichoracearum

INTRODUCTION

Pesticides play a key role in environmental pollution as well as public concerns about healthy food. Worldwide, pesticides consumption distributed as follows; 45 % for Europe, 25 % USA, and 25 % for the rest of the world [1]. In Jordan, annual pesticides usage excess 2.5 thousand tons [2]. Conventional pesticides application are labor intensive, costly, and not suitable for automation. In addition, disease detection require a qualified experts which seldom available immediately onsite. As a result, excess application of pesticides normally delivered to plant and soil. Remote-sensing technology can detect several plant physiological responses such as water stress [3] and chlorophyll content [4] and their applications hold potential for sensing leaves health. For greenhouse, a wide range of remote sensing studies are carried out nowadays to assess the platform Performance such as, vehicles design, architecture and accuracy. In fact, the requirements for remote-sensed vehicle have been designed and the specifications for behavior of navigation and operations have been defined in Hellström et al [5]. Their study showed that it is quite possible to learn and track a path previously demonstrated by an operator with an accuracy of 0.1m on flat ground. The objective of this study was to detect powdery mildew in cucumber using remotely sensing data carried on autonomous greenhouse sprayer vehicle.

MATERIALS AND METHODS

Disease background. Powdery mildew caused by Erysiphe cichoracearum (Sphaerotheca fuliginea), is a serious and common fungal disease during warm and humid conditions with overcast days. Unlike downy mildew, infestation and reproduction may occur under relative humidity as low as 46%. The optimal temperature for the fungus infection 27°C. Symptoms develop primarily in 2-3 week old leaves and stems. Younger leaves are almost immune to powdery mildew. The disease characterized by white powdery-like growth, especially on the upper side of leaves and stems. Early infection symptoms are lesions turn yellow to brown and dry out resulting in early leaf-fall of the older leaves. The disease spread rapidly in wet
weather and infected field might wipe out in only few days. However, fruit infection is rare. Management practices to control this disease include maintaining a healthy vigorous crop, removing volunteer cucurbit crops and weeds around the field, sanitation, and weekly fungicidal sprays. Recent data indicates that sodium bicarbonate (Household baking soda) and potassium silicate sprays may effectively control powdery mildew, as well as other cucumber diseases.

**Imagery acquisition and analysis.** Images used for this research were collected from two different resources; the first ones were taken from a live environment at The University of Jordan, while the other group were collected from a dataset at UCI Machine Learning Repository. A SONY camera model DSC-H55 was used to capture images with a resolution of 2592×1944 pixel. An overall set of 59 images were utilized to assess the accuracy of the results. Each captured image was converted to a 3D matrix containing RGB values for each pixel.

The camera was carried using a robot made by The University of Jordan in both faculty of Agriculture and IT (Fig. 1). The camera and nozzle were installed at the end of a robotic arm. When the vehicle detected a plant, it stopped and the robotic arm scanned the plant from the bottom up. During the scanning process, the camera captures the images for each single leaf; and the robotic arm controlled the distance between the nozzle and the infected leaf to confirm the pest in the scope of the spray nozzle.

A microcontroller was used to control the robot vehicle speed; camera motion; camera image capturing; sprayer operations and spraying nozzle, transferring data between the chip and a computer fixed on the vehicle. MATLAB (R2014b) was used for image processing and programming environment.

![FIGURE 1](image)

Unmanned system used in the study.

During image pre-processing, we removed image noise and isolating colors of interest (i.e. green spectrum, white spectrum) by switching between different color models, and testing different values of color ranges. The difference between the original photo and the processed one is shown in Figure (2a, 2b). Afterwards, the resulting image is converted from RGB into grey scale for filtering as shown in (Figure 2c). A flat morphological top-hat structuring filter is applied to correct the image illumination a shown in (Figure 2d). In order to detect infected leaf regions, the image was transformed from grayscale into monochrome to isolate white spots within leaf black regions; therefore, Otsu’s binarization algorithms that involve iterating through all the possible threshold values and calculating a measure of spread for the pixel levels each side of the threshold. The aim is to find the threshold value where the sum of foreground and background spreads is at its minimum, i.e. the intraclass variance of the black and white pixels (Figure 2e). However, in order to preserve the white regions of the disease, hole filling and noise addition using salt and pepper was applied and then applying a 2D median filter over it (Figure 2f). Finally, the resulting image of previous step is converted into the L*, a*, b color model to apply on additional step of noise filtering where image was first transformed into monochrome scale, then filtered similarly to previous step, then used to mask the image in (Figure 2a) producing the image displayed in (Figure 2g). Previous steps are repeated for another round of filtering to decrease the remaining noise. The final processed image is shown in (Figure 2h).

**RESULTS AND DISCUSSION**

Several research studies has been conducted to detect pests infection such as the use of combined information from thermal and stereo visible light images using machine learning techniques for tomato powdery mildew [6]. Aji et al. [7] proposed an application of image processing and machine learning to identify three palm oil diseases based on visual appearances in the early cultivation stage. The diseases are Anthracnose, Hawar Leaf, and Leaf Spot. However, leaf imaging process required a white and clean background to capture the picture. Interestingly, in this research, no shooting conditions (e.g. must be captured in white background, sunlight, etc.) were required to capture the images.

In order to validate our experimental results, considering that the goal was to automatically identify whether a leaf is infected, each of the resulting images is used to extract a set of features that used later to classify the image, then validated against the human judgment of the infection in the image. Image features can be summarized as follows: entropy, mean, standard deviation of the image after the first step of filtering, entropy, mean, standard deviation of the image after the second step of filtering, 2D correlation coefficient of the resulting
image, Mean Squared Error (MSE) of the resulting image, Peak-SNR of the resulting image, and SNR of the resulting image. In order to evaluate the image quality for infection classification, we refer to the confusion matrix shown in Table 1 as a primary source for accuracy estimation in classification problems. Based on this confusion matrix, the three-different criterion used are listed in Table 2.

**TABLE 1**  
Confusion matrix

<table>
<thead>
<tr>
<th>Predicted</th>
<th>Infected</th>
<th>Not-Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Not-Infected</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

**TABLE 2**  
Evaluation methods

<table>
<thead>
<tr>
<th></th>
<th>Actual Rate</th>
<th>Hit Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>(A+D) / (A+B+C+D)</td>
<td>D / (B+D)</td>
</tr>
<tr>
<td>Actual Rate</td>
<td>D / (B+D)</td>
<td></td>
</tr>
<tr>
<td>Hit Rate</td>
<td>D / (C+D)</td>
<td></td>
</tr>
</tbody>
</table>

Accuracy identifies the percentage of the total number of predictions that were correctly classified where actual rate shows the percentage of predicted infected among actual infected images. Finally, the hit rate shows the percentage of predicted infected in actual infected and actual non-infected. Several classification algorithms have been tested (i.e. using Weka Machine Learning Toolkit) showing various results. Specifically, Random Forest (RF), Support Vector Machine (SVM), and Instance Based k-nearest neighbor (IBk), and Multilayer Perceptron (MLP) were applied, among others, and reported as shown in (Figure 3). All algorithms were run a 10-fold cross validation, and then measures were calculated according the resulting confusion matrix. The figure shows that at the three evaluation measures IBK and RF performed better than MLP and SVM, which performed poorly in comparison.

Our results showed that reading the previously extracted image features after each processing step of our work helped some classification algorithms to detect infected papers accurately. It is noticeable that SVM detected all non-infected leaves correctly; however, inaccurately identified 9 out of 14 infected leaves as non-infected regardless of the algorithm configurations set in the experiment. With a

![Figure 2](image-url)

**FIGURE 2**  
(a) The original image, (b) HSV Model output. (c) The greyscale image. (d) Applying flat morphological top-hat structuring filter. (e) Monochrome image (f) after adding noise, filling holes and applying 2-D filter. (g) After using the L*a*b model & adding noise, filling holes and applying 2-D filter. (h) Output of the first step
smaller error, same phenomena appeared with MLP where all non-infected leaves were detected, 13 infected were detected correctly, and 1 non-infected instance was identified as infected.

Benefits of an autonomous greenhouse sprayer include both increasing accuracy and precision with spraying, which would contribute to more efficient use of resources and decreased health risks associated with human exposure to dangerous chemicals.

Overall, it sounds possible to detect powdery mildew infection in cucumber plants using remote sensing approach. It also sounds interesting that extracting image features after each image processing step helped achieve high infection detection accuracy. In addition, the accuracy of classification methods were not similar and depended on dataset class distribution and feature types. Accordingly, this method can be applied in manufacturing agricultural instruments that can detect the disease on certain leaves, and spray them if necessary. A possible extension to this work can be identifying the area of the infection, it’s location, and the distance from the camera, or an automatic sprayer, can be a challenging, yet valuable, addition to this work.

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REFERENCES


FIGURE 3
A classification measures comparison between different classifications algorithms


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APPARENT PERMEABILITY FOR SHALE GAS
CONSIDERING ADSORPTION AND FLOW PATTERNS:
TAKING THE LONGMAXI SHALE GAS RESERVOIR OF
SICHUAN BASIN AS AN INSTANCE

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ABSTRACT

The flow pattern is unique in a certain range of pore size divided by the Knudsen number. In order to characterize permeability of nano-pore in shale gas reservoir more accurately, and solve the issue of apparent permeability calculation, the formulas of nano-pore permeability are put forward considering the influence of adsorption gas and pore size distribution, taking the chang7 shale gas reservoir of Ordos Basin as an instance. After the calculated results were compared and analyzed, the conclusions are obtained as follows: (1) Pore size is the main factor to determine the flow pattern; (2) There are three main flow pattern in the shale reservoirs nano-pore, slip flow, Fick diffusion and transition diffusion, meanwhile Darcy percolation and Knudsen diffusion do not exist; (3) Flow pattern has great influence on apparent permeability and adsorption has a greater impact in a high pressure condition (greater than 20 MPa).

KEYWORDS:
Shale gas, Apparent permeability, Nano-pore, Adsorption thickness

INTRODUCTION

With the help of advanced experimental means, nano-pore can be observed directly. F. Javadpour [1] found diameter of pores and grooves less than 5 nm using AFM; according to research of Guo et al., volume ratio of pores which diameter is less than 15 nm reaches more than 60%, which diameter is less than 100 nm reaches more than 80% [2]. Nano-pore is so widely distributed in reservoir that Darcy percolation theory is not completely suitable for permeability calculation of shale gas reservoir, and the main reasons include two aspects: (1) adsorbed gas exists and does not participate in the flow within a certain period of time, and it makes nano-pore become smaller; (2) diameter of gas flow channel is nanometer, then molecular effect is obvious and flow patterns are complex.

In order to obtain a more accurate characterization of permeability in nano-pore, the effective pore diameter is obtained with application of adsorption law to calculate adsorption thickness, and the weights of various flow patterns’ influence on apparent permeability are set as the proportion of different ranges of pore volume in nano-scale pore system. Considering the influence of the adsorption and various flow patterns on permeability, a method is obtained which is relatively more accurate to calculate apparent permeability.

MATERIALS AND METHODS

The division of flow pattern. Knudsen equation [3, 4] is as equation followed:

\[ K_n = \frac{\lambda}{d}, \lambda = \frac{k_B T}{\sqrt{2\pi \delta^2 \rho}} \times 10^{21} \]  

(1)

Where, \( \lambda \), mean free path of gas molecules, nm; \( d \), pore diameter, nm; \( k_B \), Boltzmann constant, 1.3805 \( \times 10^{-23} \) J/K; \( \delta \), molecular collision diameter, about 0.4 nm [5].

Then equation (1) can be transformed as equation followed:

\[ d = \frac{k_B T}{\sqrt{2K_n \pi \delta^2 \rho}} \times 10^{21} \]  

(2)

The critical diameters, \( d_{0.001}, d_{0.01}, d_{0.1} \) and \( d_{10} \) respectively as \( K_n = 0.001, 0.01, 0.1, 1.0 \) determine the flow pattern as Table 1.
TABLE 1

Division of Flow Pattern by Knudsen Number

<table>
<thead>
<tr>
<th>$K_s$</th>
<th>$d$</th>
<th>Flow pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_s &lt; 0.001$</td>
<td>$d &gt; d_{0.001}$</td>
<td>Darcy percolation</td>
</tr>
<tr>
<td>$0.001 &lt; K_s &lt; 0.01$</td>
<td>$d_{0.001} &lt; d &lt; d_{0.001}$</td>
<td>Slippage</td>
</tr>
<tr>
<td>$0.01 &lt; K_s &lt; 0.1$</td>
<td>$d_{0.1} &lt; d &lt; d_{0.01}$</td>
<td>Fick diffusion</td>
</tr>
<tr>
<td>$0.1 &lt; K_s &lt; 10$</td>
<td>$d_{0.01} &lt; d &lt; d_{0.1}$</td>
<td>Transition diffusion</td>
</tr>
<tr>
<td>$K_s &gt; 10$</td>
<td>$d &lt; d_{0}$</td>
<td>Knudsen diffusion</td>
</tr>
</tbody>
</table>

TABLE 2

The diffusion coefficients

<table>
<thead>
<tr>
<th>Diffusion</th>
<th>Formula</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fick diffusion</td>
<td>$D_{\text{fick}} = \frac{k_B T}{6\pi \mu r_p}$</td>
<td>Storch - Eins equation</td>
</tr>
<tr>
<td>Knudsen diffusion</td>
<td>$D_{\text{Knudsen}} = \frac{2r}{3}\left(\frac{SRT}{\pi M}\right)^{0.5} \times 10^6$</td>
<td>Molecular kinetic theory</td>
</tr>
<tr>
<td>Transition diffusion</td>
<td>$\frac{1}{D_{\text{transition}}} = \frac{1}{D_{\text{fick}}} + \frac{1}{D_{\text{Knudsen}}}$</td>
<td>Bosanquet equation</td>
</tr>
</tbody>
</table>

TABLE 3

Apparent permeability

<table>
<thead>
<tr>
<th>Flow pattern</th>
<th>Apparent permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slippage</td>
<td>$k_{\text{app,slip}} = \frac{r^2}{8} \left[1 + \left(\frac{8\pi RT}{M}\right)^{0.5} \mu \left(\frac{2}{\pi\rho_{\text{avg}}^2} - 1\right)\right] \times 10^6$</td>
</tr>
<tr>
<td>Fick diffusion</td>
<td>$k_{\text{app,fick}} = \frac{Mk_B}{6R\pi r_p \rho_{\text{avg}}} \times 10^{21}$</td>
</tr>
<tr>
<td>Transition diffusion</td>
<td>$k_{\text{app,transition}} = \frac{4\mu}{3\rho_{\text{avg}}^{0.5}} \left(\frac{2M}{\pi TR}\right)^{0.5}$</td>
</tr>
<tr>
<td>Knudsen diffusion</td>
<td>$k_{\text{app,Knudsen}} = \frac{1}{\rho_{\text{avg}}} \left[\frac{6\pi Re_{\text{fick}}}{Mk_B} \times 10^{21} + \frac{3}{4\mu} \left(\frac{\pi TR}{2M}\right)^{0.5}\right]^{-1}$</td>
</tr>
<tr>
<td>All patterns</td>
<td>$k_{\text{app,all}} = \frac{W_{\text{Darcy}}}{k_{\text{app,Darcy}}} + \frac{W_{\text{slip}}}{k_{\text{app,slip}}} + \frac{W_{\text{fick}}}{k_{\text{app,fick}}} + \frac{W_{\text{transition}}}{k_{\text{app,transition}}} + \frac{W_{\text{Knudsen}}}{k_{\text{app,Knudsen}}}$</td>
</tr>
</tbody>
</table>

Calculation of apparent permeability. Mass flux. F. Javadpour [1] put forward the calculation using total mass flux as equation followed:

$$J_{\text{total}} = J_{\text{d}} + J_{\text{D}} = \frac{q \rho_{\text{avg}}}{A} = \frac{k_{\text{app,avg}} \Delta p}{\mu L} \times 10^3 \quad (3)$$

where, $J_{\text{total}}$, $J_{\text{d}}$, $J_{\text{D}}$, respectively the total, the seepage, the diffusion mass flux, kg / (m² · s) ; $q$, volume flow, m³/s ; $A$, cross area of flow channel, m² ; $\rho_{\text{avg}}$, average fluid density, kg / m³ ; $k_{\text{app}}$, apparent permeability, $\mu m^2$.

According to Hagen-Poiseille equation:

$$J_{\text{d}} = \frac{-r^2 \rho_{\text{avg}} \Delta p}{8\mu L} \times 10^9 \quad (4)$$

Roy et al. [9] put forward the equation:

$$J_{\text{D}} = \frac{MD \Delta p}{RTL} \times 10^6 \quad (5)$$

where, $D$, diffusion coefficient, m² / s . The diffusion coefficients of Fick diffusion, Knudsen diffusion and Transition diffusion can be written as Table 2.

As for the slippage, Brown et al. [10] put forward the corrected coefficient F as equation followed:

$$F = 1 + \left(\frac{8\pi RT}{M}\right)^{0.5} \mu \left(\frac{2}{\pi \rho_{\text{avg}}^2} - 1\right), J_{\text{d,slip}} = FJ_{\text{d}} \quad (6)$$

Apparent permeability. According to the Mass flux method, apparent permeability of different flow patterns can be obtained. However, the flow patterns exist in the certain range of pore diameter at the same time. The weights of various flow patterns’ influence on apparent permeability are set as the
proportion of different ranges of pore volume, named as \( W_{Darcy} \), \( W_{slip} \), \( W_{Fick} \), \( W_{transition} \) and \( W_{Knudsen} \). The apparent permeability can be written as Table 3.

The effective pore radius. Assuming the adsorbed gas molecules absorbed on the pore wall evenly, formula of adsorption thickness can be written as equation followed.

\[
r_e = r_i - h_{add}, \quad h_{add} = \frac{V_{add}}{S} \times 10^9 \tag{7}
\]

where: \( V_{add} \), volume of adsorbed gas under reservoir conditions, \( m^3/kg \); \( h_{add} \), thickness, nm; \( r_e \), effective pore radius, nm; \( S \), surface to mass ratio, \( m^2/kg \).

In the presented work [4], calculations of adsorption layer thickness are obtained using equation (7) and different adsorption laws as Table 4 showed. Langmuir calculation and Polanyi calculation can be used in this paper.

Where, \( V_m \), Langmuir constant, \( m^3/kg \); \( P_L \), Langmuir pressure constant, MPa; \( p \), pressure, MPa; \( \varepsilon \), adsorption potential, \( J/mol \); \( p_h \), saturated vapour pressure, MPa; \( T \), temperature, K; A, B are constant, and they can be obtained according to fitting of Polanyi adsorption characteristic curve; C, D are constant, and they can be obtained according to fitting of FHH adsorption characteristic curve.

RESULTS AND DISCUSSION

The apparent permeability under different pressures (5-50 MPa) and temperatures (298.15 K, 303.15 K, 308.15 K, 313.15 K, 318.15 K) condition will be discussed using the data of Longmaxi shale formation, Sichuan, China. Fig. 1 shows the distribution of pore diameter in Longmaxi formation [5].

![FIGURE 1](image)

The pore diameter distribution of Longmaxi shale formation [5]
Critical diameter of flow pattern. Calculate the value of $d_{0.001}$ and $d_{10}$ under different temperature and pressure conditions as an example. The curves can be obtained as Fig. 2 and Fig. 3.

It can be seen from Fig. 2 and Fig. 3:

1. The temperature has little influence on the critical diameter, and the temperature of 318.15 K is chosen because it is closest to the actual temperature in shale reservoir.

2. Darcy percolation does not exist in the nano-pore ($d<100$ nm) of Longmaxi formation because the all values of critical diameter $d_{0.001}$ under different conditions are greater than 100 nm which does not exist in the nano-pore; Knudsen diffusion does not exist for the diameter of gas molecular is about 0.38 nm, while the all values of critical diameter $d_{10}$ under different conditions are less than it. So, in fact, gas molecular can not get in the pore which Knudsen diffusion needs. It means that there are three main flow pattern in the Longmaxi formation shale reservoirs nano-pore, slip flow, Fick diffusion and transition diffusion.

3. The pressure has great influence on the critical diameters, thus, with the decrease of the pressure, the distribution of flow patterns changes sharply. However, the pressure in shale reservoir changes slowly in a certain period. So pore size is the main factor to determine the flow pattern.

Apparent permeability. Fig. 4 shows the curves of apparent permeability of three kinds of single flow pattern, respectively $K_{\text{app,slip}}$, $K_{\text{app,Fick}}$, $K_{\text{app,Transition}}$, apparent permeability of all flow patterns, $K_{\text{app,all}}$, and apparent permeability with adsorbed gas and all flow patterns, $K_{\text{app,all,Lang}}$ and $K_{\text{app,all,Pol}}$. 
Compared the results with permeability experiment data of the Longmaxi shale gas reservoir of Sichuan Basin in References. The experiment data of WANG Ji-lin [6-7] shows the permeability is $(1.0-8.0)\times10^{-3}$ mD. And the experiment data of Cui Yaxing [8] shows the permeability is $(3.0-5.0)\times10^{-3}$ mD. The data has the obvious similarity. The discussion is obtained as followed:

1. The apparent permeability decreases as the pressure increases. The main reason is that as the pressure increases, slippage coefficient $F$ and diffusion coefficient decrease, density and adsorbed thickness increase.

2. Flow pattern has great influence on apparent permeability. There are two orders of magnitude difference between slippage apparent permeability, $K_{app,slip}$, with Fick diffusion apparent permeability, $K_{app,Fick}$, and Transition diffusion apparent permeability $K_{app,Transition}$. The apparent permeability is more accurate considering the flow pattern distribution.

3. Adsorption has a greater impact on the permeability in a high pressure condition (greater than 20 MPa).


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POINT DENSITY ANALYSIS WITH COGNITIVE MAPPING TECHNIQUE: ISTANBUL-HISTORICAL CITY CENTER

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²Landscape Architect, Istanbul, Turkey

ABSTRACT

There are some basic requirements such as observing with personal experience, perceiving and realizing the detail and using imagination in the perception of the space. On the basis of these requirements, the detail about the space is interpreted by processing in a cognitive process with all experiences that the perceptor has. In the study; it is tried to reveal the perceptibility level of the environment in order to create a healthy space organization that meets the needs and expectations of the visitors who use the space. On the purpose of this, Sultanahmet Square and its surrounding which carry the traces of different civilizations and cultures that belong to thousands of years and have the urban texture with its physical and functional change and transformation in addition to the identity of the architectural and social texture, trade and tourism center have been selected as the research area in Istanbul. Question and Cognitive Mapping Technique has been conducted in the study. 229 visitors have been asked to mark the most frequently used/ unused spaces and the most popular/unpopular spaces in the area by using face-to-face interviews and the visitor data Then, Point Density Analysis has been conducted with these maps and Density Map has been prepared with the preferences with the help of ArcGIS Program. As a result, the obtained density maps and the opinions of the visitors have been evaluated and the results of spatial analysis have been detailed.

KEYWORDS:
Urban Space, Perception, Cognitive Maps, Density Map, Historical City Center

INTRODUCTION

To evaluate the behaviors of the visitors and to reference their requests are important in order to increase the quality in the historical city centers. To manage the quality, which is shaped in parallel with the structure, needs and desires of the society in the areas that include the history and historicity concepts is important to carry the cultural heritage to the next generation [1]. Urban design is used as an effective tool in order to raise the urban quality.

The concept of quality in human-space relation is directly related to how the space meets the needs of the visitor and how it affects the behaviours of the visitors. In this context, subjective evaluations on the perception of people in spatial quality have been measured by the feeling of satisfaction of the person [2]. Space visitors should meet some psychological requirements such as comfort, pleasure, discovery, prestige, social relations, activity and peace [3]. Undoubtedly that, requirements will vary depending on the type of the space and the intended use. Moreover, these requirements can vary in terms of different living conditions and personal characteristics (age, gender, status, cultural background) [4]. For this reason Mazumdar (2003) [5] has emphasized the necessity of examining the visitor profile to determine the special quality and has discussed that people in different gender, age (children, young and old people) and ethnic group have different needs and expectations. Perception of space and recognition of qualifications cause to evaluate, select, decide and respond to the behavioral responses of the person [6]. The responses to the environment on the basis of the objectives and motives are revealed by spatial behaviors. Visitor’s satisfaction can be measured and space readability can be ensured by interpreting the behaviours of the user [7]. Urban reading concept can also be defined as understanding, analyzing or evaluating. Spatial readability is the most important factor in an area, it has an important place to perceive and experience the space and to develop the design plans in urban design applications [8, 9].

In particular, the readability of urban spaces in historic city centers is important for both urban identity and quality criteria. To evaluate the behaviour of the visitors and to reference their request and perception to the space are necessary to increase the quality in historic city centers in the landscape planning and design approaches. Wrong or incomplete urban design applications negatively affect quality characteristics such as accessibility, functionality and perceptibility; it reduces the readability of spatial quality from the point of view of the visitors.
MATERIALS AND METHODS

Visitor Data. The answers obtained from the Question Technique have been received with the help of SPSS Program. In the case of high level of significance (Asymp.Sig.) of the obtained results (p<0.05), the relation is evaluated [10].

Cognitive Maps. It is used in the graphical description of people how they perceive and organize the spaces in their mind [11]. The most common way to use cognitive maps is to give an empty paper to people and want them to draw the maps of environment that includes factors, which are important for people. This process is sometimes standardized by the rough maps that the certain features are submitted and the details are wanted to be filled by people. Maps are sometimes given to the people to identify the specific regions on the basis of characteristics and people are wanted to mark the factors that are indicated [12]. In some Cognitive Maps, people are wanted to draw the places that correspond to their mental World in response to the feature in the question. The obtained drawings are converted into appropriate data that are useful for statistical analysis [13]. With the results obtained from the Cognitive Map Technique, each point on the plan is overlaid and the most marked points are evaluated, and each narration is overlaid by the actual plan and analyzes that test the accuracy of the drawing can be made [14]. With the Cognitive Map Technique, the points on the maps that are marked by each visitor can be interpreted to be more or less preferred places is overlaid. Such maps are called Density Maps. The Density maps show where point features are concentrated. Density maps are used to show the concentration of a variable within a given geographic radius. Important information required for density analysis; spatial information such as the number of available points, the size of search area, and the proximity of the points to each other [15]. The each point is distributed throughout the study area, and a density value is calculated for each cell in the output raster. A circle is drawn around each raster cell centre using the search radius. In a density calculation, points that fall within the search area are summed, and then divided by the search area size (circle) to get each cell's density value [16] (Figure 1).

Nowadays the “Spatial Analyst” which has ArcGIS extension takes the basis facilitates to make statistical analyses. By the help of the program, the density surfaces are being produced on a two-dimensional geographical area with the spatial points. These density surfaces can be transformed into maps by classifying and coloring can be created (Figure 2). The most preferred/Unpreferable or Visual/Non visual points are being found with the Visitors Density Map prepared with this method.

![Grid Cell On Usage Map](image1)

**FIGURE 1**

Classifying and Coloring of Density Map [15].
The urban squares are an important urban life focus that has revealed the identity and personality of our cities throughout history [18]. Sultanahmet Square and its surrounding is a traditional city center with a 2700-year history, a multi-layered character that served The Eastern Roman, Byzantine, Ottoman Empires and The Republic of Turkey (Figure 3). Nowadays, it has become a space in tourism industry which is mostly driven by entertainment, recreation and traditional commercial spaces. It has a very strong potential in terms of tourism function with its spaces which have important and historical values at universal level such as The Palace of Topkapı, Blue (Sultan Ahmed) Mosque, Hürem Sultan’s Bathhouse, Hagia Sophia Museum, the Monolith, the Obelisk, the Column, German Fountain, etc. [19].

The level of perceptibility of the environment has been put forward by people in Sultanahmet Region known as the most important historic urban texture of Istanbul in the study. The obtained analyzes and the findings are aimed to provide data for spatial quality studies in the future and to guide them for similar studies. While determining the sample size, some reasons such as difficulties in the face to face interview, marking on the plan, the number of questions to be exhausting for the interviewer and the participant, the reluctance of the visitors have been taken into consideration in order to reduce the wrong information. This study has been applied 229 visitors in the research area.

**RESULTS AND DISCUSSIONS**

Demographic Characteristics of Visitors. Firstly, 5 questions about gender, age, nationality, education and occupation have been analyzed.

**Gender.** 118 of 229 visitors were male (51.53%), 111 were female (48.47%). According to the results; both male and female visitors have used the space equally and it is seen that there is a lot of male visitors with little difference (Figure 4).

Age. 88 of the visitors (38.43%) were between the ages of 18-30, 84 (36.68%) were between 31-45 years, 36 (15.72%) were between 46 and 60 years, and 12 (5.24%) were 61-75 years old, 8 (3.49%) were under the age of 18 and 1 (0.44%) were over the age of 75. According to the results; the visitors between the ages of 18 and 30 use the space more than the visitors over 75 years. As a result, everyone from all ages uses the space (Figure 5).
Nationality. 154 of the visitors (67.25%) were Turkish Citizen and 75 (32.75%) were foreign (Figure 6). According to the results; Turkish citizens use the space more than others. The reason for this result is that Turkish citizens prefer for the area for use because of its proximity to workplaces, worship, use for recreational purpose and etc.

Education. 134 of the visitors (58.51%) were university graduates, 66 (28.82%) were high school graduates, 22 (9.61%) were secondary school graduates and 6 (2.62%) were primary school graduates. 1 (0.44%) were illiterate (Figure 7). According to the results; it is seen that the visitors who visited the space are mostly university graduates. Illiterate visitors are less in the research area.

Occupation. 32 of the visitors (13.97%) were personnel/staff, 31 of the visitors (13.54%) were architects/engineers, 30 of the visitors (13.10%) were students, 26 of the visitors (11.35%) were self-employed, 17 of the visitors (7.42%) were housewife, 17 of the visitors (7.42%) were salesman/marketing specialists, 16 of the visitors (6.99%) were doctors, nurses, pharmacists, etc., 15 of the visitors (6.55%) were retired, 12 of the visitors (5.24%) were public relations and communication specialist, 12 of the visitors (5.24%) were accountant/financial advisor/banker etc., 7 of the visitors (3.06%) were teachers, 5 of the visitors (2.18%) were academician, 3 of the visitors (1.31%) were pilot/hostess, 3 (1.31%) were tour guide, and 3 of the visitors (1.31%) were from other occupation group (Figure 8). According to the results; personnel or technical staff use the space first, then architects/engineers and students use the space.

![FIGURE 6](image6.png)
**FIGURE 6**
Nationality of Visitors

![FIGURE 7](image7.png)
**FIGURE 7**
Education Level of Visitors

![FIGURE 8](image8.png)
**FIGURE 8**
Education Level of Visitors

![FIGURE 9](image9.png)
**FIGURE 9**
Research Area Map & Example Questionnaire
Map for Visitors Who Mark Over It
Perceptual Characteristics of Visitors. 4 questions were asked to understand the perception of the space by asking 229 participants to mark the spaces as “The Most Preferred Points in the Research Area”, “Unpreferable Points in the Research Area”, “Points that have visual control (wide and uninterrupted perspective) in the Research Area”, and “Points that have not visual control in the Research Area”. Visitors have marked the results over the prepared questionnaire maps (Figure 9).

Density Analysis of The Most Preferred Points. According to the answers of 229 visitors, the most preferred spatial points in the Sultanahmet Region are shown in Figure 10.

The result of Point Density Analysis that has been conducted by ArcGIS Program has been found as Output Cell Size 7,36948782675292 and Radius 61,412399; point values between 0 and 0.001856781 have been obtained. The Density Map has been revealed by using point values preferences in 5 different scales. In accordance with the obtained results, it is seen that the visitors prefer the large pool area around Ayasofya Square. Then the resting area beside Blue Mosque, German Fountain, Column, Monolith and Obelisk have been preferred. It is seen from the results that the visitors prefer the surrounding of the magnificent architectural values for use. The most preferred area is the pool area which offers resting in the area, allowing integration with the green space and providing shade especially in the summer months.

Density Analysis of Unpreferable Points. According to the answers of 229 visitors, the unpreferable points in the region are shown in Figure 11. The result of Point Density Analysis that has
been conducted by ArcGIS Program has been found as Output Cell Size 8,45034763401056 and Radius 70,419564; point values between 0 and 0,001199339 have been obtained. The Density Map has been revealed by using point values preferences in 5 different scales.

According to the obtained results, it is understood that the visitors do not prefer to be at the points that include boundaries and enters of the space. These points are the areas that do not allow activities such as rest, sitting and waiting there.

In the results, it is seen that the restaurants and shops around the north of Marmara University Rectorate Building constitute the quietest points in the area. Dalbostı Street entrance does not show a full square, the green area around it cannot be integrated into the square and the curved entrance prevents visual dominance and so on. That’s why, it is understood that it is not preferred by visitors.

### Density Analysis of Points That Have Visual Control

The points that visitors think that visual control is achieved in the area are shown in Figure 12. The result of Point Density Analysis that has been conducted by ArcGIS Program has been found as Output Cell Size 7,39770917492447 and Radius 61,647576; point values between 0 and 0,002026274 have been obtained. The Density Map has been revealed by using point values preferences in 5 different scales.

According to the results, it is understood that the visitors have chosen the row benches near the Blue (Sultan Ahmed) Mosque as the point where visual domination is most intense and the area is perceived best. Fountain is the second area that is chosen. The data have showed that the areas where the Blue Mosque and the Hagia Sophia Museum can be perceived together with the slope, trees or structures by the visitors.

![FIGURE 12 Points That Have Visual Control and The Density Map of Visual Points](image1)

![FIGURE 13 Points That Have Not Visual Control and The Density Map of the Points That Do Not Have Visual Control](image2)
Density Analysis of Points That Have Not Visual Control. The points that visitors think that visual control is not achieved in the area are shown in Figure 13. The result of Point Density Analysis that has been conducted by ArcGIS Program has been found as Output Cell Size 8.38937195379058 and Radius 69.911433; point values between 0 and 0.00206274 have been obtained.

The Density Map has been revealed by using point values preferences in 5 different scales. The results show that the space in the entrance which connects the Hagia Sophia Museum to Bab-i Ali Street, is inadequate to provide visual control by the visitors and to provide a historical perspective of the area. Visitors did not prefer the points where cultural values could not be seen in historic places, where the cultural values of the area could not be perceived, a successful spatial organization could not be achieved and the historical perspective could not be seen continuously. Therefore, the results of the Not Visual Control Density Map that do not have visual control and the Density Map of Unpreferable Points are similar in the study.

CONCLUSION

Historic urban centres’ constitute the most meaningful parts of the whole city as the spatial reflection of cultural, social, religious, architectural, aesthetic, economic and political signs created by the ancient societies. In these places, it should be ensured that the changes occurring depending on the needs of the society are kept under the criteria that will not harm the historical character. Parallel to the developments by the era, realistic and comprehensive strategies should be formed, where the social demands are taken into account, correctly analysed and met, while the historical and cultural qualities are highlighted and evaluated, and the urban vitality and continuity are ensured. The Sultanahmet Region, which hosts different civilizations and cultures, with its geographical location, physical and social texture characteristics, transportation relations, trade centre identity and touristic character, has its own unique values on a global scale and has the current problems in providing integration of new living conditions with the historical urban texture, is the most important destination area of Istanbul-Historic Square. In this study, it is tried to be understood about the concept of perceptibility such as integrity, understanding, interpreting and understanding the needs, demands and expectations of visitors on the map, understanding the whole system texture from the space parts, experiencing, identity, feeling of belonging to the space, authenticity, historical, cultural and aesthetic values, physical form and uses and accessories.

According to the Most Preferred Points Analysis, it is seen that the fountains around the pool area, the Obelisk, the Column, the Monolith, and the German Fountain and the Blue Mosque in front of the east gate are preferred by the visitors. According to the Unpreferable Points Analysis, it was understood that the visitors did not prefer to have the area boundary and the points forming the entrances. In addition to this, in the Visual Control Analysis; the fact that the fountain is preferred as one of the most important points of the pool environment reveals the necessity of reorganization and improvement works related to this area. The age difference among the visitors in the Sultanahmet Square and its surroundings is noteworthy. Undoubtedly, in these areas, which have undergone many changes in the stratified history, and have the traces of the cultures it has lived for centuries; it should be ensured that the area ensures tourist use while embracing visitors of all ages in resting area. In urban space studies, it should be possible to make analysis by taking into account visitor characteristics, visitor expectations and requests. A more holistic urban design approach should be adopted, which eliminates negativity in these areas, contributes to historical and cultural continuity, transforms the space into a place to go for visitors, maintains the balance of modernization and environmental protection, maintains the originality and keeps the development under control and esthetical concern in these areas. The right direction should be made in order to find quality in historic city centres’, to maintain a balance between the present and future values and to create good living conditions. This guidance requires interdisciplinary work. Undoubtedly, the greatest task belongs to landscape architects.

On the basis of this study, it is thought that it will create different topics by shedding light on similar scientific studies about the perception of the environment by the inhabitants of the city.

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EXPERIMENTAL STUDY ON COALBED METHANE (CBM) DISPLACEMENT BY MIXED CARBON DIOXIDE AND NITROGEN

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ABSTRACT

In order to describe displacement effect on coalbed methane (CBM) by CO₂ and N₂, the paper takes displacement and replacement efficiency as evaluation parameters. There are 5 gases being taken for indoor displacement experiment on coal samples adsorbed CH₄, i.e. CO₂, N₂ and three mixed gases with different ratio (CO₂:N₂=1:1, CO₂:N₂=1:4 and CO₂:N₂=1:9) under 1.5MPa, 2.5MPa, 3.5MPa, 4.5MPa and 5.5MPa controlled gas injection pressure. Following rules are obtained from the experiments: (1) In terms of displacement efficiency, as the injection pressure increases, displacement efficiency of different gases will increase first and then decrease. (2) In terms of replacement efficiency, replacement efficiency of N₂ shows slowly decrease as injection pressure increase, while replacement efficiency of CO₂ shows increase at first and then decrease as injection pressure increase. (3) Taking coal samples saturated with CH₄ under 2.5MPa pressure as an example, the best displacement pressure shall be 2.5-3.5MPa; (4) When gas injection pressure is relatively low (lower than 2.0 MPa), the displacement and replacement efficiency of N₂ is higher than that of CO₂, however, under relatively high pressure (higher than 2.0 MPa), the displacement and replacement efficiency of N₂ is lower than that of CO₂. (5) For a mixed gas with certain mix ratio, for example (CO₂: N₂=1:1), replacement of CO₂ and displacement of N₂ will produce synergistic effect under a certain pressure range (2.5-3.5MPa).Its average displacement efficiency is 86.14%, average replacement is 30.61%, which is higher than average CO₂ displacement efficiency 83.06% and average N₂ displacement efficiency 83.39%; It is lower than average CO₂ replacement efficiency 34.92%, but higher than average N₂ replacement efficiency 20.78%.

KEYWORDS:
Coalbed methane (CBM), Displacement experiment, Carbon dioxide, Nitrogen, Synergistic effect

INTRODUCTION

Coalbed methane (CBM) is a high quality clean energy. At the same time, under certain conditions, it is also a potential safety hazard for coal mines. The technology of stable and increase production of coalbed methane (CBM) has always been a difficult point restricting the development of coalbed methane (CBM). With success application of improving coalbed methane (CBM) recovery by CO₂ displacement in the United States [1], this technology has provided new ideas for CO₂ gas storage and coalbed methane (CBM) development. Many scholars have conducted extensive researches on coalbed methane (CBM) production increase technologies by CO₂ and N₂ displacement, which had resulting many successful field tests in Poland, Japan, Canada, Netherlands and China. Based on a large number of laboratory experiments and field test data, it is generally believed that the mechanism of coalbed methane (CBM) production increase by gas injection mainly includes two aspects, namely displacement and replacement. Since adsorption capacity of coal to CH₄, CO₂ and N₂ is different, and CO₂ is with the strongest adsorption ability, CH₄ could be displaced by CO₂ due to competitive adsorption effect. At the same time, gas absorption increase in coal will produce expansion effect, resulting decrease of coal permeability and affecting production of coalbed methane (CBM) [8-13]. Therefore, how injected CO₂ and N₂ mixed gas will affect increase production of coalbed methane (CBM) has become the focus of researches. After the mixed gas is injected, the partial pressure of CH₄ is reduced and desorption begins. In order to avoid CH₄ absorbed back in coalbed after desorption, as well as coalbed permeability decrease caused by large amount CO₂ injection, continuous injection of mixed gas is needed to make the displacement mechanism work. In view of the above process, an indoor laboratory evaluation on effect of coalbed methane (CBM) displacement by CO₂ and N₂ gas mixture was carried out to determine the optimal gas-displacing coalbed methane (CBM) option.
MATERIALS AND METHODS

Coal samples preparation. Dafosi coalbed of Jurassic Yan'an formation in western Binxian county, Xianyang city, Shaanxi province is taken as source of experimental coalbed samples. The following are detailed data of coal samples: Average formation pressure is 2.5MPa, average formation temperature is 33°C, gas content of the sampling coalbed is relatively high, between 6.89~16.69m³/t, average 11.55m³/t. Gas composition in the coalbed is mainly CH₄, gas density is 55.31~89.8%, average 75.76%; N₂ density is 9.79~41.39%, average 22.41%; CO₂ density is 0.32~4.65%, average 1.83%.

Porosity of the coal samples measured by vacuum pressurized saturated formation water method is generally distributed in range of 6.05%~10.24%, average porosity is 8.18%. Permeability of the coal samples are between 0.23~0.65mD, average permeability is 0.45mD. Porosity and permeability of the coal is relatively small, indicating the samples belong to compacted coal.

Pulverize the coal samples by a pulverizer, and sieve them with different meshes. Screen 10~120 mesh coal particles and mixed them by a certain proportion (Table 1). Put the well-mixed samples into a 100.0cm length, 4.0cm diameter sand-filling pipe, load with 30MPa overburden pressure until the samples are compacted. Then inject low pressure non-adsorption helium gas and test. According to the test result, permeability of the sand-filling pipe is 0.55mD, which is close to permeability of the original coal sample and meets the experimental requirements.

Scheme of experiment. Taken 5 gases separately for displacement experiment on coal sample adsorbed CH₄, i.e. CO₂, N₂ and three mixed gases with different mix ratio (CO₂:N₂=1:1, CO₂:N₂=1:4 and CO₂:N₂=1:9) under 1.5MPa, 2.5MPa, 3.5MPa, 4.5MPa and 5.5MPa controlled gas injection pressure. And two indexes displacement efficiency and replacement efficiency is taken as evaluation parameter. Displacement and replacement efficiency is defined as follows:

Displacement efficiency

$$\eta = \frac{V_{out}}{V_{add}} \times 100\% \quad (1)$$

Among above formula: \(\eta\): Displacement efficiency; \(V_{add}\): CH₄ volume adsorbed in coal samples, mL; \(V_{out}\): Volume of CH₄ displaced from coal samples, mL.

Replacement efficiency

$$\theta = \frac{V_{out}}{V_{in}} \times 100\% \quad (2)$$

Among above formula: \(\theta\): replacement efficiency; \(V_{in}\): Volume of gas injected into coal samples, mL; \(V_{out}\): Volume of CH₄ displaced from coal samples, mL.

<table>
<thead>
<tr>
<th>Mesh</th>
<th>10~20</th>
<th>20~40</th>
<th>40~60</th>
<th>60~80</th>
<th>80~100</th>
<th>100~120</th>
<th>120~160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion</td>
<td>10%</td>
<td>10%</td>
<td>25%</td>
<td>25%</td>
<td>20%</td>
<td>5%</td>
<td>5%</td>
</tr>
</tbody>
</table>

![Schematic diagram of displacement experimental device](image)

**FIGURE 1**

Schematic diagram of displacement experimental device

1, 2, 4, 6, 7, 11, 12, 13, 14-Control valve; 3, 10-Pressure sensor; 5-Pressure sensor and flowmeter; 8-Backpressure valve; 9-Thermostat box; 15-Submersible pump

9201
Device of experiment. Displacement experiment device consists of a gas injection system, a sand-filling pipe sample chamber system, an intermediate container gas distribution system, a vacuum pumping system, a temperature control system, a drainage gas collection system, and a gas concentration system with gas chromatography analysis.

Procedure of experiment and calculation method. (1) Procedure of experiment. ① Open the three interfaces of sand-filling pipe, connect vacuum pump, set temperature of thermostat box to 80°C, and vacuuming for 24 h;
② Switch thermostat box temperature control system off, open the thermostat box to let sand-filling tube cool off to room temperature for 3 hours; Then set temperature of thermostat box to 33°C, close it and stand still in constant temperature for 2 hours, turn vacuum pump off;
③ Inject CH4 into intermediate container from the CH4 cylinder. Open the valve between intermediate container and sand-filling pipe after pressure is constant to make CH4 enter sand-filling pipe with coal samples. Make it saturated for 12h. During this operation, the control pressure shall be stabled around 2.5 MPa basically (which is average reservoir pressure);
④ Discharge free gas in sand-filling pipe by drainage gas recovery method. Collect gas by a bottle filled with water and measure volume of water discharged;
⑤ Displace CH4 gas in coal samples by gas in the gas injection cylinder. Check flow meter No. 5 for volume of injected gas. Collect gas by drainage gas recovery method. Press gas into gas sampling bag by submersible pump and fill water into the gas bottle.
⑥ Measure volumetric concentration of CH4 gas in sampling bag by portable gas chromatography; When displacement starts, measure once every 10min; When concentration of CH4 is found below 100%, measure once every 1min. In case 3 successive numerical changes shows less than 5%, it is known equilibrium state is reached, and the displacement of CH4 volume could be calculated.
⑦ Change type of gas and the way of displacement, repeat the experiment.

(2) Calculation method. According to experimental data, displacement and replacement efficiency could be calculated as follows:
① Intermediate container CH4 gas balance formula:

\[ P_1V_1 = z_1nRT \]  

Intermediate container gas balance formula after coal samples in sand-filling pipe saturated by CH4:

\[ P_1(V_i + V_j) = z_1nRT \]  

The following equation can be obtained by formula 3 and formula 4:

\[ V_2 = \frac{(z_2P_2 - z_1P_1)V_1}{z_1P_2} \]  

Among above formula: \( P_1 \): Initial CH4 pressure in intermediate container, MPa; \( V_i \): Volume of intermediate container; \( z_i \): Initial CH4 compression factor in intermediate container; \( n \): The amount of CH4 substance in initial intermediate container, mol; \( R \): Thermodynamic parameters, 8.31441J/(mol·K); \( T \): Absolute temperature, K; \( P_2 \): Pressure in intermediate container and sand-filling pipe when coal samples are saturated by CH4, MPa; \( V_2 \): Pore volume in sand-filling pipe, mL; \( z_2 \): CH4 compression factor when coal samples are saturated by CH4 in intermediate container and sand-filling pipe.
② Convert volume of CH4 in sand-filling pipe into volume under standard conditions:

\[ P_1V_2 = z_1\eta_1RT \]  

\[ P_2V_3 = z_1\eta_1RT \]  

\[ V_3 = \frac{z_2P_2V_2}{z_1P_3} \]  

Among above formula: \( \eta_1 \): The amount of CH4 substance in sand-filling pipe, mol; \( P_3 \): Gas pressure under standard conditions, 0.1MPa; \( V_3 \): CH4 gas volume under standard conditions, mL; \( z_3 \): CH4 gas compression factor under standard conditions, 1.
③ Residual CH4 volume in sand-filling pipe after free gas is discharged:

\[ V_4 = V_3 - V_5 = V_3 - \frac{m_1}{\rho_1} \]  

Among above formula: \( V_4 \): Residual CH4
volume in sand-filling pipe after free gas is discharged, mL; \( V_5 \): Volume of free CH\(_4\) gas, mL; \( m_1 \): Weight of water discharged by free CH\(_4\) gas, g; \( \rho_1 \): Density of water, g/cm\(^3\).

4. Volume of displaced CH\(_4\):
\[ V_6 = V_4 \cdot \alpha \]  

Among above formula: \( V_6 \): Volume of displaced CH\(_4\), mL; \( V_4 \): Total gas volume in gas sampling bag, mL; \( \alpha \): Volume concentration of CH\(_4\) in gas sampling bag, %.

6. Calculation of displacement efficiency \( \eta \):
\[ \eta = \frac{V_6}{V_4} \times 100\% \]  

7. Calculation of replacement efficiency \( \theta \):
\[ \theta = \frac{V_5}{V_6} \times 100\% \]  

Among above formula: \( V_8 \): Volume of injected gas under standard conditions, mL.

RESULTS

Carry out displacement experiments on 5 coal samples that saturated with CH\(_4\) by 5 different gases. Set different injection pressure during the experiments, i.e. 1.5MPa, 2.5MPa, 3.5MPa, 4.5MPa and 5.5MPa. Experimental parameters are shown in Table 2 and experiment results are shown in Figure 3.

It can be seen from Figure 3(a) that:

1. Changing trend of displacement efficiency on CH\(_4\) adsorbed in coal samples by different gases is the same, i.e. displacement efficiency will increase and then decrease along with increasing displacement pressure. The highest displacement efficiency appears when displacement pressure is in range of 2.5-3.5 MPa.

When displacement pressure increases, it will improve flow of displacement gas, resulting increase of displacement efficiency at the beginning. However, the continuously increase of pressure also makes CH\(_4\) desorption more difficult from pores of coal samples. The higher the pressure is, the less desorption of CH\(_4\) will shown, so the displacement efficiency gradually decreases with increasing pressure.

2. When pressure is relatively low (lower than 2.0 MPa), N\(_2\) is with highest displacement efficiency and CO\(_2\) with the lowest displacement efficiency under the same pressure. When displacement pressure is 1.5MPa, displacement efficiency of N\(_2\) is 76.01%, and displacement efficiency of CO\(_2\) is only 68.19%. When pressure is gradually increased, displacement efficiency of CO\(_2\) is with the fastest growth, reaching a peak of 83.96% at 2.5 MPa, while displacement efficiency of N\(_2\) is relatively slow, reaching a peak of 85.03% at 3.5 MPa. As the pressure increases further, displacement efficiency of CO\(_2\) decreases rapidly, but decrease rate of CO\(_2\) displacement efficiency is relatively slow. At 5.5 MPa, displacement efficiency of CO\(_2\) reduced to 71.31%, while displacement efficiency of N\(_2\) is only 61.71%.

<table>
<thead>
<tr>
<th>Gas number</th>
<th>G1 CO(_2)</th>
<th>G2 N(_2)</th>
<th>G3 (CO(_2):N(_2)=1:1)</th>
<th>G4 (CO(_2):N(_2)=1:4)</th>
<th>G5 (CO(_2):N(_2)=1:9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>injection pressure 1.5 MPa</td>
<td>M1-1.5</td>
<td>M2-1.5</td>
<td>M3-1.5</td>
<td>M4-1.5</td>
<td>M5-1.5</td>
</tr>
<tr>
<td>Coal sample number injection pressure 2.5MPa</td>
<td>M1-2.5</td>
<td>M2-2.5</td>
<td>M3-2.5</td>
<td>M4-2.5</td>
<td>M5-2.5</td>
</tr>
<tr>
<td>Coal sample number injection pressure 3.5MPa</td>
<td>M1-3.5</td>
<td>M2-3.5</td>
<td>M3-3.5</td>
<td>M4-3.5</td>
<td>M5-3.5</td>
</tr>
<tr>
<td>Coal sample number injection pressure 4.5MPa</td>
<td>M1-4.5</td>
<td>M2-4.5</td>
<td>M3-4.5</td>
<td>M4-4.5</td>
<td>M5-4.5</td>
</tr>
<tr>
<td>Coal sample number injection pressure 5.5MPa</td>
<td>M1-5.5</td>
<td>M2-5.5</td>
<td>M3-5.5</td>
<td>M4-5.5</td>
<td>M5-5.5</td>
</tr>
</tbody>
</table>
When pressure is relatively low, CH₄ desorption is mainly controlled by pressure, and competitive adsorption of CO₂ to CH₄ does not affect displacement much. Since displacement pressure is not sufficient to allow gas enter smaller pores, the strong adsorption capacity of CO₂ makes pores in matrix of coal samples plugged and permeability decreases, resulting displacement efficiency smaller than that of N₂. As pressure increases, desorption of CH₄ is suppressed to a certain extent, and displacement effect caused by competitive adsorption of CO₂ is gradually obvious. The higher the CO₂ content is, the more obvious the displacement efficiency is droved by replacement effect. Therefore, as pressure increases, CO₂ displacement efficiency is with fastest growth. Under higher pressure, CO₂ displacement efficiency is higher than that of N₂.

(3) In range from 2.0 to 4.5 MPa, the mixed gas displacement efficiency curve is higher than single CO₂ or N₂ displacement efficiency curve, indicating that displacement efficiency of mixed gas is higher than that of single gases under this
pressure range. Among all curves, curve of L3 and L4 with the mixing ratio CO₂ and N₂ of 1:1 and 1:4 are the most obvious. Displacement efficiency of L3 reaches 86.61% at 2.5 MPa, which obviously exceeds displacement efficiency of single CO₂ or N₂ under the same conditions.

Gas injected into the coal sample displaced and replaced CH₄ adsorbed on pore wall of the coal sample, this is the main mechanism of increasing coalbed methane (CBM) production by gas injection. As the adsorption capacity of CO₂, CH₄ and N₂ gradually decreased in coal samples, replacement of CO₂ is more obvious under the same pressure and temperature, while displacement is the main effect of N₂. Under medium pressure conditions (2.0-4.5 MPa), on the one hand, compared to amount of free CH₄ desorption under low pressure conditions, amount of free CH₄ desorption is suppressed to a certain extent. In order to displace CH₄, competitive adsorption of CO₂ is required. The higher the CO₂ content is, the greater the contribution of displacement will be. On the other hand, in order to avoid desorbed CH₄ absorbed back to coal samples, it is necessary to displace CH₄ by N₂ since N₂ is with weaker adsorption capacity than CH₄. The higher the N₂ content is, the more contribution of displacement will be. Mixed gas displacement efficiency of synergistic effect from displacement and replacement is better than displacement effect of single gas.

It can be seen from Figure 3(b) that:

(1) There are different characteristics on CH₄ replacement efficiency changing trends in coal samples according to different gases. As pressure increases, CO₂ replacement efficiency increases at first and then decreases. Curve L3 with higher CO₂ content also shows the same trend. Replacement efficiency of N₂ continually decreases as the pressure increase and then its tends become slow gradually. Replacement efficiency curves L4 and L5 with higher N₂ content also show the same changing trend. Replacement efficiency of mixed gases are generally between displacement efficiency of CO₂ and N₂. According to different proportion of CO₂ and N₂ content, replacement gas efficiency curve of mixed gases are quite different with CO₂ or N₂ displacement efficiency curves.

(2) When pressure is relatively low (lower than 2.0 MPa), replacement efficiency of N₂ is significantly higher than that of CO₂. When displacement pressure is 1.5 MPa, replacement efficiency of N₂ is 29.17% while replacement efficiency of CO₂ is only 23.04%. As pressure increases, replacement efficiency of N₂ decreases smoothly, and replacement efficiency of CO₂ increases rapidly. Replacement efficiency of CO₂ reaches a peak of 36.64% when displacement pressure increased to 3.5MPa. When displacement pressure reaches to 5.5 MPa, displacement efficiency of CO₂ reduces to 27.82% while replacement efficiency of N₂ is 17.43%.

Analysis of curves in Figure 3(b): Mechanism of CO₂ and N₂ injection in coal samples on CH₄ production is different. Under low-pressure conditions, a large number of CH₄ gas molecules starts desorption, displacement effect of N₂ plays an important role on coalbed methane (CBM) production, but contribution of CO₂ replacement is not that obvious. What’s more, due to strong adsorption capacity, matrix micropores plugging of coal samples were decreased, which made CO₂ replacement efficiency less than that of N₂. Although displacement pressure increase could help injecting gas into smaller pores, in order to increase the displacement pressure, it will inevitably increase injection volume. High pressure will also make CH₄ molecule not easily desorbed; hence replacement efficiency of N₂ will gradually decrease as displacement pressure increases. However as pressure increases, CO₂ molecules could enter more pores and spread wider. Although pressure increase could cause decrease on amount of free CH₄ desorption to some extent, the competitive adsorption of CO₂ could help to displace more CH₄. Thus CO₂ displacement efficiency increases gradually at beginning of displacement pressure increase. As pressure rises further, increase rate of gas injection is greater than increase rate of CH₄ produced by replacement. Therefore, CO₂ replacement efficiency gradually decreases as the pressure increase.

**CONCLUSION**

5 gases was taken for displacement experiment on coal samples adsorbed CH₄, i.e. CO₂, N₂ and three mixed gases with different mix ratio (CO₂:N₂=1:1, CO₂:N₂=1:4 and CO₂:N₂=1:9) under 1.5 MPa, 2.5 MPa, 3.5 MPa, 4.5 MPa and 5.5 MPa controlled gas injection pressure separately. Displacement and replacement efficiency are the key parameters for evaluating the experiment results. The following rules are obtained by comparing change of displacement and replacement efficiency in each experiment:

(1) Injection pressure of various gases has different effects on displacement and replacement efficiency. In terms of displacement efficiency, as the injection pressure increases, displacement efficiency of different gases will increase first and then decrease. In terms of replacement efficiency, replacement efficiency of N₂ shows slowly decrease as injection pressure increase, while replacement efficiency of CO₂ shows increase first and then decrease as injection pressure increase. Therefore, it is not true that the higher the displacement pressure is, the more coalbed methane (CBM) will be developed. Although pressure increases will help gases spread wider at some extent, it also suppresses desorption of CH₄. Taking coal samples saturated with CH₄ under 2.5 MPa pressure as an example, con-
Considering influence of pressure on displacement and replacement efficiency, the best displacement pressure shall be 2.5-3.5 MPa. In this pressure range, average displacement efficiency of the 5 injected gases is 84.16%, and average replacement efficiency is 26.44%.

(2) Under same injection pressure conditions, different injection gases show different displacement and replacement efficiency. When gas injection pressure is relatively low (lower than 2.0 MPa), replacement of CO$_2$ is not obvious, displacement and replacement efficiency of N$_2$ are higher than that of CO$_2$. As pressure of gas injection increases, amount of free desorbed CH$_4$ gradually decreases, and contribution of CO$_2$ replacement effect to CH$_4$ development is showing out gradually. Under relatively high pressure (higher than 2.0 MPa), displacement and replacement efficiency of CO$_2$ is higher than that of N$_2$. Under a certain pressure range (2.5-3.5 MPa), replacement effect of CO$_2$ and displacement effect of N$_2$ will generate synergistic effect with a certain proportion of mixed gas (volume ratio of CO$_2$ and N$_2$ is 1:1 or 1:4 respectively). Taking mixed gas (CO$_2$:N$_2$=1:1) as an example, the average displacement efficiency is 86.14%, average replacement efficiency is 30.61%, which is higher than average CO$_2$ displacement efficiency 83.06% and average N$_2$ displacement efficiency 83.39%. It is lower than average CO$_2$ replacement efficiency 34.92%, but higher than average N$_2$ replacement efficiency 20.78%.

**ACKNOWLEDGEMENTS**

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PHYSICAL AND CHEMICAL PROPERTIES OF AEOLIAN AND DUNE SAND OF THE REGION 31-33° N, 4-9° E AT SOUTHERN ALGERIA

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ABSTRACT

This study aims to examine the physical and chemical properties of dune and aeolian sands in the region of southern Algeria. The sand sampling was done from three major routes along the distance of 1210 km, from 15-20 m far from the main road edge each 10 km. A total of 120 sand samples were collected seasonally from (0-20 cm) depth, during the period from autumn 2016 to summer of 2017. Adding to the physical and chemical analysis, the particles of Aeolian and dune sand samples were subjected to the X-ray diffraction (XRD) and X-ray fluorescence (XRF) to determine the chemical composition of metallic oxides and concentration of some metallic trace elements (MTE).

KEYWORDS:
Physical and chemical properties, Dune and aeolian sand, Southern Algeria, XRD, XRF, MTE.

INTRODUCTION

Sand is a granular material consisting of small particles from the disintegration of materials of mineral origin (mainly rocks) or organic (shells, coral skeletons ...) whose size is between 0.063 (silt) and 2 mm (gravel). Its composition can reveal up to 180 different minerals (quartz, micas, and feldspars) as well as limestone debris [1].

The grains of sand are light enough to be carried by the wind. By time, they accumulate and form the dunes. The sandstorm is a violent wind which loads the sand grains. The heavier grains settle first in high energy environments (river, high of a beach), the finest in lower energy environments (delta, lake, basin, creek) [2].

The density of the dry sand varies, according to its granulometry and composition, from 1700 to 1900 kg.m⁻³ (on average 1850 kg.m⁻³).

In the 1970s and 1980s, numerous studies focused on this subject, in order to be able to differentiate between natural variations linked to physical factors [3] and to distinguish possible effects of human activities.

Variations in temperature and salinity are also sources of modification of these ecosystems [4]. Pollution can also strongly modify this ecosystem.

 Sands as a draining medium can absorb large quantities of certain pollutants (in the event of a spill). Silica adsorbs easily on its surface some toxic (lead for example). The clogging or death by biocidal pollutants of sand life can cause a blackening of the sand layer (with unpleasant odor related to mercaptans and emission of greenhouse gases (CO₂, methane) and toxic gas (H₂S).

Heavy metal contaminations of sand and sand, the food chain (sand-plant-human or sand-plant-animal-human), drinking of contaminated ground water, safety and marketability through phytotoxicity, land tenure problems, indoor / outdoor air pollution [5-6].

After air and water, sand is the most used resource in the world. It represents a volume of international trade of 70 billion dollars a year [7]. More than 15 billion tons are extracted worldwide each year, a tonnage equivalent to the natural production of these sediments by rivers [7].

The size, the nature and the more or less rounded shape of its grains make it a material of sought-after quality for construction.

In masonry, sand is used as an aggregate [8] mixed with a binder such as lime or cement. In foundries of ferrous metals or light alloys, the molds can be made of sand agglomerated by resins or clays, to cast the pieces. In the kitchen, it was used in the nineteenth century for the preservation of meat. It is used as raw material of glass. It can be used to filter liquids, gases or air [9-10].

It is used as an abrasive in factories to clean metal parts. Sand is also an important element in the tourist field, when it is present on beaches and dunes where it is also an essential element for the protection of the coast.
It is also used in jet at high pressure to give the effect-faded jeans. Agricultural amendment to both increases the pH of soil that is too acidic (e.g. market gardening) and improve the texture of the soil and, of course, mineral input (calcium carbonate, for shell sand) for certain crops (e.g.: cabbage).

This study report the seasonal variations of physical and chemical properties and heavy metals level of the sand in the region of Borma-HassiMessaooud-Ouargla-Touggourt-El Oued (710 km) and Ouargla-El Hadjira-Guettarra-Guerrarra-Ouargla (500 km). The sand samples were characterized by physical and chemical analyses, X-ray diffraction and X-ray fluorescence more than 480 samples were taken and analysed until the period of autumn 2016 and summer 2017.

MATERIALS AND METHODS

Sampling methods, periods and locations. Sampling campaigns were conducted in Southern Algeria at the region three regions, the first between Ouargla-Touggourt-El Oued (OTE); the second at Ouargla-El Hadjira-El Guettara-El Guerrarra (OHG) and the third at Ouargla-HassiMessaooud-El Borna (OHB). One hundred twenty samples were collected each season for 1200 km, every 10 km and from 15 to 20 meters on the side of the road, the sample were taken until the period of autumn 2016 to summer 2017.

The sand particles were collected using aluminium spoon, scope beforehand in the oven at the temperature of 900 °C for 2 hours, about 2 kg of sand particles for each sampling point was collected in propylene boxes (as shown in the Figure 1).

Meteorological conditions. The meteorological conditions recorded at the sampling sites and until the sampling period were listed in the Table 1.

Chemical, reagents and apparatus. Hydrochloric acid 37%, Sodium hydroxide BioXtra, ≥98% (acidiometric), pellets (anhydrous), potassium chloride BioXtra, ≥99.0%, silver nitrate ACS reagent, ≥99.0% were purchased from Sigma-Aldrich Company. Electric conductivity was measured using Thermo Scientific™OrionStar™A112 benchtop-conductivity meter, allowing a range of measurement varied between 0.01 μS/cm to 200.0 mS/cm and a resolution in conductivity varying between 0.01 μS minimum, auto ranging up to 3 significant digits, purchased from Thermo Fischer Scientific. pH was measured using WTW™ inoLab™ 7110 benchtop pH meter, allowing measurement in the pH range of -2 to 20 ± 0.1 and with a resolution of -2 to 20 ± 0.01, -2 to 19.999 ± 0.005, purchased from Thermo Fischer Scientific.

Physical and Chemical analysis. Particles size distribution. The method used is in accordance with Standard NF P 94-056 (March 1996) [11], which consists of quartering, then proceed to a test portion according to the formula: 200D <P <600D (D: average diameter estimated larger grain). Weigh the sample and soak it in a container, then remove the fine particles by continuous washing (0.08 mm sieve), dry the material at 105 °C, sieve the sample and collect the refusal of each sieve and weigh it.

Determination of calcium carbonates. An excess of hydrochloric acid is applied to the soil sample and the excess is then measured by a solution of sodium hydroxide from which the carbonate content in the soil is determined exactly (NFP 15-461, 1964) [12].

FIGURE1
Detailed sites sampling
Determination of chlorides. Take about 1 kg of a representative sand sample (particles ≤ 20 μm), bring the sample to a temperature between 105 and 110 °C, then pass the sample through a 600 μm sieve, take 100 g of soil and add 200 mL of distilled water and shake for 24 hours, transfer 25 mL to a vial and titrate to neutral pH with silver nitrate (BS Bowly standard) [13].

pH. A 20 g quantity of sand was weighed, then added 100 mL of distilled water, allowed the solution rested for 24 hours and then measuring the pH.

Determination of organic matter. The method of loss on ignition was used for the determination of organic matter in sand samples. The samples were placed in a muffle furnace for 16 hours at a temperature of 375 °C, the weight loss, after calculations, gives us the organic matter content.

The highest temperatures that result in the destruction of carbonates in sand samples must be avoided, leading to an error in the estimation of organic matter. Pre-drying of the samples at 150 °C is essential for the removal of interstitial water trapped in the sand samples.

Measurement of electrical conductivity. After rinsing the electrode with distilled water or activating if necessary, the measuring instrument is first calibrated to determine the constant of the K cell. This is done by measuring EC and the temperature of the KCl solution (0.02 N or 0.1 M). Then the correction of the effect of the temperature by the correction coefficient is carried out, after which the EC and the temperature of the solution to be analyzed are measured and the results obtained are corrected.

Determination of sulphate content. The determination of sulphate contents was carried out in accordance to the BS 1377 norme [13] and following the protocol illustrated by Figure2. The contents of SO$_4^{2-}$ and CaSO$_4$ are calculated from the following formulas:

$$SO_4^{2-} = 34.3 \times (P_2 - P_1)/P_0$$

$$CaSO_4 = 184.23 \times (P_2 - P_1)/P_0$$

X-ray diffraction (XRD) analysis. The mineral identification/quantification of the collected samples of sand was accomplished using PANanalytical X’pert PRO X-ray diffractometer with a two theta/theta goniometer geometry. All samples were scanned at the start position two-theta equal to 5.0084 and the end position two-theta equal to 89.9944. The measurements were performed with the 0.0170 two-theta degrees scan step sizes and scan step time 10.3368 s, using copper Kα radiation with a nickel filter at 45 kV voltage and 30 mA current of the generator. A dynamic scintillation detector with low background, and large dynamic range was used for the collection of X-ray diffraction data from samples.

X-ray fluorescence (XRF) analysis. A Niton® XL3 model, environmental analyzer (Thermo Scientific, Munich Germany), equipped with a miniature X-ray tube 40 kV/50 A excitation source, and Ag-anode. Photon counts from the detector are amplified, digitized and processed internally by a multi-channel analyzer and concentrations display in ppm. The instrument is capable to analyzing elements in the Z range of 19 (K) to 92 (U) by using K and L shell emission lines. XRF spectra were analyzed using advanced Niton Software 7.1 version.
**Quality assurance/Quality control.** Several steps were taken to assure that the data collected in this study are of high quality including collection of field and procedural blank samples and duplicates from each collected sample. Field and procedural blanks were taken with each series of samples and processed in the same manner to the samples. Concentration of the target in the procedural blanks corresponded to less than 10% of the concentrations found in the samples and thus met the quality criteria.

Concentrations of duplicates obtained in the collected samples were in good agreement with each other (less than 20%).

The detection limits of XL3t XRF analyser, are specified following the United States Environmental Protection Agency (US EPA) protocol of 99.7% confidence level (US EP, 2005) [14]. Individual LODs improve as a function of the square root of the count test time. The detection limits for all analyses were based on a 100-s total analysis time in a regular standard soil mode.

**RESULTS AND DISCUSSION**

**Physical and chemical analysis.** The Tables 2, 3 and 4 shows the results of physical and chemical analysis at the region of Ouragla-Touggourt-El Oued (OTE); Ouragla-El Hadjira-El Guettara-El Guerrarrar(OHG) and Ouragla-Hassimessaoud-El Borma (OHB), respectively. The physical and chemical analysis including the measurement of Hydrogen potential (pH), electrical conductivity, organic matter (OM), CaSO₄, CaCO₃ and NaCl contents. The Table 5, display the particles size distribution of sand for the sites sampling.

According to the Table 2-4, the pH of the sand sample was basic, the pH seasonl range varied from 8.59 to 8.91 for OTE site, and from 8.76 to 9.20 for OHB site and from 8.41 to 8.96 for OHG site. Electrical conductivity varied seasonally from 922 to 4929, from 441 to 627, from 901 to 1170 in OTE, OHB and OHG, respectively. The high concentration of CaSO₄, 2H₂O was measured in winter season at OTE site (10.68%), while the lower one was recorded in Springer at OHB (0.96%) (See Figure 4B). The lower percentage of sulphite (SO₃²⁻) and the sulphate (SO₄²⁻) were recorded in springer at all site sampling, 0.93 and 1.12 %; 0.18 and 0.22%; 0.64 and 0.77% were recorded at OTE, OHB and OGB respectively. The higher percentage of sulphite (SO₃²⁻) and the sulphate (SO₄²⁻) were recorded in cold season (winter) at all site sampling, 1.99 and 2.39%; 0.34 and 0.40%; 0.81 and 0.97% were recorded at OTE, OHB and OGB respectively. The higher percentage of CaCO₃ contents in sand samples were recorded in Springer
season at OTE and OHG sites, when we measured, respectively 2.33 and 4.00%, however at OHB site, the highest percentage was measured in summer season (1.78 %). The lower percentage of CaCO₃ contents were recorded in the cold season for OTE and OHG site, while for OHB site the lower percentage was recorded in Springer (see Figure 4A). High percentage was recorded at OTE site, when the NaCl% varied from 3.13 to 3.69. In contrast the percentage of NaCl recorded in both site (OHB and OHG) still constant in all season, when it’s ranged from 0.02 to 0.03 at OHB site and from 0.05 to 0.06 NaCl in OHG site. Organic matter (OM) percentages recorded at all site sampling were higher at the cold season and lower at the warm season (see Figure 4C). The physical and chemical properties measured in this study varied from site to site and from season to season. These variations are related to the meteorological parameter recorded in this study (Table 1), when the precipitation has effect to increase the acidic species by the outcomes of acidic rain and affect to pH of the sand sample and the electrical conductivity. Also the organic matter (OM) contents was affected by the high speed of wind, when the percentage of OM was increased or decreased from site to site and from season to season via the long range transport.

### TABLE 2

<table>
<thead>
<tr>
<th>Physical and chemical properties of sand in the region of OTE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
</tr>
<tr>
<td>Mean</td>
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<tr>
<td>Max</td>
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<tr>
<td>SD</td>
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<tr>
<td>Win</td>
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<tr>
<td>Max</td>
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<tr>
<td>SD</td>
</tr>
<tr>
<td>Spr</td>
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<tr>
<td>Max</td>
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<tr>
<td>SD</td>
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<tr>
<td>Sum</td>
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<tr>
<td>Max</td>
</tr>
<tr>
<td>SD</td>
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</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>Physical and chemical properties of sand in the region of OHB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
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<td>Mean</td>
</tr>
<tr>
<td>Max</td>
</tr>
<tr>
<td>SD</td>
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<td>SD</td>
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<tr>
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<tr>
<td>Max</td>
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<tr>
<td>SD</td>
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<tr>
<td>Sum</td>
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<tr>
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</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>Physical and chemical properties of sand in the region of OHG</th>
</tr>
</thead>
<tbody>
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<td><strong>pH</strong></td>
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<td>Mean</td>
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<td>Sum</td>
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<tr>
<td>Max</td>
</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>
FIGURE 3
Particles size distribution of curve sand

4A

4B

4C

FIGURE 4
Seasonal variation of $\text{CaCO}_3$ (4A); $\text{CaSO}_4\cdot2\text{H}_2\text{O}$ (4B) and OM (4C)

According to the Table 5, the composition of sand varied from site to site, at OTE site, 43.05% were lemon and fine sand fractions and 51% was high sand and 5.50% was gravies. At OHB and OHG, the lemon fraction was absent at OHG and very little fraction were recorded at OHB site (0.09%). At the same time 66.44 and 53.82% were recorded respectively at OHB and OHG as high
sand fraction. Very little fraction of gravies was measured in both sites (2.67 and 1% at OHB and OHG sites, respectively). The fine sand fraction recorded at OHG and OHB were the highest (45.18%) for OHG site and the lowest for OHB site (30.89%) among those measured at the three sites (see Figure 3). This variation of sand composition was directly related to the nature of sand origin or nature. The aeolian sand was formed principally by the grosses particles; In contrast, the dune sand was formed by the fine particles.

**X-ray diffraction analysis.** The Figure 5 shows a diffractogram X-ray of sand sample. The presence of intense peak corresponding to the two theta position with the value of: 21, 27, 36, 39, 46, 50, 55, and 60 confirming that the sample is sand, where the peaks statement are related to the quartz, the main constituent of sand [15]. The X-ray diffraction analysis showed that the sand sample has a high percentage of quartz reaching 83%. The Table 6 gives the two theta position, d-spacing in angstrom with relative intensity of each peak.

**Heavy metals.** Seventeen heavy metals were detected at all site sampling (Table 7). Among heavy metals, also called element trace metallic (ETM): Molybdenum, Zirconium, Zinc, Rubidium, Vanadium, Titan, Barium, Niobium and Aluminium were recorded at all sites sampling, very high concentrations of aluminium were measured, when the ranged was from 1029 to 1524 ppm at OHB site, from 940 to 1541 ppm at OHG site, from 775 to 1722 ppm at OTE site. Other heavy metals such as: Strontium, Manganese, Uranium, Thorium, Lead and Chrome were maximum recorded at two sites sampling. Uranium and Thorium are radioelement were recorded at same site sampling with concentration varied respectively between 0 and 5.8 ppm and from to 0 to 2.1 ppm. Thorium is by product results of nuclear disintegration of Uranium with alpha particles emissions, the presence of these radioelement is very frequent in this region (Southern Algeria) [16], and this fact is related to the nuclear experimentation released at the colonial period in the last century. Iron, Calcium, Potassium, Sulfur and Phosphor as fertilizing element were also present at all sites sampling with very high concentration. Molybdenum and Strontium were recorded with high concentration at OTE site with concentration attained relatively sometimes 7.4 and 342 ppm, exceeded the value guidelines set by WHO and European Committee, when the values of 5 ppm for Molybdenum and 100 ppm for Strontium were fixed as limit concentration premises [17].

<table>
<thead>
<tr>
<th>Granulometry (%)</th>
<th>Slit</th>
<th>Fine sand</th>
<th>Coarse sand</th>
<th>Gravels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OTE</strong></td>
<td>Mean</td>
<td>3.67</td>
<td>39.83</td>
<td>51.00</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>11.00</td>
<td>58.00</td>
<td>62.00</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>4.50</td>
<td>11.94</td>
<td>11.15</td>
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<tr>
<td><strong>OHB</strong></td>
<td>Mean</td>
<td>0.00</td>
<td>30.89</td>
<td>66.44</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>0.00</td>
<td>36.00</td>
<td>70.00</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.00</td>
<td>3.48</td>
<td>2.96</td>
</tr>
<tr>
<td><strong>OHG</strong></td>
<td>Mean</td>
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<tr>
<td></td>
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<td>55.00</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.30</td>
<td>5.34</td>
<td>5.08</td>
</tr>
</tbody>
</table>

**TABLE 5**
Particles size distribution of sand samples

**FIGURE 5**
Diffractogram X-ray (XRD) of sand
### TABLE 6
Peak list with two theta position of sand sample

<table>
<thead>
<tr>
<th></th>
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<td>1334.55</td>
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<tr>
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<td>682.90</td>
<td>0.1428</td>
<td>1.18030</td>
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<td>83.8108</td>
<td>132.96</td>
<td>0.3264</td>
<td>1.15331</td>
<td>0.69</td>
</tr>
</tbody>
</table>

### TABLE 7
Heavy metals concentration (ppm) in sand samples

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>OOH</th>
<th>OOG</th>
<th>OTE</th>
<th>Guidelines value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
<td>3.6-13</td>
<td>2.8-3.6</td>
<td>00-7.4</td>
<td>5</td>
</tr>
<tr>
<td>Zirconium</td>
<td>Zr</td>
<td>99-662</td>
<td>118-146</td>
<td>19-362</td>
<td>-</td>
</tr>
<tr>
<td>Strontium</td>
<td>Sr</td>
<td>00</td>
<td>19-44</td>
<td>38-342</td>
<td>100</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>22-25</td>
<td>22-24</td>
<td>20-27</td>
<td>100</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>00-41</td>
<td>00</td>
<td>00-5.8</td>
<td>-</td>
</tr>
<tr>
<td>Uranium</td>
<td>U</td>
<td>00</td>
<td>00</td>
<td>00-5.8</td>
<td>-</td>
</tr>
<tr>
<td>Thorium</td>
<td>Th</td>
<td>0-3</td>
<td>00</td>
<td>00-2.1</td>
<td>-</td>
</tr>
<tr>
<td>Rubidium</td>
<td>Rb</td>
<td>10-15</td>
<td>5-8</td>
<td>8-15</td>
<td>-</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb</td>
<td>3-4</td>
<td>00</td>
<td>3-5</td>
<td>50</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>1503-4096</td>
<td>1274-1548</td>
<td>1047-2457</td>
<td>-</td>
</tr>
<tr>
<td>Chrome</td>
<td>Cr</td>
<td>00-18</td>
<td>00</td>
<td>00-11</td>
<td>-</td>
</tr>
<tr>
<td>Vanadium</td>
<td>V</td>
<td>00-40</td>
<td>12-17</td>
<td>15-22</td>
<td>-</td>
</tr>
<tr>
<td>Titan</td>
<td>Ti</td>
<td>874-1589</td>
<td>537-1043</td>
<td>249-1419</td>
<td>-</td>
</tr>
<tr>
<td>Calcium</td>
<td>Ca</td>
<td>2700-9335</td>
<td>4731-10679</td>
<td>12044-39013</td>
<td>-</td>
</tr>
<tr>
<td>Potassium</td>
<td>K</td>
<td>3584-4613</td>
<td>2102-2746</td>
<td>3132-5657</td>
<td>-</td>
</tr>
<tr>
<td>Sulfur</td>
<td>S</td>
<td>814-1372</td>
<td>1733-3171</td>
<td>1195-26287</td>
<td>-</td>
</tr>
<tr>
<td>Barium</td>
<td>Ba</td>
<td>99-151</td>
<td>00-81</td>
<td>93-95</td>
<td>-</td>
</tr>
<tr>
<td>Niobium</td>
<td>Nb</td>
<td>00-4.1</td>
<td>00-1.6</td>
<td>00-2.8</td>
<td>-</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Al</td>
<td>1029-1524</td>
<td>940-1541</td>
<td>775-1722</td>
<td>-</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>P</td>
<td>4175-8102</td>
<td>4574-4748</td>
<td>4189-4650</td>
<td>-</td>
</tr>
<tr>
<td>Selenium</td>
<td>Se</td>
<td>103058-120855</td>
<td>107630-117472</td>
<td>75278-107355</td>
<td>-</td>
</tr>
<tr>
<td>Chloride</td>
<td>Cl</td>
<td>14826-16511</td>
<td>15804-16172</td>
<td>14964-21953</td>
<td>-</td>
</tr>
</tbody>
</table>
Metallic oxide composition of sand. The Table 8 gives the percentage of metallic oxide (SiO$_2$, Al$_2$O$_3$, CaO, Cr$_2$O$_3$, Fe$_2$O$_3$, K$_2$O, MnO, MgO, Na$_2$O, TiO$_2$) at OTE, OHB and OHG sites sampling. Those metallic oxide were calculated as basis of metallic element corresponding and multiplicity by corresponding factor of each element as indicated in the Table 8. Selenium Oxide (SiO$_2$) is the main constituent its account between 80 and 88% at OHG site, between 78 and 89% at OHB site, and between 51 and 81% at OTE site. The second main metallic oxide contributor is the calcium oxide, when its account between 13.8 and 40.9% at OTE site, between b 5.3 and 12.3% at OHB site, and between 3 and 10% at OHG site. The others metallic oxides such as: Al$_2$O$_3$, Fe$_2$O$_3$, K$_2$O and TiO$_2$, accounts together between 5.5 and 8.3%, between 7.8 and 13.7% and between 4.3 and 10.02% at OH, OHG and OTE and sites, respectively. The rest of metallic oxide such as Cr$_2$O$_3$, MnO, MgO and Na$_2$O are inexistent or represent very little contribution (Figure 6).

According to the composition of metallic oxides listed in the Table 8 and based on the previous work on mechanical testing of concrete specimens formulated with sand dune showed that the dune sand can be used successfully to constitute a skeleton of a concrete when a judicious choice is made on its composition [18].

<table>
<thead>
<tr>
<th>Element</th>
<th>Oxide</th>
<th>Factor</th>
<th>OHG</th>
<th>OHB</th>
<th>OTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>SiO$_2$</td>
<td>0.6743</td>
<td>80-88</td>
<td>78-89</td>
<td>51-81</td>
</tr>
<tr>
<td>Al</td>
<td>Al$_2$O$_3$</td>
<td>0.52952</td>
<td>0.8-1.4</td>
<td>0.8-1.2</td>
<td>0.0-1.3</td>
</tr>
<tr>
<td>Ca</td>
<td>CaO</td>
<td>07147</td>
<td>5.3-12.3</td>
<td>3.10</td>
<td>13.8-40.9</td>
</tr>
<tr>
<td>Cr</td>
<td>Cr$_2$O$_3$</td>
<td>0.68420</td>
<td>0.00</td>
<td>0-0.3</td>
<td>0.0-0.02</td>
</tr>
<tr>
<td>Fe</td>
<td>Fe$_2$O$_3$</td>
<td>0.69943</td>
<td>1.4-1.8</td>
<td>1.6-4.2</td>
<td>1.1-2.7</td>
</tr>
<tr>
<td>K</td>
<td>K$_2$O</td>
<td>0.83015</td>
<td>2.8-3.7</td>
<td>4.6-6.5</td>
<td>4.1-7.3</td>
</tr>
<tr>
<td>Mn</td>
<td>MnO</td>
<td>0.77446</td>
<td>0.00</td>
<td>0-0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Mg</td>
<td>MgO</td>
<td>0.60304</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Na</td>
<td>Na$_2$O</td>
<td>0.74186</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ti</td>
<td>TiO$_2$</td>
<td>0.5994</td>
<td>0.5-1.4</td>
<td>0.8-1.6</td>
<td>0.2-1.4</td>
</tr>
</tbody>
</table>
CONCLUSION

The seasonal variation of physical and chemical properties of sand samples collected at the three site sampling in southern Algeria showed some variation, which is related to the meteorological parameters and the origin of the sand collected, this latter has as origin aeolian at some site sampling and as origin dune at other site.

Among the toxic heavy metals detected in almost sites sampling: Molybdenum and strontium were recorded with high concentration exceeded the value guidelines set by WHO and European Committee. Zirconium was the most abundant heavy metals when exceed 660 ppm in some sand sample.

Thorium by product of Uranium degradation was sometimes present in sand samples, these latter were related to the nuclear experimentation released at the colonial period in the last century.

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yacine.moussaoui@gmail.com
STUDY ON ENVIRONMENTAL POLLUTION AND GOVERNANCE IN RURAL DEVELOPMENT

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²Western Environmental Resources Legal Construction Research Center, Chongqing University, Chongqing, 400044, China

ABSTRACT

Environmental pollution in China rural has become a major topic of public debate and political concern. At the same time, few rural areas are subjected to measurements of environmental quality, and the phenomenon of cross-flow of sewage still exists. As the state is vigorously promoting the construction of new rural in China, it is of great practical significance to study the environmental pollution in and governance in rural. Domestic sewage is a key source of surface water pollution in rural areas of developing countries, especially in China, where a large amount of highly concentrated nutrients is directly discharged into the receiving waters. Therefore, in this study, the status of environmental pollution is present and governance strategy is prosed. Establishing and improving laws and regulations on environmental protection, strengthening research and planning and strengthening environmental protection management should be conducted. This will provide a useful reference and guidance for rural environmental pollution and governance.

KEYWORDS:
Rural, Environmental Pollution, Governance, Domestic sewage

INTRODUCTION

China's environmental crisis is one of the most urgent challenges of the country. The nation’s rapid economic development over the past three decades has come at the expense of severe environmental pollution [1]. For example, China is the world's largest greenhouse gas emitter with more than 9 billion cubit meters of carbon dioxide emissions per year. The air quality of its major cities continuously fails to meet acceptable international health standards. Among 338 Chinese cities with air quality monitoring data in 2017, average PM2.5 concentrations amounted to 4.3 times the WHO guideline.2 The problems of water and soil pollution are equally widespread and serious. Such environmental deprivations pose a major threat to China's economic development and national health [2]. According to an estimate of China's Ministry of Environmental Protection, environmental pollution costs the country more than 3% of its GDP per year.

Domestic sewage is considered to be a major pollution source in rural areas of developing countries [3-5]. In such areas with a less developed infrastructure for wastewater treatment in which wastewater is not treated, domestic sewage containing high levels of substances, such as nitrogen, phosphorus, and pathogens, is often directly discharged from open drainage ditches to nearby water bodies [6]. This has caused the eutrophication of the surrounding surface water in developing countries, especially in China [7]. Therefore, the identification of suitable domestic sewage treatment approaches in these rural areas, especially nitrogen and phosphorus removal, has become an urgent issue [8].

Many ecological engineering approaches have been developed to treat rural domestic sewage worldwide, such as constructed wetlands (CWs), retention ponds, and subsurface filtration technologies [9, 10]. Many studies have confirmed that pollutants in domestic sewage, such as chemical oxygen demand (COD), nitrogen, phosphorus, heavy metals, and pathogens, could be effectively removed by CWs [11]. Vymazal [12] reviewed the nutrient removal mechanisms of various types of CWs. Similarly, retention ponds and subsurface filtration technology have also been confirmed to be effective methods to remove nutrients in rural areas [13]. However, the effectiveness of nutrient removal via these approaches is highly variable and depends on a range of factors, such as the temperature, discharge, wetland plants, and pollutants concentrations [14]. In addition, the pollutants accumulated in sediments, filter materials, and decaying wetland plants could be released and re-enter receiving waters, which may lead to the release of the retained nutrients, thus, becoming a source of nutrients of the water column [15]. Furthermore, although all these approaches have significant advantages over typical wastewater treatment plants in terms of the nutrient removal and economic costs, the land requirements for these approaches may be the most limiting factor to their application, especially in hilly areas [16]. Therefore, it is challenge that how to reduce the land
FIGURE 1
Status quo of environmental protection
requirement for domestic sewage treatment via ecological engineering approaches in rural areas, especially in hilly areas of developing countries.

The worldwide movement of public participation in environmental governance started in the 1960s and 1970s. The United States was the first to get the public involved in environmental management. In 1979, the Chinese government promulgated the first law, the Environment Protection Act (Trial), which stipulated the rights and obligations of the public in environmental governance. In 1993, the State Environmental Protection Administration first proposed that “public participation” be required in environmental assessment. At present, the research on public participation in environmental governance is usually conducted from the following two perspectives: most scholars study the public's environmental participation behavior, while others come from the legal point of view, advocating for the right of citizens to participate in environmental protection.

MATERIALS AND METHODS

A comprehensive literature search was conducted in July 2019 using the keywords “Rural***”, “environment”, and “pollution” in ISI Web of Knowledge (http://webofknowledge.com) and Scopus (http://scopus.com) databases. Articles published during 2012–2018 were then screened by country and only studies on environment of China were selected for further analysis. A total of 100 articles affiliated with China were sorted and retrieved from the total (global) for detailed analysis.

RESULTS AND DISCUSSION

Public participation behavior. In the 1990s, China established an environmental petition system for local bureaus of environmental protection (BEPs) to hear and solve public complaints about environment-related problems. Complaining by letter is one of the most commonly used forms of petition from ordinary citizens; 2,768,473 letters were received on environmental matters by BEPs during the years 2006–2010. The number of letters related to environmental issues is a direct indicator of the participation of citizens regarding environmental issues. The greater the number of letters, the higher the degree of public participation is in the local environment.

Environmental protection in rural development, it is not only a matter of concept, it is more manifested in the way people behave (Fig. 1). This behavior is a common action between public participation and local governments. Environmental protection is not simply a matter of destroying existing rural development areas. At present, the rural areas that have been developed in China have more or less ecological degradation and ecological resources destruction. Under such circumstances, it may be difficult to assume the responsibility of the entire environmental protection only by relying on the conscious awareness of the public participation. In addition, the rural development in China is relatively late, and there are also many problems in the process of development.

The main cause of the pollution of the public's own living environment. Since China's economic reform in the 1980s, it has already suffered from black smoke from high levels of coal burning by winter heating units and gasoline consumption by vehicles. But the pollution challenge grew worse after the 1980s, when the government launched a new economic development strategy. In the 21st century, China's air pollution problems became particularly acute, because of consumption of coal took off. China's combination of coal burning has increased a number of pollutants, including sulfur dioxide and nitrogen oxides, but for simplicity we will focus much of our discussion on sulfur dioxide. With the rapid progress of China's economy, the living standard of households is also improving. However, at the same time, the environmental protection of households has not improved adequately. And the environmental participation policies promulgated by the government were not readily available. For the reasons given above, there is also a large amount of household waste water and domestic garbage.

Measures to promote the environmental governance in rural development. (1) Establishing and improving laws and regulations on environmental protection. Whether managers or participants should take the scientific development concept as a guide and establish a correct awareness of environmental protection, we must put environmental protection at the top of all work, preferring to develop at a slower pace with less immediate economic benefits. At the same time, it is necessary to introduce and revise relevant laws and regulations in a timely manner, and to strengthen the management of environment with the system as the starting point, and to achieve legal governance. In particular, the environmental assessment system must be strictly implemented.

(2) Strengthening research and planning. It is necessary to strengthen the planning work in the early stage of rural development. It is necessary to fully investigate and understand the rural environment, humanities and customs. When planning, we must fully consider the factors of environmental protection, and should be rationally laid out and focused. Develop step by step, do functional partitioning, do not blindly establish projects and construction.
(3) Strengthening environmental protection management. It is necessary to strengthen monitoring work, pay attention to changes in the environment at any time, and take measures. It is necessary to properly handle pollutants such as garbage and sewage, strictly control the construction of relevant supporting facilities, and achieve green construction and green management. It is impossible to blindly construct artificial landscapes and destroy the original natural environment.

CONCLUSIONS

As the largest developing country in the world, China has environmental and resource constraints on development that have become increasingly prominent. The public is a direct victim of environmental pollution. Therefore, the public should also be the participant in environmental governance.

In this study, the status of environmental pollution is present and governance strategy is prosed. Establishing and improving laws and regulations on environmental protection, strengthening research and planning and strengthening environmental protection management should be conducted. The public environmental participation policy has played a catalytic role in the governance of environment pollution. This indicates that the Chinese governments' policies of public environmental participation are relatively successful and are effective in slowing down the deterioration of environmental quality. This will provide a useful reference and guidance for rural environmental pollution and governance.

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RESEARCH ON APPLICATION OF LIMIT LEARNING METHOD AND LEAST SQUARE SUPPORT VECTOR MACHINE IN ECOLOGICAL EMISSION PREDICTION

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ABSTRACT

The discharge of ecological environment is an important index to restrict economic development. Effective controlling and optimizing the discharge of ecological environment is an important measure to protect the ecological environment at present. In order to accurately measure NOx emission from pulverized coal fired boilers, a maximum learning method (ELM) was proposed to optimize least squares support vector machine (LSSVM) for predicting ecological emission. To overcome the shortcomings of over-fitting of traditional extreme learning machine, it was proposed a novel NOX emission prediction model (ELM-LSSVM) based on the combination of extreme learning machine and least squares support vector machine (LSSVM). This method used the improved maximum learning method to optimize the LSSVM parameters, established the optimization model, and predicted the different NOx emissions of coal-fired boilers. The learning samples of NOx traffic were obtained by phase space reconstruction, and the least squares support vector machine was introduced to improve the limit learning. The training set of NOx traffic was learned, and the performance of the model was tested by simulation experiments. Finally, taking 330MW coal-fired boiler of a thermal power plant as the research object, the NOx prediction model of ELM-LSSVM was established. The simulation results showed that the soft sensor model has higher prediction accuracy and generalization ability, and it can effectively predict NOx emission.

KEYWORDS:
Ecological environment, ELM, LSSVM, optimization model

INTRODUCTION

Energy and environment are important problems restricting human development. By 2050, China’s energy structure, dominated by coal, will remain unchanged. The proportion of coal in total energy will not be less than 50%, and the proportion of thermal power generation will be very difficult to be less than 50%. In order to achieve the goal of environmental friendliness and energy conservation in China, coal-fired power plants must be paid attention to pollutant emissions. The ecological environment of flue gas discharged from thermal power industry is one of the main pollutants. Because the ecological environment is affected by many factors, it is difficult to artificially predict the emission characteristics of the ecological environment. Therefore, the prediction of the emission characteristics of ecological environment has become an important research issue.

Most studies on eco-environmental emission prediction were used small sample data. But the original data is very large, the data are still sampled to reduce the training sample size in the process of modeling. So it has two shortcomings, on the one hand, it is easy to lose useful information by using small sample data, on the other hand, the generalization performance of the model is degraded when the model is moving away from the modeling time. The application of extreme learning method to the prediction of ecological environment emissions can solve the problems well, it can realize the optimal treatment of ecological environment emissions and form an effective treatment mechanism.

Artificial Neural Networks and Support Vector Machines (SVM) were the most widely used artificial intelligence technologies in the optimization of power plant boilers [1]. Jia used ANN algorithm to model NOX emission, and used genetic algorithm to optimize the performance of ANN algorithm [2]; Li combined RBF neural network with genetic algorithm to build a hybrid model of coal-fired boiler efficiency and NOx emission [3]; Luo established a prediction model of NOx emission and power plant boiler efficiency by using BP neural network with the experimental data of optimizing combustion characteristics [4]. Liu modeled the NOx emission characteristics of boilers used genetic algorithm to optimize the parameters based on SVM [5]. Liu combined SVM with Ant Colony Optimization (ACO) to model Nox emission [6]. Lv carried out
feature extraction and analysis based on the Partial Least Squares PLS and Least Squares Support Vector Machine (SVM) algorithms. Zhou Hao et al. [7]. Zhou used Core Vector Machine (CVM) build NOx emission model on large-scale data. Using combustion optimization technology to model and predict the emission characteristics of NOx can effectively reduce emissions [8].

Artificial Intelligence (AI) technology was a typical representative of neural network and it can support vector machine (SVM) because it had good ability to deal with non-linearity [9]. It also can solve the problem of NOx modeling better. In the process of NOx model prediction, supporting vector machine (SVM) had attracted more and more attention in NOx emission modeling due to the weakness of neural network in fitting and generalization. In view of the characteristics of NOx emission prediction, such as complex non-linearity, multi-dimensional and multi-dimensional models, it was need to solve the structural risk minimization problem due to few data samples. Least Square Support Vector Machine (LSSVM) was an excellent learning method. In the training process of LSSVM, it is necessary to determine the “super-parameters” of the model [10]. The Super-parameters are very important to establish effective LSSVM prediction model. How to obtain “super-parameters” of the model [10]. The training process of LSSVM, it is necessary to determine the “super-parameters” of the model [10].

The structure of SLFN was shown in Figure 1. For N arbitrarily different samples \( \{(x_i, t_i) \mid i = 1,2, \ldots, N\} \), where,

\[
x_i = [x_{i1}, x_{i2}, \ldots, x_{in}]^T \in \mathbb{R}^n,
\]

\[
t_i = [t_{i1}, t_{i2}, \ldots, t_{im}]^T \in \mathbb{R}^m.
\]

The mathematical model of standard single hidden layer feed forward neural network with L hidden nodes and G(x) as excitation function was shown in formula 1.

\[
\sum_{j=1}^{L} \beta_j g_i(x_j) = \sum_{j=1}^{L} \beta_j g(w_j x_j + b_j) = Y_j
\]

where, \( w_j = (w_{j1}, w_{j2}, \ldots, w_{jn})^T \) is the weight of connecting the input sample and the first hidden node, \( b_j \) is the bias of the second hidden node, \( \beta_j = (\beta_{j1}, \beta_{j2}, \ldots, \beta_{jn})^T \) is the weight of connecting the second hidden node and the output sample, \( g(x) \) is the excitation function, it can be “Sigmoid”, “RBF” or “Sin”. \( Y_j = (y_{j1}, y_{j2}, \ldots, y_{jn})^T \) is the jth output sample of SLFNs.

If the actual output of the network was equaled the expected output, the above equation can be abbreviated as follows:

\[
\hat{\beta} = T
\]

where,

\[
H = H(\alpha_1, \alpha_2, \ldots, \alpha_{n_2}, b_1, b_2, \ldots, b_L, x_1, x_2, \ldots, x_{n_1})
\]

\[
= \begin{bmatrix}
  h(x_1) \\
  \vdots \\
  h(x_{n_1})
\end{bmatrix} = \begin{bmatrix}
  g(\alpha_1 \bullet x_1 + b_1) \cdots g(\alpha_{n_2} \bullet x_1 + b_1) \\
  g(\alpha_1 \bullet x_2 + b_2) \cdots g(\alpha_{n_2} \bullet x_2 + b_2) \\
  \vdots \\
  g(\alpha_1 \bullet x_{n_1} + b_L) \cdots g(\alpha_{n_2} \bullet x_{n_1} + b_L)
\end{bmatrix}_{n \times L}
\]

\[
\beta = \begin{bmatrix}
  \beta_1^T \\
  \vdots \\
  \beta_L^T
\end{bmatrix}_{L \times n}
\]

\[
T = \begin{bmatrix}
  t_{11} \\
  \vdots \\
  t_{m1}
\end{bmatrix}_{m \times L}
\]

\[
H = HP = \hat{\beta}
\]

Thus, the least squares optimal solution of linear systems over W was shown in formula (5).

\[
\hat{\beta} = H' T
\]

where, \( H' \) represents the Moore-Penrose generalized inverse of the output matrix H of the hidden layer.

---

**FIGURE 1**

Structural diagram of SLFN

---

**EML LEARNING MODEL**

Extreme Learning Machine (ELM) was first proposed by Huang et al. [13]. This method was developed in single-hidden Layer Feed forward Neural Network (SLFN). ELM’s hidden layer nodes were generated randomly without substitution optimization. The only free parameter to be learned in ELM was the weight between the hidden layer and the output layer. Therefore, ELM was a linear parameter model which can be reduced to solving linear systems.
was defined as follows: LSSVM regression algorithm was developed on the basis of SVM. It used equality constraints instead of inequality constraints in SVM to transform the quadratic programming problem into the solution of linear equations, thus it can avoid the SVM solving quadratic programming problem in dual space. LSSVM was faster than SVM in learning speed and more deterministic in training results because of its small amount of computation. LSSVM overcame the over-learning problem through the principle of structural risk minimization, and it had became an important technology in the field of regression analysis [14, 15]. LSSVM regression algorithm was described as follows:

Training data set \((x_i, y_i), i = 1, 2…l\), \(x_i \in \mathbb{R}^n, y_i \in \mathbb{R}\). The objective function of LSSVM was defined as follows:

\[
\min_{\omega, \xi} J(\omega, \xi) = \frac{1}{2} \omega^T \omega + \frac{\gamma}{2} \sum_{i=1}^l \xi_i^2 \tag{6}
\]

The constraint condition is

\[
y_i = \omega \phi(x_i) + b + \xi_i, i = 1, 2…l . \tag{7}
\]

Among them, \(\gamma\) is the regularization parameter; \(b\) is the constant bias; \(\omega\) is the weight vector; \(\xi_i\) is the fitting error of \(i\)-th sample. By introducing Lagrange multiplier \(\alpha > 0\), the above optimization problems were transformed into unconstrained optimization problems.

\[
L(\omega, b, \xi, \alpha) = J(\omega, \xi) - \sum_{i=1}^l \alpha_i \left(\omega \phi(x_i) + b + \xi_i - y_i\right) \tag{8}
\]

Among them, \(\gamma\) is the regularization parameter; \(b\) is the constant bias; \(\omega\) is the weight vector; \(\xi_i\) is the fitting error of \(i\)-th sample. By introducing Lagrange multiplier \(\alpha > 0\), the above problems were transformed into unconstrained optimization problems.

According to Karush-Kuhn-Tucker (KKT) condition, the equation (7) was optimized, and the linear equations of predictive model coefficients were obtained.

\[
\begin{bmatrix}
0 \\
\epsilon_1^T \\
\Omega + l / \gamma
\end{bmatrix}
\begin{bmatrix}
\alpha \\
\beta \\
\gamma
\end{bmatrix} =
\begin{bmatrix}
0 \\
y \\
\epsilon_1
\end{bmatrix} \tag{9}
\]

Where, \(\epsilon_1\) is the \(N\)-dimensional vector composed of 1; \(y\) is the output vector of training set; Support Vector Value \(\alpha\) is the model parameter of LSSVM; \(\Omega\) is a symmetric matrix of \(N \times N\). As follows:

\[
y = [y_1, \ldots, y_l]^T \quad \epsilon_1 = [1, \ldots, 1]^T \quad \alpha = [\alpha_1, \ldots, \alpha_l]^T
\]

\[
\Omega_y = \phi(x_i) \cdot \phi(x_j) = K(x_i, x_j), i, j = 1, \ldots, l
\]

According to formula (9), the parameters of the model were as follows:

\[
\begin{bmatrix}
\alpha = \Phi^T \gamma^{-1} y - \Phi^{-1} \epsilon_1 \\
b = \epsilon_1^T \gamma^{-1} y \\
\Omega = \Phi + \gamma^{-1} \epsilon_1
\end{bmatrix} \tag{10}
\]

Finally, the mathematical model of LSSVM can be obtained.

\[
y(x) = \omega^T \phi(x) + b = \sum_{i=1}^l \alpha_i K(x, x_i) + b \tag{11}
\]

Where, \(k(x_i, x_j)\) is a kernel function, which maps data samples from input space to high-dimensional feature space and performs linear fitting in high-dimensional feature space. According to the theory of functional theory, any symmetric function satisfying mercer condition can be the most kernel function [16]. Common kernels had the following forms: linear kernels, polynomial kernels and RBF kernels. RBF kernel function only needs to determine one parameter, which can directly reflect the distance between two data. Experiments showed that it had good performance and strong applicability [17]. Therefore, RBF kernel was used as the kernel function of LSSVM in this paper.

### Table 1: ELM Algorithm

<table>
<thead>
<tr>
<th>ELM Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input sample: (N = {(x_i, t_i)</td>
</tr>
</tbody>
</table>

ELM have the following advantages: 1. ELM has few parameters to adjust, and its learning ability is only related to the number of hidden layer nodes; 2. The same structure of ELM can be used for feature learning, regression, classification and clustering (two or more classes); 3. It avoids the problems that traditional gradient-based algorithms need to face, such as inappropriate learning rate, local minimum and over-fitting.
\[ K(x_i,x_j) = \exp(-(x_i - x_j)^2 / 2\delta^2) \] (12)

**ELM-LSSVM Prediction Model.** Because ELM was used empirical risk minimization criterion to model, and it was also used training error minimization criterion to measure the quality of the model, so it was easy to appear “over-fitting” phenomenon, and the generalization ability of the model was poor. A model with excellent performance should be considered both empirical risk and structural risk, and we should make a reasonable balance between them to improve generalization ability. To this end, the structure risk minimization principle of LSSVM was introduced into ELM to generate ELM-LSSVM. In ELM-LSSVM, formula (1) was changed as follows:

\[
\min \frac{1}{2} ||\beta||^2 + \frac{1}{2} \sum_{i=1}^{N} \delta_i^2 \\
\text{s.t. } \delta_i = y_i - h(x_i), \delta_i \geq 0, i = 1, 2, \ldots, N
\] (13)

Where, \( ||\beta||^2 \) and \( \delta_i^2 \) respectively express experience and structural risk.

For the conditional extreme problem of equation (11), the Lagrange multiplier was introduced to be:

\[ L_{ELM} = \frac{1}{2} ||\beta||^2 + \frac{1}{2} \sum_{i=1}^{N} \delta_i^2 - \sum_{i=1}^{N} \alpha_i (\delta_i - \beta h(x_i)) \] (14)

In the formula (14), \( \alpha_i \) is a Lagrange multiplier, and there are:

\[
\begin{align*}
\frac{\partial L}{\partial \beta} &= 0 \Rightarrow \beta = \sum_{i=1}^{N} \alpha_i h(x_i) \\
\frac{\partial L}{\partial \delta_i} &= 0 \Rightarrow \alpha_i = \zeta \cdot \delta_i \\
\frac{\partial L}{\partial \alpha_i} &= 0 \Rightarrow y_i - h(x_i) - \delta_i = 0
\end{align*}
\] (15)

The following linear equations are obtained:

\[
\begin{bmatrix}
0 & I_y^T \\
I_y & \Omega + I / \zeta
\end{bmatrix} \begin{bmatrix}
\alpha \\
y
\end{bmatrix} = \begin{bmatrix}
0 \\
y
\end{bmatrix}
\] (16)

In conclusion, ELM-LSSVM considered both empirical risk and structural risk, which decreased the probability of over-fitting and improved the generalization performance.

The steps of ELM-LSSVM network traffic modeling were as follows:

Step1: Collecting data on ecological emissions.

Step2: When the emission value changes greatly, it will have a negative impact on ELM-LSSVM training. Therefore, the pretreatment of ELM-LSSVM was carried out.

\[ x'(i) = \frac{x(i) - E_x}{\sigma_x} \] (17)

In the formula, \( x(i) \) and \( x'(i) \) are the original and normalized values respectively, while \( E_x \) and \( \sigma_x \) are the mean and standard deviation.

Step 3: Phase-space reconstruction of ecological emission data was carried out to obtain learning samples of ELM-LSSVM.

Step 4: Inputting the training set into ELM-LSSVM learning, and establishing one-step or multi-step prediction model of ecological emission data.

Step 5: The prediction accuracy and error of the forecasting set of ecological emission data were analyzed.

**RESULTS**

When collecting data in the experiment, each group of samples was obtained under the same stable condition. Maximum learning method combined with least squares support vector machine (LSSVM) model parameters was used to predict NOX emission from a 330MW coal drum boiler. The prediction results were compared with ELM-PSO, ELM-BBO and ELM optimization methods. In the multi-condition test of NOX emission, the specific data of various operating parameters affecting the combustion characteristics of boilers, such as power generation load, coal feeder speed, primary air and so on, were showed in Table 2.

The relative errors between the training results of the extreme learning machine and the emission of NOX were showed in Fig. 2 and Fig. 3. From Fig.3, it can be seen that the relative error of training was mostly within (+5%). Fig. 4 and Fig. 5 were the relative errors of the test results and NOx emissions of the extreme learning machine. The trend of NOx emission forecast value was basically consistent with the trend of measured value, and the relative error of measurement was within (+3%). At the same time, compared with ELM-PSO and ELM-BBO based on NOX emission prediction algorithms, the relative error of prediction was 6.3% and 5.9% respectively. The prediction accuracy of NOX emission based on ELM was not much different from that based on ELM-LSSVM in this paper. However, due to its single hidden layer structure, the learning speed of limit learning machine was faster than that of ELM-PSO, ELM-BBO and ELM. The variation of errors was small, which showed that the Extreme Learning Machine had strong generalization ability.

The 25 training data and the other 3 groups of forecast data were predicted by regression, and the predicted values were showed in Fig. 3. In order to verify the superiority of this algorithm in modeling,
the algorithm was compared with PSO-ELM, BBO-LSSVM, WOA-ELM and standard ELM. The absolute value of prediction error of each method was showed in Fig. 6.

It can be further found that the data in samples 17, 18 and 19 did not participate in the training of the model, and the error value was relatively large compared with other training samples, which conformed to the principle of system modeling. Further, it can be found that ELM-BBO and ELM-POS had similar predictive performance, while ELM had poor predictive performance, especially for the three groups of future samples that did not participate in training. The performance of ELM-LSSVM model was better than that of ELM model.

### TABLE 2
Part of the original data of boiler operation

<table>
<thead>
<tr>
<th>Working Condition</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>...</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load /MW</td>
<td>329</td>
<td>320.7</td>
<td>324</td>
<td>...</td>
<td>332</td>
</tr>
<tr>
<td>Speed of four coal feeders /r/m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>334</td>
<td>334</td>
<td>333</td>
<td>...</td>
<td>335</td>
</tr>
<tr>
<td>B</td>
<td>340</td>
<td>341</td>
<td>339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>403</td>
<td>363</td>
<td>464</td>
<td></td>
<td>420</td>
</tr>
<tr>
<td>D</td>
<td>429</td>
<td>389</td>
<td>311</td>
<td></td>
<td>428</td>
</tr>
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<td>Two wind speed /m/s</td>
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<td>34.1</td>
<td>46.7</td>
<td>37.8</td>
<td>...</td>
<td>35.5</td>
</tr>
<tr>
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<td>35</td>
<td>45</td>
<td>38.6</td>
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<td>37.8</td>
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<tr>
<td>BC</td>
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<td>35</td>
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<td>35.6</td>
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<tr>
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<td>33.4</td>
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<td>36.6</td>
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<td>DE</td>
<td>32.9</td>
<td>40.1</td>
<td>37.5</td>
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<tr>
<td>Burn-out air openness /%</td>
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<td>50</td>
<td>...</td>
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<tr>
<td>OFA up</td>
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<td>50</td>
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<tr>
<td>SOFA</td>
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<td>Exhaust gas temperature /%</td>
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<td>57.37</td>
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<td>Har</td>
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<tr>
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<tr>
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<td>1.21</td>
</tr>
<tr>
<td>War</td>
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<td>9.5</td>
<td>8.4</td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>Aar</td>
<td>24</td>
<td>18.6</td>
<td>21.4</td>
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<tr>
<td>Oxygen content /%</td>
<td></td>
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<td></td>
</tr>
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<td>5.45</td>
<td>6.25</td>
<td>5.34</td>
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<td>8.4</td>
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<td>6.7</td>
</tr>
<tr>
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<td>18.6</td>
<td>21.4</td>
<td></td>
<td>23.4</td>
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<td>Coal quality characteristics /%</td>
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<tr>
<td>Qar/10^3kj/kg</td>
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<td></td>
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</tr>
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<td>I</td>
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<td>25.4</td>
<td></td>
<td>27.4</td>
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<tr>
<td>IV</td>
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<td>27.3</td>
<td>26.5</td>
<td></td>
<td>27.6</td>
</tr>
<tr>
<td>Primary wind speed /m/s</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>NOx discharge mg/m^3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGURE 2</td>
<td>Predicted and measured values of NOx emissions after ELM-LSSVM training</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGURE 3</td>
<td>Relative error of NOx emission prediction after ELM-LSSVM training</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOx discharge

585.9  876.15  624.65  ...  627.5
In the process of eco-emission, the characteristics of NOx emission from coal-fired boilers are affected by many factors, and the relationship between them is complex. It is very difficult to predict and control the NOx emission. In this paper, the least squares support vector machine (LSSVM) method and the maximum learning method was used to establish the prediction model of NOx emission. In order to improve the prediction accuracy and generalization ability of LSSVM, extreme learning machine “over-fitting” and so on. Taking 330MW pulverized coal fired boiler of a power plant as the test object, the ELM algorithm was used to optimize the Super-parameters of LS-SVM and establish the prediction model of NOx emission. The model was trained and tested by using the relevant data which were collected by DCS as training samples and test samples. The simulation results showed that the prediction model of NOx emission had better accuracy and stronger generalization ability based on EML-LSSVM.

CONCLUSION

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ACKNOWLEDGEMENTS

REFERENCES


RESEARCH ON THE DEVELOPMENT OF AGRICULTURAL E-COMMERCE UNDER THE ENVIRONMENT OF "INTERNET +"

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ABSTRACT

Since the implementation of the “Internet +” strategy for the national development, many new agricultural businesses and e-commerce companies have effectively explored the “Internet +” modern agriculture field, resulting in the rapid development of agricultural e-commerce industry. However, compared with other fields, development level of agricultural e-commerce is still lagging due to its unique characteristics and the operational system, and, thus, the operation and promotion effects are not satisfactory. Based on the characteristics of agriculture, it is imperative to solve the individualized problems in the development of e-commerce with Internet technology, innovative thinking and channels. In the given context, this paper uses the case study method to conduct an empirical analysis of the development of agricultural e-commerce through surveying relevant agricultural data. While comparing the industrial value chain model, a systematic analysis of the overall operation of the agricultural e-commerce industry value chain and the status of each subject have been proposed. The main problems in this paper are: for example, the agricultural industry value chain is long and scattered, the leading role of the dominant enterprises is not sufficient, and the logistics distribution and marketing strategies cannot meet the needs of agricultural e-commerce development. The study stresses on agricultural enterprises to use "Internet +" as a means to promote intelligent and data-based production management, integration of industry chain resources with industry e-commerce platforms, and building of brands through network integration marketing.

KEYWORDS:
"Internet +", Agricultural, E-commerce, Industry Value Chain

INTRODUCTION

Since the implementation of the “Internet +” development strategy, e-commerce has become a kind of business transaction with no geographical restrictions, transmitting information to consumers and merchants, and completing transactions safely. It has allowed farmers to avoid risks and reduce sales costs in transactions, then increase the income. Many new agricultural business entities and e-commerce companies have effectively explored the “Internet +” modern agriculture field, and agricultural e-commerce has developed rapidly. Agricultural e-commerce across China has ushered in the best development opportunities. The development opportunities for agricultural e-commerce are enormous. The strength of all parties joining the agricultural e-commerce is increasing day by day, bringing about a huge turning point in the development of the agricultural economy, the rapid circulation of agricultural products and increase in the farmers' income. The scale of agricultural e-commerce development continues to increase, and the model continues to deepen. However, compared with other fields, due to its unique industry characteristics and mode of operations, the level of agricultural e-commerce development is relatively lagging behind, as compared to other industries, and the synergy of operation and promotion is not satisfactory. Therefore, based on the characteristics of agriculture, it is imperative to solve the individualized problems in the development of e-commerce with Internet technology, thinking, and channels.

At the same time, the research on small and medium-sized agricultural enterprises' e-commerce can not only enhance the theoretical grasp of e-commerce in the agricultural field, but also promote the wide application of agricultural e-commerce, and promote the efficient and low-cost operation of the agricultural markets, increasing agriculture efficiency and increasing farmers' income. This is also of great significance for the implementation of the national "Internet +" modern agricultural policy, the promotion of innovative agricultural modernization, the solution of China's "three rural issues," and the short-term development of the balanced development of the national economy and urban areas.

Based on the above background, this paper intends to start from the rapid development of China's "Internet +" and e-commerce and the practical needs of the transformation and development of traditional agricultural enterprises, by summarizing
the e-commerce business model and marketing strategy adopted by a regional agricultural enterprise in Inner Mongolia in the e-commerce attempt. The agricultural e-commerce contains some problems, such as industrial value chain is long and scattered, logistics distribution and marketing strategies cannot be combined with the actual development of agricultural e-commerce and other practical issues, and the optimization development strategy of agricultural e-commerce based on "Internet +" environment is proposed. That is, using the "Internet +" as a means to use the industry e-commerce platform to integrate industrial chain resources, promote production management intelligence, data analysis, and build brands through network integration marketing. The paper also put forwards feasible suggestions and measures to combine the most traditional agriculture with the latest Internet, and seek the development of agricultural e-commerce with "Internet + "; and put forward feasible suggestions and measures to combine the most traditional agriculture with the latest Internet, and seek the development of agricultural e-commerce with "Internet + ". So that farmers can obtain relevant policy information and market conditions in the first time, which helps to solve employment, increase income, and promote rural economic and social development.

"Internet +". In the past few years, Germany has proposed the Industry 4.0 strategy, the United States has developed a plan to develop the Industrial Internet, and the Chinese government has also proposed to develop an "Internet +" action plan. As the Chinese economy enters a new phase, a rare opportunity for China. At one time, Chinese economy is entering a new phase of development and at the same time, China faces challenges in core technology and industrial security [1]. The "Internet +" program means the use of information connectivity and "information energy" to promote a deeper and integrated development of the virtual economy and the real economy and to enable human society to enter a new social and economic development model, namely the Internet economy [2].

By understanding the Internet +, Liu J and colleagues [3] discussed the intrinsic meaning of "Internet +" and compared the differences between "Internet +", Internet, Industry 4.0, Industrial Internet, and explained the connotation of "Internet +". Zeng J [4] proposed the "Internet +" action plan as a strategy to accelerate network construction and Internet application, promote ICT traditional industry reform, improve competition efficiency, and regulate market competition, realizing the convergence of Internet + strategy and network. The Grey stone corporation of Nanjing city has also predicted the prospect of "Internet +" in future especially in the second half of 21st century. Wang G [5] analyzed the nature and characteristics of “Internet +” and listed the three characteristics of Chinese Internet development. From the fields of communication, finance, transportation, medical care and education, it analyzed the integration and development of the Internet with other traditional industries. Wang Z [6] and others looked at the characteristics of "Internet +" from six different perspectives and described possible applications in manufacturing, finance, commerce, transportation, healthcare, and education.

With the rapid development of Internet technology in China, "Internet +" has been combined with all key areas of life. On the one hand, "Internet +" has greatly increased industry and employment innovation. On the other hand, "Internet +" has made the market more competitive. Some traditional industries have been in recession or even disappeared in the fierce market competition. A large number of new companies are also struggling in the fierce competition [7].

Leisure agriculture is an important carrier and development direction of traditional agricultural transformation and upgrading, and "leisure agriculture + Internet" is the direction of transformation and upgrading of leisure agriculture. At present, under the catalysis of the Internet, the business model of leisure agriculture is undergoing profound changes. However, "leisure agriculture + Internet" still faces some explicit problems. The transformation and upgrading of "Internet +" leisure agriculture is a complicated system engineering. It is necessary to construct a public policy support system, a human resources support system, a planning and service support system by the logic of industrial evolution [8].

Agricultural e-commerce. Agricultural e-commerce is an important part of the Internet and modern agriculture [9]. In the current rapid development of e-commerce, Zhao Z [10] explored the impact of e-commerce on agricultural product marketing, while analyzing its advantages and constraints, and likely problems, and proposed a new marketing strategy for agricultural products.

D Souza D J [11] proposed an e-commerce framework as a platform for rural agricultural development and improvement in India; established rural knowledge centers in villages and established agricultural India knowledge portals.

Wang S Q [12] introduced the development status of e-commerce in Hebei Province, China. It is proposed to improve the construction of network infrastructure, strengthen the construction of agricultural standardization system, raise awareness of e-commerce, and strengthen the training of e-commerce talents [13]. Based on a detailed study of the agricultural trade process in Hebei, a multi-intelligence system was used to construct an agricultural e-commerce transaction model. The model consists of a buyer agent, seller agent, third-party
agent, negotiation agent, a transaction agent, and a database agent. Users can automatically exchange information with sub-agents created by the system for the corresponding roles. Realize the automation and intelligence of agricultural e-commerce transactions.

E-commerce used to improve the efficiency of agricultural products circulation, which can become a new growth point for agricultural and rural development, and also an innovative driving force for farmers' sustainable and rapid growth in income. Yu and Zhang [14] used the analytic hierarchy process to analyze the factors that restrict the development of agricultural products e-commerce, and proposed countermeasures and suggestions.

Zhu Q [15] studied the application of computer algorithms in agricultural products e-commerce, introduced the e-commerce and accurate mining algorithms of agricultural products, constructed the characteristic information of consumer preference model, and constructed the agricultural product feature model and recommendation algorithm. In-depth research on accurate recommendation algorithms was conducted in four aspects. Based on the characteristics of agricultural products, optimizing the traditional product recommendation process can help to improve the accuracy of recommendations.

E-commerce of fresh products is a new sales model for freshly produced goods, which can solve the problem of "hard to buy, hard to sell" for fresh products. Given the characteristics of e-commerce of fresh produce, Jing-Min Lu et al. [16] listed the necessary conditions for logistics cooperation and the principles that must be followed, and proposed supply chain collaboration and business cooperation to promote the healthy and sustainable development of e-commerce.

To solve the problem that the existing agricultural e-commerce platform has poor liquidity, the platform cannot meet the regional and effective demand of agricultural products and make the mobile Internet technology better applied to the agricultural field, Wang Q et al. [17] designed and realized mobile agricultural electronics business system. The system is a vertical e-commerce platform focused on quality agricultural products. Adopting the new group purchase operation mode, combined with crowd-funding, fully considers the unique characteristics of agricultural products.

"Internet +" modern agriculture. "Internet +" modern agriculture means that modern agriculture realizes information exchange, resource sharing, and online trade through the use of Internet information platform. With the help of computer equipment and mobile terminals, we can realize the rapid and efficient operation of production, processing, and sales of agricultural products, and a series of transactions around the core of agricultural production and supply chain management.

With the implementation of the "Internet +" action plan, e-commerce has received more attention during the economic transition. The new strategic emerging industries have attracted the attention of governments at all levels. The rapid transformation of global Internet technology, the rapid development of "Internet + agriculture" is a direct manifestation of rural e-commerce, and has become an important driving force for promoting rural economic development and improving farmers' living standards. Xu G [18] summarized the "Internet + Agriculture" as the basic process of agricultural product circulation system reform, focusing on the characteristics and advantages of fresh agricultural products in Guizhou Province, combined with the bottleneck of the shortboard and new cold chain logistics development. A proposal was made to establish an integrated e-commerce platform based on the cold chain standardization of Guizhou agricultural products. Zhang YQ [19] analyzed the development atmosphere of agriculture on the Internet, and studied two e-commerce models. Based on the comparative analysis of two typical e-commerce models and operational mechanisms, this paper put forwards the five suggestions about optimization of e-commerce construction and development of agricultural products. Liang H E [20] took Nanjing as an example to illustrate the achievements of rural e-commerce development under the background of "Internet +". The problems existing in the development of rural e-commerce are analyzed, and corresponding countermeasures are proposed. Under the "Internet +" wave, agricultural products e-commerce plays an important role in reducing circulation costs, increasing farmers' income, and promoting large-scale agricultural production. Fujian agricultural products e-commerce has begun to take shape and has been rapidly developed through continuous innovation and practice. Chen R [21] proposed a business to promote moderate scale operation of agriculture, accelerate the building of rural infrastructure, promote the clustering of agricultural enterprises, deepen the reform of the rural financial system, and cultivate agricultural e-commerce talents. Weixian Li [22] expounded the problems in the "Internet + agriculture" e-commerce operation and development, explored the new e-commerce operation mode of the Internet and characteristic agriculture and analyzed the development trend of modern agricultural e-commerce. Cao HX [23] elaborated the integration process and status quo of "Internet +" and modern agriculture from the concept of "Internet +" and typical Case of "Internet + Agriculture", providing more reference and interaction for the development of "Internet + Modern Agriculture". Liang R. [24] proposed the following measures for the development of the new industrial pattern of modern agriculture based on "Internet +". Adapting top-level design to guide coordinated development;
strengthening the “Broadband China Strategy” and accelerating infrastructure construction; implementing new farmers’ cultivation actions to improve the quality of rural compound technical talents; establishing an online banking platform, broadening financing channels; and innovating modern agricultural sales models and regulatory measures.

Research hypothesis. Internet + "modern agriculture is not a simple combination of "Internet" and "modern agriculture". Instead, it integrates Internet technology into all aspects of agricultural development, expands the extension of agricultural production through Internet technology and online sales channels, and various economic management models under the agricultural production. It includes online sales and promotion of agricultural products, logistics configuration, and emergency operations. With the innovation and development of Internet technology, the agricultural industry relies more on e-commerce to promote its all-round development of research, production, and sales. Agricultural e-commerce promotes the transformation and upgrading of traditional agriculture to modern agriculture and promotes the integration of the Internet and modern agriculture.

Li L [25] proposed the management of the green supply chain of agricultural products and the development of a new industrial infrastructure based on “Internet +”. Sheng-Jun Li [26] combined the technical characteristics and the characteristics of the modern agricultural industry, and proposes that “Internet + modern agriculture” should adopt the modern agricultural, industrial chain structure to integrate Internet technology and adopt the multi-layer structure of “application technology + network service + cloud service”. The cloudy structure of “public cloud + private cloud” and the main auxiliary structure based on modern agricultural application network technology. Therefore, with the aid of the entire industrial chain, “Internet + Modern Agriculture” has achieved sustainable and win-win system integration. Liu J et al. [27] reviewed the current situation of “Internet +” modern agricultural construction in Jiangxi province and reviewed the regularity of offline and summary itself. The logical system of the development of "Internet + modern agriculture” was put forward.

Internet-plus modern agriculture is the process of applying various process, structure and agricultural sectors to the advanced information technology of the Internet through human-machine relationships, promoting the application of modern technology, modern industrial equipment and modern agricultural management concepts and methods to promote scientific, commercial, intensive, and industrialized agriculture, achieving high-yield, high-quality, high-efficiency, and ecological goals.

Therefore, this study uses Internet thinking to propose an "Internet +" e-commerce development optimization strategy that promotes plant management upgrades, integrates planting industry chain resources, and develops e-commerce development strategies. Combined with the theory of network marketing, it proposes to establish an e-commerce management service department, establish a quality and safety traceability system, build a unified regional brand, optimize product structure, enrich e-commerce transaction mode, expand e-commerce transaction and delivery channels, and carry out network integration marketing promotion. The specific scenarios are as follows:

(1) Promote agricultural product management upgrade with "Internet +". Utilize the Internet and Internet of Things technologies to improve the level of planting management intelligence and the integration level of planting services output to build a big data platform. Use the "Internet +" means to explore the cloud planting model, use big data, cloud computing and Internet of Things technologies to achieve close contact between self-supporting planting bases, planting farms, and large planting households, and implement full-process cooperation to promote automation, intelligence, and standardized planting, so that the line becomes the front desk of the growers.

(2) Integrate agricultural product industry chain resources with "Internet +". Under the trend of Internetization of financial and insurance services, as the core component of the industrial value chain it provides financial institutions with big data on aquaculture industry, strives for credit guarantee funds, and provides financial services such as secured loans to farmers and peasants through the open service platform of the Internet, and promotes the cooperation between agricultural enterprises and farmers. Encourage deep cooperation between farmers and enterprises, not only can stabilize the supply of goods, but also expand the market share of enterprise products, establish barriers to competition, and realize the benefits of the industrial value chain.

(3) Based on "Internet +" to develop e-commerce development optimization measures for agricultural enterprises. With the "Internet +" thinking, the enterprise organization structure, production and sales process will be upgraded, the e-commerce model will be innovated, and specific optimization strategies will be proposed to create a safe and reliable modern and integrated e-commerce platform, and independently develop high-value-added core products. To realize the electronic sales of products in the whole industry chain, coordinate the information flow, capital flow and logistics of the industry value chain, and finally form a complete, effective and efficient entire industry’s value chain e-commerce system, and ex-
plore and enhance the comprehensive competitive advantage of the enterprise.

MATERIALS AND METHODS

Research object. Agricultural enterprises are the main business entities of agricultural e-commerce and are the intermediate links connecting farmers, rural areas, agriculture and industry, cities and markets. Under the "Internet +” environment, the main products of agricultural e-commerce online transactions are fresh agricultural products, feed additives, premixes, various services, and meat products. The specific e-commerce model can be divided into agricultural products e-commerce, feed e-commerce, meat and milk e-commerce, agricultural products storage and transportation e-commerce, agricultural products processing e-commerce.

This paper selects a comprehensive agricultural product enterprise located in Hohhot, Inner Mongolia Yinshan Mountain Range. The enterprise is an agricultural product enterprise with modern organic agricultural products as the main body and agricultural and animal husbandry by-products transformed into deep processing, planting, and breeding. It mainly sells green ecological and organic agricultural products. Its products include yellow millet, flaxseed powder, high-quality oatmeal, oatmeal, special oat powder, cistanche, cynomorium, miscellaneous grains, cold fresh beef and sheep, dried meat, dairy products and other agricultural and sideline products.

Research measures and methods. (1) Establish a quality and safety traceability system to create a unified regional brand. Integrate quality traceability management system, networked control system, establish Quick Response (QR) code quality traceability management system, achieve the goal of anti-counterfeiting and anti-smashing goods in the enterprise perspective, realize scan traceability, product information transparency and enhance in connecting retail terminal consumers Consumer trust. By developing good internal agricultural production, we will integrate regional product resources through cloud planting to form a unified regional product – “organic agricultural products”. Further, Help will be provided to the farmers in rural areas with view to solve market problems, environmental protection, food safety, capital and other issues, promote the transformation and upgrading of traditional agricultural industries to modernization, and promote the cultivation of productive areas in poverty-stricken areas; bear responsibility for food safety, control of the entire industry chain, standardization operations, product safety, fresh Healthy, traceable.

(2) Integrate and optimize product structure to achieve bidirectional extension of product lines. The strategy of agricultural e-commerce products is: cultivating good varieties independently upstream, optimizing product structure in the middle reaches, and integrating organic agricultural products and agricultural and side products into the scope of e-commerce business in the downstream. Based on local crops and feed ingredients, we will form a production system for independent agricultural products, occupy the commanding heights of the products, support pollution-free social planting, feed production, nutrition and food safety control, and extend the product line. By the requirements of “high efficiency, ecology, brand” agricultural standardization development, carry out pollution-free standardization demonstration production, actively apply to the authoritative certification body for pollution-free agricultural products, green meat and poultry, organic agricultural products certification, and strive to use pollution-free, green and organic food signs. Improve brand authority, enhance customer perception, enhance customer trust, and smooth product circulation.

(3) Develop B2C mode to reduce intermediate links. In combination with the new product structure, it is recommended to continue to develop retail sales of production materials and services for small-scale growers and continue to penetrate the market. Develop e-commerce between the seller's enterprise and the buyer's individual, establish direct contact between the enterprise and the target consumer, on the one hand, through direct online communication, improve service quality, close the relationship between the enterprise and the customer, and cut the needs and expenses of the offline personnel. On the other hand, lessen the intermediate trading links and reduce transaction costs.

(4) Promote Customer-to-Factory model and reduce business risk. According to the different procurement terminals, a dual order model is implemented to build an order farming industrial chain jointly. On the one hand, the online pre-sale service is opened. Customers send orders through an e-commerce platform, customize personalized products, companies organize production according to orders or use fresh produce recently produced for delivery, which can ensure the preservation and quality of products, achieve zero inventory, reduce intermediate links and increase revenue. On the other hand, according to the needs of the market, production orders are issued to professional cooperatives, growers, so on, and packaging and distribution are uniformly carried out after the order is completed. Through this model, the blindness of production is reduced, the business risk is reduced, and the difficulties of buying and selling difficulties of the retail investors are solved. At the same time,
the relationship between the production links of the industrial value chain is closely coordinated, and
the coordinated development of the agricultural industry value chain is realized.

(5) Try Online To Offline mode, online sales, physical experience and delivery. Relying on
direct sales stores, we provide customers with intuitive product display, eliminate customers’ big pur-
chase doubts, guide customers to online consumption, and establish small and precise direct sales
stores in large cities in target sales areas. After receiving the order, the mobile APP client or the
WeChat public account will rely on the storage resources for a short-time express delivery service
according to the location of the consumer. Alterna-
tively, relying on the company's direct sales stores
and large-scale business super, after the customer
pays online, the product is self-raised. Direct-
operated stores can also purchase products from the
loyal consumers of the B2C channel to the e-
commerce platform after a period of publicity and
sales, and develop the offline product-oriented
stores into product experience centers.

(6) Expand e-commerce trading channels
and develop mobile APP business. Provide the
official website of the enterprise website and coop-
erate with the third-party e-commerce platform.
Tmall fresh, Jingdong home, kitchen, Yi Guo fresh
and other third-party e-commerce platform has rich
experience in marketing, logistics, services, infor-
mation technology and internet finance. Therefore,
while developing internal e-commerce, we should
also explore cross-field cooperation with third-party
e-commerce companies in the field of agricultural
products retailing, open enterprise stores in third-
party fresh-selling platforms, join online supermar-
kets, and mainly produce fresh agricultural products.
Utilize their customers, outlets, logistics and other
resources to expand the retail channel, and deliver
product information and services to target consum-
ers faster and more intuitively, and improve service
quality and user experience while controlling costs.

RESULTS AND DISCUSSION

The effect of integrating and optimizing product structure on agricultural enterprises.
After optimizing the industrial structure, the company bases on local crops and feed ingredients to
form a variety of independent agricultural products, separates the distribution system of production
systems from the operation of self-produced products, concentrates on developing its own production
and sales capabilities, optimizes product structure, and deepens the depth of product mix, as Figure 1
shows. On this basis, it supports pollution-free planting, feed production, nutrition and food safety
control, extending the product line of the company and occupying the commanding heights of the
product, and improving brand authority and en-

Enrich the e-commerce transaction model
and reduce the cost of capital management.
Combine the optimization of agricultural product
structure, use big data, cloud computing and Inter-
et of Things technology to achieve a close contact
between self-supporting planting bases and large
planting households, implement full-process coop-
eration, promote automation, intelligentization,
standardization and standardized breeding. Carry
out remote services, product promotion, and quality
supervision, so that the line becomes the front desk
forces as the leading factor, through the cooperation of pesticide products, veterinary drug products, equipment distributors and other products. The company will jointly build an e-commerce platform to break the closeness of the self-built platform and reduce homogenize low-end competition and reduce production costs. Through the e-commerce platform, the farmers, farmers, and farms are supported in all aspects of capital technology, production materials, and information services. Guide the growers and plantation fields to adjust the production plan in time, form a scale effect, and direct sales to the enterprise through the e-commerce platform.

As the company's leading products, organic oatmeal, and red-skinned potatoes sold nearly 6,000 tons (As shown in Table 1) in the whole network in 2016, which is nearly 3,000 tons higher than the original sales. The transaction price is also 5 yuan/kg higher than the original price. It can be seen that the agricultural e-commerce market has broad prospects.

The enterprise builds an agricultural industry e-commerce platform with its product supply chain as its core and serializes the entire industrial chain of the agricultural production industry. The downstream entities of the supply chain can realize information, communication, and transaction through

![FIGURE 2
Optimized agricultural e-commerce industry value chain model](image)

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Comparison of sales of corporate e-commerce platform and traditional model in 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agricultural product name</strong></td>
<td><strong>Sales (t)</strong></td>
</tr>
<tr>
<td>Organic Oatmeal Rice</td>
<td>1457</td>
</tr>
<tr>
<td>Red potato</td>
<td>3569</td>
</tr>
<tr>
<td>Small pumpkin</td>
<td>1350</td>
</tr>
<tr>
<td>Yellow millet</td>
<td>3050</td>
</tr>
<tr>
<td>Oatmeal flour</td>
<td>2460</td>
</tr>
<tr>
<td>Flax seed powder</td>
<td>560</td>
</tr>
<tr>
<td>Cold fresh beef jerky</td>
<td>4325</td>
</tr>
</tbody>
</table>

The above results show that the agricultural e-commerce sales model based on "Internet +" has higher sales volume and a higher sales price than the original traditional sales model.
the platform. The plantation, cooperatives and the company exchange products and services through the online mall, an e-commerce platform.

Through the implementation of e-commerce-based cooperation between enterprises and growers, this part of agricultural distribution products has broken the geographical limitations of traditional distribution, reduced the price increase of lower-level distributors, and lowered the sales price. Moreover, it is possible to carry out informatization and data management of the distribution products, saving energy, reducing the input of workforce, material resources, and financial resources, and saving costs. Drive the company's products to reduce trading links, drive the integrated marketing of industry chain products, achieve bulk product transactions, reduce corporate costs, and expand corporate income sources.

**CONCLUSION**

At present, China's agricultural industry is transforming into specialized, large-scale, improved seed, facility, branding, service socialization and deep processing. Agricultural e-commerce has broad prospects and rapid development. Therefore, to survive and grow in the fierce e-commerce market competition, agricultural enterprises must scientifically formulate development strategies, integrate resources, adjust products, strengthen management, and adopt modern marketing concepts as a guide to adopting flexible and diversified business models to win the competitive market advantage.

The above research results show that the agricultural e-commerce model based on "Internet +" can significantly increase the sales volume and sales price of agricultural products of agricultural enterprises. At the same time, the agricultural products and services can be supplied to target consumers more rapidly and intuitively, improving service quality and buyer experience while controlling costs. In other words, this model can promote the transformation and upgrading of traditional agriculture to modernization, and drive farmers to become rich in poverty-stricken areas; solve the problem of farmers' difficulties in buying, selling and lending, and realize the coordinated development of the agricultural industry value chain. It can also improve product circulation efficiency and enhance the shopping experience while reducing intermediate trading links and reducing transaction costs. Improve service quality and user experience based on cost control.

This paper puts forward the strategy of "Internet +" to promote the upgrading of agricultural product cultivation management, integrate agricultural planting industry chain resources and formulate e-commerce development strategy from the perspective of "Internet +" e-commerce optimization development strategy. From the perspective of specific countermeasures for the development of e-commerce, this paper proposes targeted solutions, including, but not limited to; transforming e-commerce thinking, establishing an e-commerce management service department; building a quality traceability system to create a unified regional product brand. Integrating and optimizing product structure, extending the product line; Enriching e-commerce transaction mode, reducing capital management costs; expanding e-commerce transaction channels, developing mobile applications (APP) business; carrying out network integrated marketing promotion and expanding market opportunities. It provides some enlightenment and reference for the development of agricultural e-commerce and other agricultural enterprises to conduct e-commerce. In response to the above research results, this study proposes the following recommendations:

1. Use the Internet to rationally arrange the internal organization of the agricultural enterprise and arrange the production and operation activities of the enterprise.

2. Establish an e-commerce center, equip with high-quality e-commerce professionals as full-time operators, responsible for technical maintenance of the e-commerce platform, order processing, online customer service and communication and coordination with production, sales, distribution and other departments. Provide personalized service to customers, provide online service technical support for buyers, and ensure better website conversion rate.

3. In to the vast rural areas for large professional households, family farm operators, to carry out a variety of forms, targeted, high-level participation in e-commerce business training. Introduce the development history and leading position of agricultural e-commerce based on the "Internet +" environment, explain the operation mechanism and security control methods of the online enterprise mall, improve the actual online transaction operation capability, and promote online transactions while promoting and promoting enterprises.

4. Quantitative analysis method is used to further analyze the impact of consumer experience on the development of enterprise e-commerce from the perspective of the purchase intention of enterprise target customers. Through the analysis of successful agricultural e-commerce models, summed up the experience of learning; Exploring the policy environment required for the development of enterprise e-commerce, and proposing the management service functions that the government should perform in promoting the development of e-commerce.
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DETERMINATION OF NUTRITIONAL STATUS OF GRASS IN SOME PARKS WITH SOIL AND PLANT ANALYSIS: THE CASE OF ESENLER COUNTY, ISTANBUL PROVINCE

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ABSTRACT

In this research, soil and plant samples were taken from 20 parks which have a grass area and located in Esenler county of Istanbul, were analysed of macro elements (N, P, K, Ca and Mg) and the nutritional status of the grass was evaluated by statistical analysis. The average N content of the soil samples is 0.102%, according to this value they were classified as “sufficient levels of nitrogen”. On the other hand, the average P, K, Ca and Mg contents of soils were 11.45, 67.45, 7 978.91 and 726.18 mg kg⁻¹. According to the results of the statistical analysis, the relationship between all parameters analyzed between the soil used in the research is “significant” (P <0.01). Total N contents of plant samples were determined as the lowest level 20.29% and the highest level 31.69%. Then P, K, Ca and Mg contents of the plant samples from macro elements an average value were respectively 0.46%, 3.06%; 0.88% and 0.36%. The relationship between all parameters analysed of grasses used in the research is statistically “significant” (P <0.01). According to the analysis of soil and plant in the grass areas in the parks, the applications such as fertilization, irrigation, spraying is extremely important in terms of healthy growth and longevity of grass. As a common opinion, the application of N, P and K is usually carried out in order to fertilization of the parks in Istanbul. Therefore, essential macro elements were taken into consideration in this study.

KEYWORDS:
Grass, soil analysis, plant analysis, Esenler county, macro element

INTRODUCTION

Especially in big cities such as İstanbul, in addition to the current works the physical conditions of the environment can affect our health positively or negatively at the same time. In addition to the feelings of happiness, which one we are getting with the breath within a green area, ornamental plants, trees, artificial ponds, the lawns we walk with bare feet play a very positive role in reducing the stress and distress of life also. Grass fields also produce oxygen like forests, protect the soil against erosion, improve the air quality which one we breathe and affect the psychological disorders in urban life positively. While Turkey's population was 73 722 988 in the year 2010, it increased by about 8.2 million at 82 003 882 in 2018. Similarly, the population of the province of Istanbul was 13 255 685 in 2010, while in 2018 there was an increase of approximately 2 million as result 15 067 724 people [1]. As the food need of growing population, as well as the need for green space should be taken into account by city planners and agricultural experts.

The province of Istanbul has the advantage in terms of number and size of parks and gardens. There are a total of 47 large-scale parks in Esenler, Istanbul. These parks are reported to have a total of 270 areas. 168 decares of this parking area in the district of Esenler covered with grass. Other places usually consist of hard ground, fitness, pool, tree, shrubbery and ornamental plants [2]. Due to the fact that our country has different climatic zones, it is very important to determine the types and types of grass that can adapt to each region. A choose of the right kind of grass, the way how to plant and use the grass, what kind of soil they need to grow and what needs to be met must be well-known [3]. A researcher, grass plants contains an average value of about 75% water, the remaining 25% of the portion is called dry matter and 16 macro and micro plant nutrients were emphasized [4]. The missing of these elements should be provided to the grass plants by fertilization. The nutrients removed from the soil by periodically lawn grass plants are reported as 45 kg N / da, 12 kg P / da and 30 kg K/da in a year [5]. Some researchers argued that increasing the amount of nitrogen applied to the soil, increased intake P, K, Ca, Mg and S [6]. According to Nyborg et al. [7], the application of 11.2 kg N alone to the meadow plant, with the application of 1.1 kg S, a 5-fold increase in the amount of dry meadow grass product was stated. In a study, it
was reported that the optimal N administration time
in the grass was in the active growth period [8]. It is
stated that heavy nitrogen fertilizer application in
cool climate grass makes the plants more sensitive
to cold, drought, diseases and pests. In general,
because of grass areas in parks are continuously
mowed, fertilized and watered, so they need more
plant nutrients than agricultural plants. In grass
areas, firstly N and then P fertilization are extreme-
ly important. Average 45 kg of N, 12.5 kg P and 30
kg K per year per decares can be get from soil with
continuous mowing [9]; the need of 1 m² of carpet
grass has been reported as 15-35 g of pure nitrogen
in the year [10, 11].

For this reason, plant analyses are also useful
for the identification of important problems for
grass plants and for the creation of fertilization
programs. In this study, it is aimed to determine to
present the current macro element nutritional status
of the lawn with soil and plants analysis results and
to determine the results obtained statistically, from
the grasses from parks which are located in the
district of Esenler in Istanbul.

MATERIALS AND METHODS

This research was carried out by taking soil
and plant (plant) samples from 20 of the total num-
er of grass areas. Surface soil samples (0-20 cm
depth) and the plant samples were collected from
the parks, Esenler, Istanbul, Turkey, during 2018
[12]. The spatial distributions of soil and plant
samples are shown in Figure 1, an example of sam-
pling for soil and plant is shown in Figure 2. The
random method based on the variability of the parks
was used for the collection of soil and plant
samples.

![FIGURE 1]
Spatial distributions of sample locations.

![FIGURE 2]
Sampling of plant and soil.
TABLE 1
Analysis methods of soils and plants.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameters</th>
<th>Units</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Total N</td>
<td>%</td>
<td>Macro kjeldahl [12, 13]</td>
</tr>
<tr>
<td></td>
<td>Available P</td>
<td>mg kg⁻¹</td>
<td>Olsen (Sodium bicarbonate) [14, 15]</td>
</tr>
<tr>
<td></td>
<td>Extractable K</td>
<td>mg kg⁻¹</td>
<td>Ammonium acetate [16]</td>
</tr>
<tr>
<td></td>
<td>Extractable Ca</td>
<td>mg kg⁻¹</td>
<td>EDTA titration [16]</td>
</tr>
<tr>
<td></td>
<td>Extractable Mg</td>
<td>mg kg⁻¹</td>
<td>EDTA titration [16]</td>
</tr>
<tr>
<td>Plant</td>
<td>Total N</td>
<td>%</td>
<td>Micro kjeldahl [12, 13]</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>mg kg⁻¹</td>
<td>Vanadomolybdophosphoric yellow color [17]</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>mg kg⁻¹</td>
<td>Flame photometer [12]</td>
</tr>
<tr>
<td></td>
<td>Ca, Mg</td>
<td>mg kg⁻¹</td>
<td>ICP-OES [12]</td>
</tr>
</tbody>
</table>

TABLE 2
Average total N amount of soils and plants according to LSD test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil Sample No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N in soil</td>
<td></td>
<td>0.16 b</td>
<td>0.13 c</td>
<td>0.06 cf</td>
<td>0.23 a</td>
<td>0.16 b</td>
<td>0.08 de</td>
<td>0.07 cf</td>
<td>0.06 fg</td>
<td>0.06 fg</td>
<td>0.23 a</td>
</tr>
<tr>
<td>Total N in plant</td>
<td></td>
<td>23.99 o</td>
<td>28.48 c</td>
<td>27.47 h</td>
<td>31.69 a</td>
<td>27.71 g</td>
<td>27.16 i</td>
<td>28.06 f</td>
<td>26.22 l</td>
<td>21.8 r</td>
<td>30.7 b</td>
</tr>
</tbody>
</table>

There is no 0.01 difference between the averages of the same letter. Soil and Plant LSD (1%) = 2.213978.

FIGURE 3
Relationship between total N content of soils.

The analysis of soil and plant samples used in this study was carried out in 3 replications in the laboratory and this was taken into consideration in statistical analysis. The different soil and plant parameters tested as well as methods adopted to analyse is shown on the Table 1.

The values obtained from the study will be subjected to variance analysis according to the MSTAT package program and the significance of the differences between the environments will be tested by LSD [18].
RESULTS

Total Nitrogen (N) in Soil and Plant. The statistical evaluation of the total N content of soil samples used in the study is shown in Table 2 and Figure 3.

As a result of the average obtained from the soil samples taken from different parks in Table 2, the factor taken in terms of N was found to be statistically significant. The total N content of the soil samples was measured as 0.102%, which is classified as sufficient [19, 20, 21]. Besides the fact that the total N content of soils in the agricultural areas is very low, the fact that the parks contain such as sufficient N levels can be caused by intensive fertilization in parks and also by the use of organic fertilizers (usually compost, peat, etc.). When the agricultural lands of Turkey are examined in terms of total N, it is generally 85% insufficient [22].

The statistical evaluation of the N contents of the plant samples used in the study is shown in Figure 4. The element N was found to be in high level in all of the grass leaves used in the research.

Phosphorus (P) in Soil and Plant. The statistical evaluation of available P contents of soil samples used in the study is shown in Table 3 and Figure 5.

When the soil samples were examined in terms of P contents, it was found that the lowest 0.05 mg kg⁻¹ (very few) and the highest were 36.85 mg kg⁻¹ (more) [19, 20]. The highest value is found in the sample no 5 (in the park soil) and the lowest value in the sample no 15. In the soil samples of the parks, the variable P element can be caused by the fact that the fertilization programs do not match each other.

![N (Plant) Relationships between N content of plants.](image)

![TABLE 3](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil Sample No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available P in soil</td>
<td></td>
<td>3.51 p</td>
<td>12.62 h</td>
<td>5.771</td>
<td>0.53 r</td>
<td>36.85 a</td>
<td>2.97 q</td>
<td>4.29 o</td>
<td>9.78 j</td>
<td>9.76 j</td>
<td>14.63 c</td>
</tr>
<tr>
<td>Soil Sample No</td>
<td></td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>13.55 g</td>
<td>6.98 k</td>
<td>24.34 c</td>
<td>9.95 t</td>
<td>0.05 s</td>
<td>13.65 f</td>
<td>21.07 d</td>
<td>4.92 n</td>
<td>28.46 b</td>
<td>5.23 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Plant Sample No</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>P in plant</td>
<td></td>
<td>3200.0 p</td>
<td>4300.0 k</td>
<td>6200.0 a</td>
<td>3900.0 m</td>
<td>4500.0 h</td>
<td>3800.0 n</td>
<td>5500.0 c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Sample No</td>
<td></td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>5700.0 b</td>
<td>4700.0 g</td>
<td>4700.0 g</td>
<td>4300.0 k</td>
<td>4700.0 g</td>
<td>4400.0 t</td>
<td>4900.0 e</td>
<td>4400.0 t</td>
<td>3600.0 o</td>
<td>4000.0 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is no 0.01 difference between the averages of the same letter. Soil LSD (1%) = 2.213978; Plant LSD (1%) = 1.248495
FIGURE 5
Relations between available P contents of soils.

FIGURE 6
Relationships between P content of plants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil Sample No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable K in soil</td>
<td>abcde fghj klmnopqrst uvwxyz</td>
</tr>
<tr>
<td>1</td>
<td>77.24</td>
</tr>
<tr>
<td>Soil Sample No</td>
<td>abcd efghij klmnopqrstuvwxyz</td>
</tr>
<tr>
<td>11</td>
<td>92.64 abc</td>
</tr>
<tr>
<td>Parameter</td>
<td>Plant Sample No</td>
</tr>
<tr>
<td>1</td>
<td>20700.0</td>
</tr>
<tr>
<td>K in plant</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>32300.0</td>
</tr>
<tr>
<td>Plant Sample No</td>
<td>18</td>
</tr>
<tr>
<td>There is no difference between the averages of the same letter. Soil LSD (1%) = 28.584; Plant LSD (1%) = 3.34010</td>
<td>0</td>
</tr>
</tbody>
</table>

9244
A statistical evaluation of the P contents of plant samples used in the study is shown in Figure 6. The optimum P contents of the plants in vegetative growth periods vary between 0.3% and 0.5% on the principle of dry matter. In case the content of phosphorus exceeds 1% in plants according to dry matter principle, symptoms of “poisoning” occur [25]. According to the results of the study, the maximum P value of the 20 grass samples used in the study was measured with the average % 0.62 in the sample no 6.

**Potassium (K) in Soil and Plant.** A statistical evaluation of the varying K content of soil samples used in the study is shown in Table 4 and Figure 7. All of the soil samples are insufficient for the K content and this need to be taken into consideration in the fertilization programs. Potassium is an important element that is indispensable for all living things including plants [25]. According to the results of the research, soil samples were found to be “insufficient” by the K element. When the soil samples were examined in terms of K content, the lowest was found to be 32.47 mg kg⁻¹ (very few) and the highest was 130.60 mg kg⁻¹ (less). The highest value was found in example 14 (in park soil) and the lowest value was found in sample 12.

A statistical evaluation of the K content of plant samples used in the study is shown in Figure 8. According to the results of the plant analysis, potassium is “high” level in all samples.

The average K content of the plant samples is 3.06% and it is classified as “high” because it is above 1.80-3.00% limit value [12]. The lowest K value in the plant samples taken from the grass areas was measured in the sample with the number of 9 with 0.66%, while the highest K value was determined in the sample no 7 with 16.00%. According to the results of a study, it was emphasized that the K content of the tea wastes were higher than the stable manure and could be easily used in landscaping areas [25].

![K (Soil)](image1)

**FIGURE 7**

Relationships between varying K contents of soils.

![K (Plant)](image2)

**FIGURE 8**

Relationships between K content of plants.
### TABLE 5
Extractable Ca amount of soils and plants according to LSD test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil Sample No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable Ca in soil</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Soil Sample No</td>
<td>8088.9 7534.7 7037.0 7366.5 9023.1 8712.94 8174.9 9233.8 8170.5 8545.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plant Sample No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca in plant</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Plant Sample No</td>
<td>8800.0 6200.0 8700.0 8700.0 7900.0 8800.0 9700.0 7800.0 8200.0 7400.0</td>
</tr>
</tbody>
</table>

There is no difference between the averages of the same letter. Soil LSD (1%) = 7.001214; Plant LSD (1%) = 3.271148

![Graph 9](image9.png)

**FIGURE 9**
Relationships between varying Ca content of soils.

![Graph 10](image10.png)

**FIGURE 10**
Relationships between Ca content of plants.
**Calcium (Ca) in Soil and Plant.** The statistical evaluation of the varying Ca content of soil samples used in the study is shown in Table 5 and Figure 9.

In case of examination soil samples were examined in terms of Ca content, the lowest was found as 5895.65 mg kg⁻¹ (excess) and the highest was 10185.40 mg kg⁻¹ (too much) [19, 20]. The highest value was found in sample 11 (in park soil) and the lowest value was found in sample 19.

A statistical evaluation of the Ca content of the plant samples used in the study is shown in Figure 10. The average Ca content of the plant samples is 0.88% and it is classified as “high” because it is above the “0.25-0.50%” limit value. The lowest Ca value in the plant samples taken from the grass areas was measured with 0.49% in the sample number 20 and the highest Ca value was determined in the sample 18 between 1.14 %. The average Mg content of the plant samples is 0.36% and it is classified as “high” because it is above the “0.13-0.30%” limit value.

**Magnesium (Mg) in Soil and Plant.** A statistical evaluation of the varying Mg content of soil samples used in the study is shown in Table 6 and Figure 11.

When the soil samples were examined in terms of Mg content, the lowest was found to be 375.95 mg kg⁻¹ (sufficient) and the highest was 1521.52 mg kg⁻¹ (too much) [19, 20]. The highest value was found in sample no 11 (in park soil) and the lowest value was found in sample no 19. A similar situation that arises in calcium has also arisen here for soil sample no 19.

**TABLE 6**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil Sample No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable Mg in soil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1   2   3   4   5   6   7   8   9   10</td>
</tr>
<tr>
<td></td>
<td>604.81</td>
</tr>
<tr>
<td></td>
<td>Soil Sample No</td>
</tr>
<tr>
<td></td>
<td>11  12  13  14</td>
</tr>
<tr>
<td></td>
<td>15  16  17  18  19  20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mg in plant</th>
<th>Plant Soil No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1   2   3   4   5   6   7   8   9   10</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
</tr>
<tr>
<td></td>
<td>Plant Soil No</td>
</tr>
<tr>
<td></td>
<td>11  12  13  14</td>
</tr>
<tr>
<td></td>
<td>15  16  17  18  19  20</td>
</tr>
</tbody>
</table>

There is no difference between the averages of the same letter. Soil LSD (1%) = 240.2099; Plant LSD (1%) = 2.101531

**FIGURE 11**

Relationships between varying Mg content of soils.
A statistical evaluation of the Mg content of plant samples used in the study is shown in Figure 12. The lowest Mg value in the plant samples taken from the grass areas was measured in the sample no 20 with 0.26%, while the highest Mg value was found in the samples no 10 and 7 with 0.44%.

**DISCUSSION**

**Total Nitrogen.** According to the results of both soil and plant analysis of the research area, the presence of sufficient and high N element is considered as expected especially in terms of colour, in the grass. As is known, N-fertilization is frequently performed to create a bright green colour on the lawn and even to protect it. According to various academic studies [23, 24, 25], plant colour is usually used as an indicator in nitrogen fertilization. Therefore, the amount of nitrogen fertilization and time of application are very important in the maintenance of all kinds of grass areas. In line with this information, it is possible to say that sufficient fertilizers are made in terms of N element in the research subject parks and even more N is used in some of them.

According to Kacar [24], the increase in plant growth as a result of nitrogenous fertilizer application undoubtedly increases the requirements of micro nutrient elements. Another reason for Zn deficiency in rice is the excess nitrogenous fertilizers. Therefore, the fact that this issue is taken into consideration in the fertilization of the grass reveals it show that it may lead to possible micro nutrient requirements and there may be deficiencies related to it [26]. The total N content of the plant samples was measured as 26.09% and this value was classified as, “high” class [12]. The highest total N value in the plant samples was tested with 31.69% in the sample no 4, whereas the lowest value was tested in the sample no 19 with 20.29%. As in the soil samples, the plant samples are also sufficient for the total N content. Organic materials have very positive effects on the N content of plants [27]. Knowledge about this interaction is important to understand N and organic matter processes. In many publications, the effects on concentrations in plants, is used as the main parameter to assess nutrient interactions [19, 22, 28, 29].

**Phosphorous.** According to the results of the research, it was found that both soil and plant samples were adequate in P element maintenance. According to Kacar [25], the amount of phosphorus applied in increasing amounts to the development medium varies in the P fractions in the plant and, depending on the amount of P applied, the amount of P in the plant's P fractions also increases. The results of the study and this information are similar. According to Luo et al. [30], the nutrients that interfere with Fe nutrition of variable plants follow the sequence P>K>Mg>N>Ca. From this point, it is understood that the macro and micro elements in the plants are in relation with each other. The average P content of the plant samples is 0.46% and it is “sufficient” because it is between “0.25% and 0.60%” [12]. The lowest P value in the plant samples taken from the grass areas was measured at 0.32% in the example 4, while the highest P value was found in the sample no 6 at % 0.62. According to the results of a study by Eisele [10], the most effective materials on the total P content of the plants were found to be stable and tea waste.

**Potassium.** According to Gűneş et al. [19], the growth of plants in the case of “deficiency” of K in the soil is initially less affected in the starting, and then stops completely. In general, the leaves are
sometimes darkened or blushed colour while maintaining their colour. Accordingly, rather than the analyses lack of over-interpretation of the K according to the colour situation, in addition to this the soil analyses, the analysis of the plant from time to time in grass areas will lead to the prediction of major problems and the subsequent elimination of the potential problem by taking necessary measures. Fertilizer containing element N and P is used extensively in the fertilization of turf fields. However, if K and other macro-micro nutrients are missing in the soil, they should be applied in the field of lands without discriminating for the needing level showed by field and soil analysis. Salman and Avcioglu [31] found that 10 g/m²/month NPK fertilizer dose was the best for the fertilization of cool climate grass in Izmir conditions According to Sun et al. [32], K is an important cation due to its high availability in plant tissues and its physiological and biochemical functions. Therefore, the concentration of potassium in the plant is quite high compared to other cations. According to the research results this fact confirms that although K is low in the soil samples, it confirms the high occurrence of plant samples.

**Calcium.** This result observed in this park should be taken into account in the creation of further fertilization programs, otherwise potential problems due to high pH and lime content in the grass may occur. In the soil and plant samples which are detected, the Ca element was in high level. According to Kacar [25], the crop plants remove a relatively large amount the Ca element from soil. This is because the soil solution contains an excess of Ca²⁺. The high level of plant samples in terms of Ca can be caused by the high density of grass plants. According to Kacar (2012), Ca²⁺ uptake is closely related to root genetics of plants. This event has also been proven by research results. According to Barber [33], calcium is one of the nutrients that is transported to the root domain by mass flow and diffusion.

**Magnesium.** One of the essential macro elements for growing and growing plants is magnesium [25]. According to the research results, both Ca and Mg contents were found to be “high” in both soils also. According to Sun et al. [32], high Mg concentrations in the soil and in the plant, disrupts the balance of Ca/Mg in the plant causes damage. These damages are more susceptible to roots that are sensitive to Ca deficiency. Symptoms of magnesium excess occur similar to Ca deficiency, and as the result folds and curl on young leaves may occur. This situation has not been encountered in the grass leaves in the researched parks and it should not be forgotten that this academic knowledge may be necessary for the future. Mn deficiency may occur due to antagonism in Mn uptake due to high Mg concentrations in soil and plant or excessive Mg fertilization [19]. Generally, Ca and Mg nutrients are neglected in the fertilization of grass areas. Baligar et al. [34] reported that many agricultural soils of the world are deficient in one or more of the essential nutrients needed to support healthy plants.

**CONCLUSIONS**

According to the data obtained from the study, it was found that the soil samples examined were inadequate in terms of K content although they were sufficient in terms of total N and P. It has been found that the soils contain high levels of soil alkaline cations in terms of their average Ca and Mg content. According to the data obtained from the research, the total N, retractable K, Ca and Mg elements of the examined plant (plant) samples were found to be high while the P contents were determined to be sufficient. The results of the analysis of the soils and plants in the grass fields should be taken into consideration when creating the fertilization programs. Otherwise, necrosis composed of some element deficiencies, symptoms such as chlorosis, may cause the use of more grass fertilizer and pesticides. The results of this study will contribute to reveal the current nutritional potentials as a result of examining and evaluating the soil and plant analysis of grass in the park-gardens in Esenler. Plant analysis is a powerful tool for confirming nutrient deficiencies, toxicities and imbalances, evaluating fertilizer programs, studying nutrient interactions and determining the availability of macro-micro elements for which reliable soil tests have not been developed. In Turkey, grass areas, which are generally located in important landscaping areas such as parks and gardens, are planted and lawns are losing their homogeneity and grasses are lost from time to time with the pressure of weeds, food element deficiencies and some diseases. This situation is frequently encountered. However, this problem can easily be overcome by proper maintenance and repairs, especially fertilization and irrigation programs. In order to avoid this problem, soil and even plant samples should be collected and analysed at certain times from the grass areas and the results should be evaluated and applied by the plant nutrition experts and the most appropriate fertilization and irrigation programs should be developed and implemented. This is very important in providing the aesthetic and functional benefits expected from grass fields. This study aims to lead those who want to work for the preparation of the most suitable fertilization programs for grass areas. There is a need to increase the fertilizer use efficiency, that is, to obtain more yields per unit of fertilizer applied, to contribute to sustainable fertilization. Knowledge of nutrient interactions can
guide fertilization trials and optimization of fertilization strategies for quality grass and high nutrient use efficiencies. So, fertilization is one of the most significant cultural practices used to improve the yield and quality of grass in the parks.

ACKNOWLEDGEMENTS

This study has been prepared from a part of master's thesis titled “Assessment of Nutritional Status of Grass in Some Parks with Soil and Plant Analysis: In the Case of Esenler District”, completed by Özkan Vardar. The authors also thank Prof. Dr. Murat Deveci for his valuable assistance about statistical analysis.

The authors declare no conflict of interest.

REFERENCES


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e-mail: kbellitürk@nkuc.edu.tr
EFFECT OF SAPTIO-TEMPORAL VARIATION ON AVAILABILITY OF TRACE ELEMENT FROM SOIL, FORAGE TO SMALL Ruminants of Semi-Arid Environment

Humayun Bashir*, Kafeel Ahmad, Zafar Iqbal Khan
Department of Botany, University of Sargodha, Sargodha, Pakistan

ABSTRACT

Metals play vital role in many metabolic processes of plants and ruminants. Present work was carried out to assess the cobalt uptake and its transfer in food chain. Current study was conducted at semi-arid region of Pakistan, Bhakkar, selected as study site which was further split into two sites. Sampling was done in four seasons ((S1) summer, (S2) winter, (S3) autumn and (S4) spring) of year. Soil, forage and goats were selected for sampling. Amount of Co was significantly different at site x season in soil; it was maximum in season 2 at site 2 and observed minimum in season 2 at site 1. In forages, Co was significantly dissimilar in site and season. In the forages at site 1 season 4 and site 2, season 4, concentration of cobalt was 0.22 mg/kg and 0.32 mg/kg, respectively. In goats, Co was non-significantly affected in different stages but significantly affected in site, season, site x season, source and source x stage. Different indices were also studied to assess the transfer of Co to upper trophic level. Level of Co was within the prescribed limits in all parameters. This study discusses the trace element availability, uptake, toxicity according to spatio-temporal differences. The significance of this work is the use of this data in the preventive diagnosis of metabolic and production diseases. The collected data may serve as a control indicator to detect toxic hazards related to the heavy metal occurrence on animal health status.

KEYWORDS:
Semi-arid, Soil, Forage, Cobalt, Spatio-temporal, indices

INTRODUCTION

A complex synergy of soil, producers and consumers lays the foundation of animal nutrition [1]. Ruminants, through their mutualistic association with gut microbiota, transform the roughage into quality food like milk and meat. Forages have been a pivotal constituent of grazer’s diet and indirectly of human diet. Metals level in soil increase to toxic level due to presence of animal slurries and toxic level of these metals also accumulate in tissue of grazing animal [2, 3].

The appearance of mineral deficiency symptoms indicates the inadequacy of forages to fulfill the dietary requirements of ruminants. It could be accredited to the fact that the mineral requirement for animals and plants is different. Some minerals (such as Co & Se) are absorbed in smaller amounts by plants but the animal requirement is much higher. Essential and non-essential trace mineral elements are required at their acceptable concentration for the maintenance of optimum physiology of plants and, in turn, animals. Transfer of trace metals mostly depends on place of plant occurrence and accumulated in large amount in leaves than any other plant part [4, 5].

Enrichment factor (EF) decides whether the entrance of heavy metals in soil takes place from natural or man-made sources [6]. Cobalt (Co) is beneficial for plant growth and development, depending upon the concentration of metal present in the rhizosphere [7]. Industrial and agricultural processes also cause to elevate the metal levels in food chain. Wastewater also act as major source of heavy metals and increase the metal pollution [8, 9]. Cobalt (Co) is an essential micronutrient for animals and humans, but its requirement in higher plants has not been understood yet. The normal concentration of this element in plants is found to be 0.1-10 μg g⁻¹ dry weights, regarding it as a trace element in plants. Co is important as a constituent of many enzymes and coenzymes. Cobalt concentration in soil and rhizosphere decides the degree to which it affects the growth and physiology of plants. Formation of complexes of Co by interaction with other elements is also common [7]. As season affects the maturity of plants which also affect metal availability. Study sites has lot of herds of goats and it was essential to check the trace metal transfer in food chain, whether metal available in enough amount or not. With the help of this work we can easily detect the seasonal and spatial impact on metal availability and its effect on forages and livestock. If metal not present in enough amount in
goats then mineral mixture should be recommended. So current study was aimed to check the possible toxic effect of cobalt with seasonal and sites variation. It was also designed to assess the translocation of this element from soil to forages and then to goats.

MATERIALS AND METHODS

Study Site. The present research was conducted at District Bhakkar. The average annual temperature in Bhakkar is 24.6 °C. In a year, the average rainfall is 213 mm. District Bhakkar was divided into 2 sites. Site-1 consist of Tehsil Bhakkar and Tehsil Mankera. Site-2 consists of Tehsil Darya Khan and Khan Kalooer Kot (Figure 1). Each site was divided into 10 plots and Goats were divided into four groups or stages, [Does (Female), Bucks (Male), Wether (Castrated), Juvenile (6-month)]. Each group had 10 animals. Samples of three sources (Blood, Urine and Feces) from four goat stages were collected. All goats were one year old except juvenile which were 6 month old. Samples for forages and fodder (Table 1) were collected randomly from 10 plots in each site and made a one unit sample or replicate. All sampling was done in four different seasons viz., Summer, Winter, Autumn and Spring. Five replicate of each forage and fodder were collected and brought to Department of Botany, University of Sargodha for further analysis.

Soil and Forage sampling. Soil and forage sampling was done by following standard methods [10, 11].

Blood-serum, urine and feces samples collection. Blood, urine and feces samples were collected from four categories of the Goats [Does, Bucks, Wether, and Juvenile (10 in each group)] by following standard methods [12].

Sample size calculation for animals. The sample size for the ruminants used in this study was calculated by using the equation for a study comparing two means [13] as shown:

\[ N = 4r^2\left(Z_{crit} + Z_{pov}\right)^2/D^2 \]

The calculated sample size was:

\[ N = 4*0.51^2(1.96+0.842)/0.19^2, N=80 \]

Therefore, the total number of samples for the four seasons was:

80 goats x 4 seasons = 320 ruminants.

Sample preparation and analytical Procedure. Samples preparation and Co analysis of all samples was done through Atomic Absorption Spectrophotometer Perkin-Elmer AAS-5000 (Perkin-Elmer Corp., 1980) after wet digestion.

Indices. Bio-concentration factor, pollution load index and enrichment factor were calculated as reported by Cui et al. [15], Liu et al. [16] and Buat-Menard et al. [17] respectively.

Statistical analysis. Data visualization was performed with the help of combined box plots using ggplot2 package in software R. Analysis of variance (ANOVA) [14] along with least significant difference (LSD) test was applied using Statistix 8.1.

RESULTS AND DISCUSSIONS

Soil. The analysis of variance of data showed that Co concentration in soil was significantly (p-value < 0.05) affected by site x season and non-significantly affected by site and season (Table 2).
Co concentration in soil varied from 0.43 to 0.50 mg/kg in all seasons of both sites. Season 2 and 4 showed minimum Co concentration at site 1 and 2, respectively and season 4 and 2 showed maximum concentration at site 1 and 2, respectively (Figure 2). Co concentration in the present study was lower than the level (10 mg/kg) given by WHO [18]. Co concentration in soil given by Yahaya [19] was much higher than the Co values presented in this study. Work of Brady [20] on Co showed high value of Co in soil than present Co value.

Determined Co concentrations in the present study were in safe limits. Co concentration in soil is mainly due to anthropogenic and natural activities all over the world. There are many factors which determine Co level in soil such as distribution of mineral particles, age of soil, use of land, amendments in soil during land use, accumulation of Co containing dust and transport factors [21]. High level of Co in soil also cause toxicity to higher plants but few data are available related to toxicity of Co in higher plants [22]. Some studies showed that Co plays significant role in fertility of soil and it affects the fertility of soil by disturbing the soil invertebrates and microbes [23]. Low soil Co level in the present study might be due to the low anthropogenic activities and low presence of Co containing dust particles in the study area.

**TABLE 1**

List of forages and fodders with local and scientific names collected in sampling

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Summer Plants</th>
<th>Local Names</th>
<th>Autumn Plants</th>
<th>Local Names</th>
<th>Winter Plants</th>
<th>Local Names</th>
<th>Spring Plants</th>
<th>Local Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Peganum harmala</em> L.</td>
<td>Hermal</td>
<td><em>Cymbopogon jwarancusa</em> J.</td>
<td>Khawi</td>
<td><em>Convovulus arvensis</em> L.</td>
<td>Lehli</td>
<td><em>Calotropis procera</em> A.</td>
<td>Akk</td>
</tr>
<tr>
<td>2</td>
<td><em>Cymbopogon jwarancusa</em> J.</td>
<td>Khawi</td>
<td><em>Calotropis procera</em> A.</td>
<td>Akk</td>
<td><em>Chenopodium album</em> L.</td>
<td>Bathu</td>
<td><em>Cymbopogon jwarancusa</em> J.</td>
<td>Khawi</td>
</tr>
<tr>
<td>3</td>
<td><em>Cucumis melo var. Agrestis</em></td>
<td>Chibber</td>
<td><em>Ziziphus mauritiana</em> L.</td>
<td>Bairi</td>
<td><em>Parthenium hysterophorus</em> L.</td>
<td>Gajar</td>
<td><em>Ziziphus mauritiana</em> L.</td>
<td>Bairi</td>
</tr>
<tr>
<td>4</td>
<td><em>Calotropis procera</em> A.</td>
<td>Akk</td>
<td>*Citrus colycohis L.</td>
<td>Tumma</td>
<td><em>Cyperus iria</em> L.</td>
<td>Bhoian</td>
<td><em>Cucumis melo var. Agrestis</em></td>
<td>Chibber</td>
</tr>
<tr>
<td>5</td>
<td><em>Tribulus terrestris</em> L.</td>
<td>Bhakra</td>
<td><em>Euphorbia prostata</em> A.</td>
<td>Dhdak</td>
<td><em>Fimbristylis dichotoma</em> L.</td>
<td>Choti</td>
<td><em>Prosopis juliflora</em> Sw.</td>
<td>Jangli</td>
</tr>
<tr>
<td>6</td>
<td><em>Citrus colycohis</em> L.</td>
<td>Tumma</td>
<td><em>Ziziphus mauritiana</em> L.</td>
<td>Bairi</td>
<td><em>Medicago sativa</em> L.</td>
<td>Bhoian</td>
<td><em>Medicago sativa</em> L.</td>
<td>Lucern</td>
</tr>
<tr>
<td>7</td>
<td><em>Achyranthes aspera</em> L.</td>
<td>Puth Kanda</td>
<td><em>Digitaria sanguinalis</em> L.</td>
<td>Mooti Khabbal, karabara</td>
<td><em>Trifolium alexandrinum</em> L.</td>
<td>Barseem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Ziziphus mauritiana</em> L.</td>
<td>Baii</td>
<td><em>Sorghum bicolor</em> L.</td>
<td>Jowar</td>
<td><em>Ziziphus mauritiana</em> L.</td>
<td>Bait</td>
<td><em>Hordeum vulgare</em> L.</td>
<td>Jao</td>
</tr>
<tr>
<td>9</td>
<td><em>Prosopis juliflora</em> A.</td>
<td>Jangli</td>
<td><em>Prosopis juliflora</em> Sw.</td>
<td>Jangli Kikkar</td>
<td><em>Prosopis juliflora</em> Sw.</td>
<td>Jangli Kikkar</td>
<td><em>Zea mays</em> L.</td>
<td>Makai</td>
</tr>
<tr>
<td>10</td>
<td><em>Pennisetum glaucum</em> L.</td>
<td>Bajra</td>
<td><em>Medicago sativa</em> L.</td>
<td>Lucern</td>
<td><em>Cicer arietinum</em> L.</td>
<td>Channa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td><em>Zea mays</em> L.</td>
<td>Makai</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td><em>Sorghum bicolor</em> L.</td>
<td>Jowar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td><em>Cyanopis tetragonoloba</em> L.</td>
<td>Guar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td><em>Sesbania sesban</em> L.</td>
<td>Jantar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

Analysis of variance:
Mean squares of Co for soil, forage and goats

<table>
<thead>
<tr>
<th>Metal</th>
<th>Site</th>
<th>Season</th>
<th>Soil Site x Season</th>
<th>Error</th>
<th>Site</th>
<th>Season</th>
<th>Soil Site x Season</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>0.005ns</td>
<td>0.000ns</td>
<td>0.103***</td>
<td>0.002</td>
<td>0.290***</td>
<td>0.002ns</td>
<td>0.038***</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Analysis of variance of metals for site x season and source x stage**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Site</th>
<th>Season</th>
<th>Soil Site x Season</th>
<th>Error</th>
<th>Source</th>
<th>Stage</th>
<th>Source x Stage</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>3.69***</td>
<td>0.0004ns</td>
<td>0.03***</td>
<td>0.004</td>
<td>0.620***</td>
<td>0.002ns</td>
<td>0.000ns</td>
<td>0.007</td>
</tr>
</tbody>
</table>

***=significant at 0.001 levels, *=significant at 0.05 levels, ns=non-significant at above 0.05 levels
Forages. Variance analysis of Co values in forages exhibited significant (p-value < 0.05) variation in sites and site x season but non-significant variation in seasons (Table 2). Level of Co in forages was ranged from 0.22 to 0.32 mg/kg at both sites of all seasons. The highest Co level was observed in season 4, at site 2 and the lowest was observed in season 4, at site 1 (Figure 2). Co level in forages was lower than the critical level (52 mg/kg) suggested by FAO [24] but it was lower than the findings of Fardous [25]. Present findings of Co in forages were similar to the findings of Peers and Phillips [26].

Toxicity of Cobalt in plants due to high level of Co in soil is not common. Atmospheric deposition, water source and other anthropogenic activities may be cause of toxicity of Co in plants. Calcium can help to cope up Co toxicity in plants by occupying the binding sites in root cells and competing of Ca²⁺ ion for binding sites cause to reduce the toxicity of Co and many other metals [22]. Co is known as necessary element for human, animals and plants but its physiological role in higher plants is not clearly identified. Co can show severe effects on the metabolic activities of plants and it can damage plant cell and cell membrane [27]. Co has significant importance in many physiological functions but its high amount also cause to disturb the many biochemical and physiological functions [28]. Co values in forages determined in the present study was not in toxic limit and it might be due to the availability of clean water, low anthropogenic activities and safe amount of Co in soil.

Goats. Goats data about Co showed that site and site x season had significant (P < 0.05), but season had non-significant (p-value < 0.05) effect on Co concentration (Table 2). Present results showed that Co value in goats was present in between 0.28-0.43 mg/kg. Season 2 of site 1 and site 2 showed the minimum and maximum results of Co, respectively (Figure 2). Results of Co data also revealed significant (p-value < 0.05) variation in source but non-significant variation in stage and source x stage (Table 2). Level of Co varied from 0.31 to 0.41 mg/kg in all sources and stages (Table 3). The highest Co concentration was observed in blood of bucks and the lowest was in urine of juvenile stage.

According to the box plot, Co level in blood plasma was ranged from 0.37 to 0.44 mg/L in all season at both sites. Season 2 at both sites depicted the minimum and maximum level of Co, respectively. Concentration of Co in feces was ranged from 0.26 to 0.43 mg/kg. Both sites in season 2 showed the lowest and highest value of Co in feces, respectively. Co results in urine samples were present in between 0.21-0.42 mg/L at both sites in all seasons. Minimum and maximum level of Co was present in season 2 at site 1 and 2, respectively (Figure 3). Present investigation showed that the Co levels in blood samples were lower than the standard value (0.5 mg/l) reported by Schweinzer et al. [29] but higher than the findings of Yatoo et al. [30].

Cobalt is considered as essential element in ruminant diet because it is an important part of vitamin B₁₂ which has significant role in energy metabolism. Vitamin B₁₂ is produced by microorganisms of rumen in animals which require a constant supply of Co for its production from animal diet [26]. Cobalt deficiency is mostly present in animals which fulfill their feeding requirement from pastures on well-drained, sandy soils. Grasses show more Co deficiency than legumes and small ruminants are subjected to more deficiency than other animals. Grains are considered as poor source of Co and its ability of synthesis of vitamin B₁₂ is also poor [31]. For this reason, deficiency of Co is deficiency of vitamin B₁₂ in ruminants. Reduced growth, loss of weight, impaired reproductive function and immunosuppression are the sign of Co deficiency. Co toxicity is not so common in world because animal it needs to be much higher in animals than in available diet [32]. Animals provide many food material to human and play considerable role in deliver the wool for local people [33]. Cobalt values in goats investigated in the present study were in lower level and might be due to feeding on those forages which grow on sandy soils. Therefore supplementation of Co is required to overcome Co deficiency.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean concentrations and standard errors of Co in different sources and stages of goats</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Does</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Bucks</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Wether</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Juvenile</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Over all</td>
</tr>
</tbody>
</table>
FIGURE 2
Mean concentration and comparison of Co transfer from soil to forage to goats

FIGURE 3
Mean concentration of Co in blood, urine and feces in different seasons at two sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Season</th>
<th>Bio-concentration</th>
<th>Pollution Load Index</th>
<th>Enrichment Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Soil to Forage</td>
<td>Forage to Goat</td>
<td></td>
</tr>
<tr>
<td>Site-1</td>
<td>S1</td>
<td>0.582866</td>
<td>1.134615</td>
<td>0.049019</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0.630424</td>
<td>1.043623</td>
<td>0.047839</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>0.525666</td>
<td>1.200685</td>
<td>0.053386</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>0.444111</td>
<td>1.392391</td>
<td>0.054956</td>
</tr>
<tr>
<td>Site-2</td>
<td>S1</td>
<td>0.605331</td>
<td>1.421599</td>
<td>0.054772</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0.555506</td>
<td>1.558071</td>
<td>0.055214</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>0.675805</td>
<td>1.359838</td>
<td>0.050103</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>0.737</td>
<td>1.26873</td>
<td>0.048209</td>
</tr>
</tbody>
</table>

Pollution Load Index: Reference Values (mg/kg) of soil Co is 9.1, Sources: [41]
Enrichment Factor: Reference Values (mg/kg) soil of Co is 9.1, Sources: [41]
Reference Values (mg/kg) forage of Co is 50, Sources: [24]
Bio-concentration Factor. BCF of Co in forages was ranged from 0.44 to 0.73 mg/kg in all seasons at site 1 and site 2. Minimum BCF in forages for Co was available at site 1 in season 4 and maximum BCF for Co was also available in season 4 but at site 2. BCF of Co in goats was present between 1.04 mg/kg to 1.52 mg/kg at all sites in all seasons. Season 2 of site 1 and site 2 showed the lowest and highest BCF of Co in goats respectively (Table 4). Bio concentration factor describes the bioavailability of heavy metals to plant species. The BCF for cobalt in present research was lower was found less than 1 indicated poor transfer of this metal from soil to plant. The difference in BCF might be due to difference in absorbing ability of metals by plant [34]. The BCF of Co in current findings were higher than the values reported by Alghobar and Suresha [35].

Pollution Load Index. Results of Pollution load index for Co in soil varied from 0.047 to 0.055 mg/kg in all sampling seasons at both sites. Highest and lowest value of PLI was showed in season 2 at site 2 and site 1 respectively (Table 4). Pollution load index demonstrates whether the quality of soil is good for plant growth or not. The values of PLI for Co in current work were lower than the reference values of Co (9.1 mg/kg) suggested by Dutch standard [36]. Khan et al. [37] reported higher values of PLI for Co (1.31-1.73) as compared to present findings.

Enrichment Factor. EF for Co at both sites in all seasons was present between 0.08 to 0.13 mg/kg. Minimum level of EF for Co was showed at site 1 in season 4 and maximum level of EF for Co was showed at site 2 in season 4 (Table 4). Cobalt E.F of current investigation was considered deficient according to level suggested by Barbieri [38]. Present E.F of Co was also lower from the work of Rizo et al. [39] and Taha et al. [40].

CONCLUSION

The concentrations of metals in animals could reflect the level of metals in soils and the environmental concentrations of toxic element. Study showed that effects of season on the availability of elements are not much applicable but on the other side variation in sites is much noticeable. All indices were also in acceptable limits. Maximum level of metal was much available in winter (season-2) season. On the other hand overall concentration of Co was lower in ruminants and maximum in blood of bucks so there is need to mineral mixture for ruminants other than bucks. Maximum level of elements in winter might be due maturity of forages in this season.

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THE CHARACTERISTICS OF MICRO PORE STRUCTURE AND THE DIFFERENCES ANALYSE OF LOGGING IDENTIFICATION FOR DIFFERENT DIAGENETIC FACIES IN LOW PERMEABILITY RESERVOIR

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\textsuperscript{3}College of Petroleum Engineering and Environmental Engineering, Yan’an University, Yan’an, Shaanxi, China
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ABSTRACT

Based on the data from casting slices, scanning electron microscope, core photo, constant speed mercury pressure and so on, when geology parameters such as material source, clay mineral type, pore type and evolution strength of diagenesis were taken into account, the Chang 6\textsubscript{1} reservoir of Wangpanshan is divided into six different types of diagenetic facies. The dominant reservoir facies are kaolinite + chlorite cementation - intergranular pore facies, kaolinite cementation - dissolved pore + intergranular pore facies, chlorite + kaolinite cementation - dissolution pores. The study shows that the microscopic pore structure characteristics and the logging response characteristics of different types of diagenetic facies are different and the difference of micro-pore throat in each diagenetic phase is obvious, especially reflected in the shape of the capillary pressure curve and the size of the throat. The correlation between the radius of the throat and the permeability is better than the correlation between pore radius and porosity and permeability. Moreover, the pore throat radius of the dominant diagenetic facies is more uniform. The connectivity is good. Besides, the seepage ability is strong which keeps high mercury saturation under the same pressure of mercury intake. The logging response characteristics of different diagenetic facies are summed up by using several logging methods like natural gamma ray and sonic time difference, resistivity. Then the logging response identification template for each diagenetic facies is established and quantitatively identify criteria for logging response of different diagenetic facies. Finally, identification and division of vertical upper diagenetic facies of single well is realized, so as to provide important reference for prediction of sweet spot in low permeability sandstone reservoir.

KEYWORDS:
Chang 6\textsubscript{1} reservoir, diagenetic facies, constant speed mercury penetration, throat, logging curve

INTRODUCTION

The most special feature of low permeability reservoir is its pore structure, which has fine pores, fine throat or fine pores and micro throat. These two parameters play a crucial role in evaluation and development of low permeability reservoirs [1]. The constant velocity mercury injection process is similar to the static process in mercury injection process. It can distinguish pores and throat and get accurate throat and pore capillary pressure curves, which helps to make accurate evaluation of reservoir [2]. Well logging is a reflection of the macroscopic physical properties of various rocks in the formation [3-5], which can continuously record data through various rock physical information of stratum. Different lithology, pore fluid and diagenetic facies are different in the corresponding logging response characteristics [6]. Therefore, the conventional logging data can be used to identify the diagenetic facies.

Wangpanshan area of Jiyuan oilfield is mainly in the territory of Dingbian County of Shaanxi province and Ningxia Yanchi County, located in the east coast of Tianhuan depression, Yishan slope of western developed delta front underwater distributary channel and distributary Bay [7]. In the study area, the Chang 6\textsubscript{1} reservoir is the main oil producing layer, along with an average porosity of 10.93\% and an average permeability of $1.18 \times 10^{-4} \mu \text{m}^2$, which belongs to the typical low permeability reservoir and is difficult to develop. Predecessors studied the reservoir characteristics from the aspects of petrology, physical properties and sedimentation [8]. However, few studies have talked about the characteristics of micro pore structure and its logging response characteristics based on diagenetic facies. This paper is about to study the charac-
teristics of diagenetic facies reservoir micro pore structure, meanwhile, discusses the different diagenetic facies, difference micro pore structure characteristics, establish different template recognition facies logging and effective evaluate microscopic reservoir geological characteristics with the micro theory and science [9], for the efficient development of Jiyuan Oilfield by means of casting slices, SEM, constant pressure technology.

MATERIALS AND METHODS

In accordance with industry standards (SY/T5368-2000, SY/T5477-2003, SY/T6285-2011) [10-12] and results from a large number of core observation, casting thin film and scanning electron microscope analysis and analysis of the test data, the reservoir is mainly composed of fine sand (68.92%) and very fine sand (15.03%). The rock type is dominated by feldspar sandstone and the lithic feldspar sandstone is next (Figure 1).

The particle size of 0.03mm-0.5mm and the separation medium are good. The grinding degree of the skeleton particles is poor and located most are on edge. The type of pore are mainly dissolved pore - intergranular pore and intergranular pore - dissolved pore (44%), micropores are next (23%). The cementation type is dominated by enlarging - pore and pore - enlarging (58%). The content of quartz (29.94%) and feldspar (35.82%) in the detrital composition are higher, cutting is the second, following by other components like mica (7.89%). The fillings take a small amount of heterobase and autogenic ore cementation.

RESULTS AND DISCUSSION

Diagenetic facies division. Through the study of a large number of casting thin sections and scanning electron microscopy test data, combined with core photos, according to the specific types of diagenesis WangPanshan Chang 61 low permeability sandstone reservoir and the principle of dominant facies (The diagenesis and the characteristics of the diagenetic mineral assemblage obtained from the thin slices of the reservoir) [13]. The diagenetic facies of Wangpanshan Chang 61 reservoir are divided into six types of diagenetic facies, such as kaolinite + chlorite cementation intergranular pore facies, kaolinite cementation dissolution pore + intergranular pore facies, chlorite + kaolinite cemented dissolved pore facies, kaolinite cementation dissolved pore facies, illite cementation dissolved pore facies, carbonate cementation dense phase (Figure 2). The provenances are all northeast and the pore development characteristics of each diagenetic facies reservoir are different and the specific characteristics are shown in Table 1.

The kaolinite + chlorite cementing - intergranular pores in the 61 reservoir of Wangpanshan are mainly distributed in the eastern and northeastern regions of the study area and the facies developed distributary channel and underwater distributary channel in delta plain. The content of chlorite membrane is higher (2.47%) and the content of residual intergranular pore is the highest (32.68%). To a certain extent, the formation of the diagenetic.

![Figure 1: Rock type and composition diagram of Chang 61 reservoir](image-url)
FIGURE 2
Distribution of diagenetic facies in Chang 6 reservoir in Wangpanshan Area

a Kaolinite + chlorite cementation - intergranular pore facies (C146 well, 2100m)
b Kaolinite cementation - dissolution pores + intergranular pore facies (L37 well, 2285m)
c Chlorite + kaolinite cementation - dissolution pore facies (Y245 well, 2165m)
d Kaolinite cementation - dissolution pore facies (J43 well, 2042m)
e Illite cementation - dissolution pore facies (Y263 well, 2090m)
f Carbonate cementation - dense facies (Y328 well, 2055m)

FIGURE 3
Photos of scanning electron microscope of different diagenetic facies in Chang6 reservoir
### TABLE 1

**Characteristic parameters of different diagenetic facies of Chang 6 reservoir in Wangpanshan Area**

<table>
<thead>
<tr>
<th>The type of diagenetic facies</th>
<th>Lithologic characteristics</th>
<th>Cement content (%)</th>
<th>Cementation type (%)</th>
<th>Porosity (%)</th>
<th>Corrosion- al ratio (%)</th>
<th>Face rate (%)</th>
<th>Permeability $10^6 \mu m^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Rock fragments feldspar sandstone</td>
<td>(I): 5.01</td>
<td>1:2.32</td>
<td>A:32.68</td>
<td>1.57</td>
<td>4.78</td>
<td>13.19</td>
<td>0.52</td>
</tr>
<tr>
<td>2 :1.45</td>
<td>2:2.16</td>
<td>B:3.91</td>
<td>0.85</td>
<td>4.31</td>
<td>11.88</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>3:2.47</td>
<td>3:2.27</td>
<td>C:11.66</td>
<td>1.58</td>
<td>4.28</td>
<td>10.58</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>IV:2.03</td>
<td>4:1.26</td>
<td>D:31.49</td>
<td>2.17</td>
<td>3.39</td>
<td>9.11</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>V:1.96</td>
<td>5:1.13</td>
<td>E:16.42</td>
<td>2.01</td>
<td>2.61</td>
<td>8.39</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>2 Feldspar sandstone</td>
<td>(I): 4.37</td>
<td>1:1.95</td>
<td>A:1.38</td>
<td>2.10</td>
<td>2.45</td>
<td>5.78</td>
<td>0.08</td>
</tr>
<tr>
<td>II: 1.22</td>
<td>2:5.28</td>
<td>B:10.53</td>
<td>4.08</td>
<td>0.79</td>
<td>6.5</td>
<td>0.08</td>
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<tr>
<td>III: 1.29</td>
<td>3:2.50</td>
<td>C:9.16</td>
<td>2.48</td>
<td>0.79</td>
<td>6.5</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>IV:2.57</td>
<td>4:1.32</td>
<td>D:24.63</td>
<td>2.01</td>
<td>2.61</td>
<td>8.39</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>V:1.18</td>
<td>5:6.49</td>
<td>E:21.50</td>
<td>2.17</td>
<td>3.39</td>
<td>9.11</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>


### TABLE 2

**Characteristics parameters of micro-pore structure of constant rate mercury penetration of different diagenetic facies in Chang 6 reservoir**

<table>
<thead>
<tr>
<th>Diagenetic facies</th>
<th>Average value of throat radius ($\mu m$)</th>
<th>Average value of pore radius ($\mu m$)</th>
<th>Total pore mercury saturation (%)</th>
<th>Hg saturation of the total throat (%)</th>
<th>Total pore / throat volume ratio</th>
<th>Microcosmic homogenization coefficient</th>
<th>Sorting coefficient</th>
<th>Displacement pressure (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Rock fragments feldspar sandstone</td>
<td>0.49</td>
<td>153.75</td>
<td>35.12</td>
<td>40.62</td>
<td>0.87</td>
<td>0.01</td>
<td>2.36</td>
<td>0.06</td>
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<tr>
<td>2</td>
<td>0.42</td>
<td>163.59</td>
<td>26.11</td>
<td>35.59</td>
<td>0.74</td>
<td>0.02</td>
<td>0.71</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>1.04</td>
<td>147.35</td>
<td>25.20</td>
<td>45.61</td>
<td>0.56</td>
<td>0.03</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
<td>148.29</td>
<td>28.51</td>
<td>29.79</td>
<td>0.92</td>
<td>0.04</td>
<td>0.15</td>
<td>0.87</td>
</tr>
<tr>
<td>5</td>
<td>0.51</td>
<td>143.40</td>
<td>17.33</td>
<td>25.57</td>
<td>0.67</td>
<td>0.05</td>
<td>0.13</td>
<td>1.19</td>
</tr>
<tr>
<td>6</td>
<td>0.86</td>
<td>134.98</td>
<td>3.14</td>
<td>25.81</td>
<td>0.13</td>
<td>0.05</td>
<td>0.09</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Note: 1: Kaolinite and chlorite cementation intergranular pore facies; 2: Kaolinite cementation - dissolution pores + intergranular pore facies; 3: Chlorite + kaolinite cementation - dissolution pore facies; 4: Kaolinite cementation - dissolution pore facies; 5: Illite cementation -dissolution pore facies; 6: Carbonate cementation - dense facies;
Facies is the best, for the chlorite membrane hinders compaction and cementation (Figure 3a), allowing a large number of primary intergranular pores to be retained. The displacement pressure and microcosmic mean coefficient (Table 2) of the diagenetic facies are the smallest of the 6 types of diagenetic facies reservoir. From Table 2 and Figure 4, it is known that the throat radius of the reservoir of this type of diagenetic facies is the widest range, 0.5µm-1.5µm and the contribution of the radius of the throat to the permeability is 92%, which the radius ratio of the pore throat is the smallest and the average value is 210.03 µm.

Kaolinite cementing - dissolving hole + intergranular pore facies mainly develops in the north area of the study area and the central part of the southern region, which is sedimentary microfacies for underwater distributary channel. Kaolinite is the main fillage (Figure 3b) and the second is iron calcite. The reservoir space in this type of diagenesis is dominated by secondary dissolved pores produced by a large number of feldspar dissolution (47.02%). The displacement pressure of the diagenetic phase is 0.22Mpa and the microcosmic mean coefficient and the relative separation coefficient are low, which are 0.02 and 0.71 respectively. Compared to kaolinite + chlorite cementation - intergranular pore phase, the radius of the throat is small. The contribution of the throat to the permeability is concentrated in the 0.6µm-1.3µm and the distribution range of the throat is 53%. The radius ratio of pore throat is 120-570 and the average value is 345.

The chlorite + kaolinite cementation - lysis phase is more developed in the study area, which is distributed in the north-east and Western provenance areas and the content of chlorite is 2.97%. With the increase of the thickness of the chlorite film and the content of the cementation (Figure 3c), the effective porosity of the reservoir and the connectivity of the throat become worse and massive residual intergranular pores loss [14], so the main reservoir space is mainly secondary porosity produced by dissolution (31.49%). The chlorite + kaolinite cementation - the dissolution pore phase is worse than the former two, that the displacement pressure and microcosmic mean coefficient increase, even the relative separation coefficient decreases and the throat radius decreases. The distribution of pore radius is basically the same as that of the first two types of diagenetic facies and the radius of the hole and throat is higher than that of the other.

![Characteristics of throat radius of different diagenetic facies reservoir](image1)

**a Characteristics of throat radius of different diagenetic facies reservoir**

![Characteristics of pore radius of different diagenetic facies reservoir](image2)

**b Characteristics of pore radius of different diagenetic facies reservoir**
c Characteristics of pore throat radius ratio of different diagenetic facies reservoir

The reservoir space is mainly kaolinite cementation dissolved pore facies (Figure 3d) of dissolved secondary pores and illite cemented dissolved pore facies of thin throat like illite [15] existing in micro throat. (Figure 4e) is developed in non main production area. The physical properties of the two types of diagenetic facies reservoirs are poor, the average porosity is 8.56% and the average permeability is 0.22×10^{-3} \text{ μm}^2. Carbonate cemented dense phase reservoirs develop ferric calcite and ferric dolomite and other carbonate cements, which account for more than 8% of the total cementation of the diagenetic facies. The intergranular pore loss of diagenetic facies is very serious. The intergranular pore between isolated grains is very poor in connectivity with dissolved pores and the reservoir is poor (Figure 4f), so is the most unfavorable diagenetic facies in this area, which is regarded as an invalid reservoir.

d Characteristics of Permeability contribution of different diagenetic facies reservoir

Pore structure characteristic of Chang 6 reservoir in Wangpanshan Area

FIGURE 4

36 core samples from different diagenetic facies reservoirs were selected for mercury injection test at site. The experimental results were compared with capillary pressure curves of 6 typical wells in different Cheng Yan facies (Figure 5). The experimental results show that the mercury saturation of Y146 well, J68 well, C95 well, L37 well, Y63 well and Y245 well decreases in turn. The shape of the curve changes from slightly skewness to fine skewness and the slope increases and the separation gradually becomes worse. The porosity and throat decrease or decrease and the matching of pore throat becomes worse and the seepage resistance
increases and the physical properties become worse. 

Kaolinite + chlorite intergranular pore is relatively developed and pore and throat are relatively large. Pore throat matching relationship and connectivity are good and mercury penetration reaches 67.71%. (Figure 5a). The throat entry mercury saturation curve and the total pore throat entry mercury saturation curve extend well and the initial stage is gentle. This stage is the dominant pore structure segment and has good seepage and storage capacity. When the pressure reaches 1.28MPa and the pore penetration rate reaches 22.99%, the end of the pore entry saturation curve is warped, which indicates that the reservoir space is transformed from pore aggregation to throat seepage channel.

![Capillary Curves](image)

**FIGURE 5**

Characteristic of the capillary curve of different diagenetic facies
The dissolution of kaolinite cementation, dissolved pore and intergranular pore, is more developed. The slope of the Hg saturation curve of the total pore throat is slightly increased with a small amount of intergranular pore. The saturation of the pore mercury is 28.47% and the mercury saturation of the throat is 29.84% (Figure 5b). The matching relationship and the connectivity of the pore and throat are worse. The phase intake saturation of the phase is smaller than that of the previous 10% diagenetic facies and the difference of the intake mercury saturation is the main factor that leads to the smaller total mercury saturation. This also reflects the importance of throat in reservoir fluid storage and seepage process.

The mercury saturation of chlorite + kaolinite cementation-lysis phase is further reduced to 42.94%, of which the saturation of the pore mercury is 17.34% and the Hg saturation of the throat is 25.60% (Figure 5c). The pore type of the kaolinite cemented pore reservoir sandstone is single and only a few pores are dissolved and the pore and throat are not connected. The Hg saturation of the pore throat is low and the effective pore throat is small, which makes most of the fluid in the throat and become a bound fluid. The mercury injection pressure of diagenetic facies is increased significantly. The total mercury saturation is only 48.52%, of which the Hg saturation of the throat is 35.79% and the saturation of the pore mercury is 12.73% (Figure 5d), so the throat is the main contributor to the storage of fluid.

In the illite cemented dissolution pore, the throat entry mercury saturation curve is approximately parallel to the total mercury saturation curve and the elongation length is similar. This indicates that the diagenetic phase throat is the main permeability contribution channel. The Hg saturation of the throat is 25.84% and the saturation of the pore mercury is 3.05% (Figure 5e). The saturation of the mercury in the carbonate cemented dense phase is 10.01% and the saturation of the pore mercury is 0.29% (Figure 5f).

Distribution characteristics of pore radius. Shown in Figure 4b and Figure 6, the whole curvilinear form of void radius presented positive skewness. The pore radius of the carbonate cemented dense phase is between 100 µm -150 µm and the remaining pore radius of the formation of the diagenetic facies is concentrated in the 110 µm -180 µm. With the decrease of porosity and permeability, the range of pore radius is narrower in turn. The order of narrowing the range of pore radius in different diagenetic facies is non - one-to-one correspondence with the order of the difference in physical properties of different diagenetic facies, this shows that the pore radius is irregularity with the permeability and porosity parameters and Figure 6 shows that the correlation is poor.

![FIGURE 6](image1.png)

Map showing relationship between pore radius and porosity, permeability of different diagenetic facies

![FIGURE 7](image2.png)

Map showing relationship between throat radius and permeability

The distribution characteristics of the radius of the throat. According to Figure 4a, the distribution curve of the throat radius in different diagenetic and representative samples shows a normal distribution of unequal frequencies. The throat radius is large and partial low frequency distribution, on the contrary, the throat radius is small and thin partial state high frequency distribution and the smaller the throat radius is the peak normal. The experimental results show that the peak value of throat radius decreases with the increase of physical properties and the frequency increases. It shows that the denser pore type of rock is composed of composite pore assemblage to single pore and the throat volume of connected pore throat decreases and the seepage channel is concentrated and narrowed.

The correlation between the radius of the throat and the permeability is good (Figure 7). The throat radius and the permeability contribution map reflect the good separation of the laryngeal channel of the reservoir with better physical properties. The roaring channel is uniform and concentrated and has the characteristics of large throat radius, wide distribution range and low frequency distribution of curves. The throat with narrow physical properties and narrow laryngeal facies and concentrated contribution to the percolation is concentrated in a
single range. The distribution frequency is high and the curve is single peak (Figure 4d). The experimental results show that the throat especially the main contribution factors of reservoir fluid flow.

Distribution characteristics of radius ratio of pore and throat. The weaker the pore structure heterogeneity is, the smaller the pore throat radius ratio is. The larger pore is easy to be surrounded by the large throat. When the fluid is discharged from the pore, the seepage resistance is weaker. Oil and gas can be continuously discharged through these large throat and the saturation of movable fluid is high [18].

The experimental results show that (Figure 4c) the radius of the hole and throat is generally biased toward the fine partial state and the peak frequency is higher than that of the smaller one. The radius of the hole and throat is generally moving to the coarse partial state and the peak frequency is low. The radius ratio of pore throat in different diagenetic facies is concentrated between 50-800 and the pore radius map and the throat radius map show the negative skewness distribution. The better the pore throat radius of the diagenetic facies reservoir is more obvious than that of the negative skewness of the curve and the pore throat radius ratio of the poor petrographic reservoir is offset to the fine skewness. It is known that the pore throat radius is smaller than the large throat radius and the smaller the throat radius, the more obvious the control effect of pore throat radius is on the reservoir. The stronger the capillary force is, the less likely it is to develop the reservoir.

CHARACTERISTICS OF LOG RESPONSE OF DIFFERENT DIAGENETIC FACIES RESERVOIR

At present, the identification and division of diagenetic facies are mainly based on the statistical analysis of experimental data. Limited to the cost of coring, the core slice information of a region is always limited [19], so we need to use other means to evaluate and verify the diagenetic facies. Well logging technology to obtain information on the strata are mainly physical properties of rock strata and the material is full of more accurate, so it can be in the thin section analysis characteristics analysis of different logging curves of diagenetic facies based on the establishment of the diagenetic facies, diagenetic facies logging identification standards, so as to effectively evaluate diagenetic facies [20-22].

![Diagram](image1.png)

a) Kaolinite + chlorite cementation- intergranular pore facies (G54, 2276m)

![Diagram](image2.png)

b) Kaolinite cementation - dissolution pores + intergranular pore facies (J68 well, 2041 m)

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c Chlorite + kaolinite cementation - dissolution pore facies (C102, 2363m)

d Kaolinite cementation - dissolution pore facies (C95, 2227m)

e Illite cementation - dissolution pore facies (J202, 2380m)

f Carbonate cementation - dense facies (X35, 2363m)

FIGURE 8
Characteristics of logging response of different diagenetic facies
The characteristic parameters of different diagenetic facies

<table>
<thead>
<tr>
<th>Diagenetic facies</th>
<th>Range</th>
<th>SP (mV)</th>
<th>GR (API)</th>
<th>AC (um/m)</th>
<th>DEN (g/cm³)</th>
<th>RT (Ω m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>kaolinite + chlorite cementation - intergranular pore facies</td>
<td>maximum value</td>
<td>66.37</td>
<td>126.27</td>
<td>246.4</td>
<td>2.55</td>
<td>30.21</td>
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<td>minimum value</td>
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<td>60.41</td>
<td>213.7</td>
<td>2.43</td>
<td>10.23</td>
</tr>
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<td>84.25</td>
<td>229.4</td>
<td>2.47</td>
<td>18.25</td>
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<td>8.12</td>
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<td>2.51</td>
<td>13.94</td>
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<td>239.4</td>
<td>2.60</td>
<td>28.86</td>
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<td>carbonate cementation - dense phase</td>
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<td>71.35</td>
<td>62.95</td>
<td>207.4</td>
<td>2.49</td>
<td>13.09</td>
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<td>average value</td>
<td>82.04</td>
<td>65.21</td>
<td>229.2</td>
<td>2.35</td>
<td>19.14</td>
</tr>
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</table>

FIGURE 9
Cross plots of various parameters derived from logging curves for different diagenetic facies of reservoirs
Characteristics of log response. Well logging characteristics of different diagenetic facies. Influenced by lithology, the natural potential curves of kaolinite + chlorite intergranular pore facies are characterized by medium low value box type and bell type and the acoustic time difference is mainly high value. Natural gamma is mainly represented by tooth shape and its density is mostly low value. Based on the thin section, physical statistics, the diagenetic phase pore rate, porosity and permeability is good, the dynamic data of field production showed that this kind of diagenetic facies higher yield (Figure 8a). The characteristics of natural kaolinite cementation and dissolution potential curve pores + intergranular pore is the high value of the box type, acoustic time is higher, the relatively high rate of pore diagenetic phase (Figure 8b). The natural potential curve of chlorite + kaolinite cemented dissolved pore is characterized by medium low value box type and the time difference of acoustic wave is high. Natural gamma is mainly represented by tooth shape and its density is mostly low value (Figure 8c). The general performance of kaolinite cementation and dissolution curve of natural potential hole is a low value, mainly for natural gamma tooth, the diagenetic phase low porosity (Figure 8d). Illite cementation and dissolution pore phase curve of natural potential features in the low value of the box type, bell shaped, sonic with high value, the low porosity diagenetic phase (Figure 8e). Carbonate cementation tight hole phase rate of less than 5% (Figure 8f). The logging re-

Establishment of log response template. Based on the statistics of all the slice data in the study area, scatter plot analysis is further done. By using GR, AC and RT curves, different diagenetic facies can be distinguished from each other in the form of cross plot [23-25]. Based on the above comprehensive statistical analysis, we can establish different criteria for distinguishing diagenetic facies in the study area and use this standard to identify the diagenetic facies of reservoirs in the study area and analyze reservoir diagenetic facies.

On the whole (Figure 9), due to the radioactive intensity relationship of the mineral enrichment section, the clay mineral filling phase shows the characteristics of medium high gamma and gamma ray logging can be used as a basis for judging the diagenetic facies of the strata. And sonic time difference logging is less sensitive to six types of different diagenetic facies. reservoirs. Resistivity logging can assist in identifying carbonate cemented diagenetic facies and clay minerals cemented into diagenetic facies. and the results are greatly influenced by the properties of pore fluid.

Kaolinite minerals are mainly characterized by high gamma. As a result, kaolinite mineral filling phase is generally characterized by low resistivity due to the conductivity of kaolinite minerals and the sand bodies of the developmental strata of the diagenetic facies have the characteristics of medium sonic time difference. Chlorite + kaolinite intergranular pore facies reservoir due to the protective effect of chlorite film layer, more likely to retain the primary pore in the burial process, retain the primary pore is also injected to organic acid water, is also conducive to the emergence of secondary pore, expression of AC in well logging curve for sonic high the value of. The chlorite interlining + kaolinite corrosion facies reservoir has better physical properties, dissolution pores, natural gamma ray, neutron logging and reservoir density are relatively low and the difference between neutron and density and porosity is relatively small. The re-

**CONCLUSION**

(1) According to the diagenesis type and strength, diagenetic minerals and its influence on the reservoir properties, combined with interstitial material content and surface porosity, the study area of low permeability sandstone reservoirs are divided into 6 diagenetic facies which are kaolinite + chlorite cementation-intergranular pore facies, kaolinite cementation - dissolution pores + intergranular pore facies, chlorite + kaolinite cementation - dissolution pore facies, kaolinite cementation - dissolution pore facies, illite cementation - dissolution pore facies and carbonate cementation - dense phase. The pore structure becomes worse in turn and the first three are the dominant diagenetic facies of the crude oil enrichment.

(2) The experimental results of constant speed mercury pressure and the correlation analysis of experimental parameters show that there are obvious difference in micropore throat characteristics of different diagenetic facies reservoirs. The throat radius of the dominant diagenetic facies reservoir is concentrated on 0.6um-1.4um and the average value of the contribution to the permeability is 76%. Throat characteristics determine the physical
properties of reservoirs and affect the seepage channel of formation fluid. The difference between pore throat radius and pore throat radius ratio parameter is the decisive factor leading to the internal difference of microscopic pore structure.

(3) The diagenetic facies type of reservoir can be continuously divided by using the diagenetic facies logging recognition technology. The acoustic wave time difference between kaolinite + chlorite and intergranular pore is higher in the dominant diagenetic phase. The natural potential curve of kaolinite cementation - dissolution pore + intergranular pore phase is high. The cementation of chlorite + Kaolinite - dissolved pore natural gamma shows a tooth shape and the density is low. Diagenetic facies identification results and physical analysis and testing dynamic data well matched that petrographic analysis can be used as the development of low permeability sandstone reservoir is one of the methods of comprehensive evaluation and favorable reservoir development zone prediction into the logging.

ACKNOWLEDGEMENTS

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PHYTOCHEMICAL ANALYSES AND ANTIOXIDANT ACTIVITY OF A TRADITIONAL FOOD SOURCE:
DWARF NETTLE (URTICA URENS L.)

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ABSTRACT

The phytochemical composition and antioxidant capacities of the edible parts of Urtica urens, which is consumed in the East Black Sea region of Turkey, are specified. The antioxidant activity was assessed in solvents of different polarity values, which were extracted by maceration and Soxhlet apparatus methods. Antioxidant capacity of the extracts was evaluated using DPPH, Fe2+-metal chelating, superoxide anion radical scavenging, reducing power and ferric thiocyanate methods and compared to reference antioxidant standards. The levels of phenolic and flavonoid components of the various extracts were also determined by using Folin-Ciocalteu and aluminum chloride methods, respectively and by HPLC which depends on detection of various standard polyphenols in the plant. In general, the results indicate the highest antioxidant activities of the extracts were obtained by Soxhlet extraction method with polar solvents. The aerial part of the plant species is a source of nutraceuticals which have satisfactory nutrient profile and antioxidant potential.

KEYWORDS:
Urtica urens, antioxidant activity, phytochemical analyses, phenolics, flavonoids, HPLC

INTRODUCTION

The importance of plants cannot be denied in the existence of human beings from the past until today. It has been common to use plants for nutrition, healing diseases, therapeutics, preparing clothes, cosmetics, dying and so on. Plant-derived nutrients contain many bioactive components and provide numerous contributions to health. It is of particular importance to take into account the results obtained from research for a more conscious and effective use of plants.

Stinging nettle is a widely spread annual plant in nature and its small leaved species Urtica urens (U. urens) is a member of the Urticaceae family with an average size of 10 to 50 cm. It is known as the ‘dwarf nettle’ or called the ‘bird or Hatuncuk nettle’ by the local people of Turkey. It prefers to grow in wetlands with rich soil. It has spread throughout the world to South America, Europe, Africa, Asia and Australia [1].

While stinging nettle has been used for ages as a nutritious food in the world [2, 3], U. urens has a special soft taste unlike the other nettle species and is commonly consumed in the Black Sea region of Turkey as soup, salad, tea, bread, or patties, roasted or with rice.

The most common use of the nettle is for health and there is a wide range of diseases for which it is thought to provide healing. It is used as a traditional medicine mainly in the treatment of allergy, arthritis, hair loss, bladder infections, skin complaints (allergies, insect infections, eczema, acne relief, etc.), neurological disorders, detox, cancer treatment, respiratory diseases, gum inflammation, endocrine system supporter and for digestion [4].

One consequence of the natural mechanism of the living organism is the oxidation of fats. These reactions generate free radicals in the body. These highly unstable and reactive species attack whatever is in their environment, which may then cause mutations, tumors, malfunctions, etc. This process is parallel to aging and cannot be prevented completely, but may possibly be reduced. The basic function of antioxidants is to prevent chain reactions and other oxidation reactions by destroying free radicals [5]. Therefore, antioxidants are very important for health.

Polyphenols are natural chemicals found in plants. The most common forms of the polyphenols are flavonoids, phenolic acid, catechins, anthocyanins, and isoflavones. They play a powerful role in the prevention and protection of degenerative diseases, especially those like cardiovascular diseases and cancers. Polyphenolic compounds are thought to limit oxidative stress by antioxidant effect and inhibit neurodegenerative diseases by preventing DNA damage [6]. On the other hand, flavonoids are classified as plant pigments of phenolic compounds. They give the bright and vibrant colors of vegetables and fruits and are considered the most effective protectors against cancer and heart diseas-
es. They are necessary for a healthy nervous system as they inhibit the oxidation of normal cells as well as nerve cells [7, 8].

The plant U. urens, subject to this research, was collected from Karagol plateau of Giresun province, Turkey. The ecological conditions (climate, height, soil, fertility, nutrient elements, topography, etc.) of the area where the plant grows are important for its nutritive and therapeutic value [9]. There is no research in the literature on the U. urens that grows in this region or even in Turkey. However, there are in-vivo and in-vitro studies of the healing effect of the plant on some diseases and, additionally, the antinociceptive, anti-inflammatory [1, 10], antioxidant, antimicrobial [11, 12], antibacterial [13], hepatotoxicity inducing [14] and wound healing [15] properties have been demonstrated. Only a few antioxidant activity studies on a different species of the nettle (U. dioica L.) which grows in Turkey have been carried out and the high antioxidant property has been stated [16-18].

The purpose of this study is to reveal a new survey of this particular plant to scientifically justify its value and use as a food and folk medicine specifically in terms of its antioxidant activity. It is also aimed to determine the most effective way to use the plant as an antioxidant source. For this reason, the extracts prepared by maceration and Soxhlet methods with different polarity solvents were used. In this study, the antioxidant activity of the aerial part of the plant was investigated through various in-vitro methods. With all these studies, both the phenolic and the flavonoid components determination were evaluated against their gallic acid and quercetin equivalents, respectively and by comparison with the standards of HPLC as support for the determination of the antioxidant capacity.

MATERIALS AND METHODS

Plant material. The fresh leaves of the nettle were collected in July 2016, from Demirci area of Bektas-Karagol plateau (altitude: 2200 m) in Giresun, Turkey. Small-leaved nettle is grown spontaneously under natural conditions. The samples were identified by Botanist Sevda Turkis, Ph.D. (Faculty of Education, Ordu University, Ordu, Turkey). A voucher specimen (No: ESK-20412) was deposited at the Herbarium of the Department of Biology-Plant, University of Eskişehir Osmangazi/Turkey. The leaves or stem were separated from the other parts and washed and dried at room temperature. The dried leaves were ground using a coffee bean grinder to a fine powder.

Chemicals and instruments. Standards of polyphenols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), nitro blue tetrazolium chloride (NBT), phenazine methosulphate (PMS), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), 2,2-diphenyl-1-picyrylhydrazyl (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2-Amino-2-(hydroxymethyl)-1,3-propanediol (tris), resorcinol, linoleic acid, ammonium thiocyanate, (NH4SCN), ferrous and ferric chloride (FeCl2 and FeCl3) were purchased from Sigma–Chemical Co. (St Louis, MO, USA). DL-α-Tocopherol, L-(+)-ascorbic acid were from Alfa Aesar (Karlsruhe, Germany). Folin–Ciocalteu reagent, ethanol reagent grade, gradient grade hydrochloric acid (HCl, 37%), ethylenediaminetetraacetic acid disodium salt (EDTA), potassium ferricyanide (K3Fe(CN)6), trichloroacetic acid (TCA), nicotinamide adenine dinucleotide (NADH), polyoxyethylene sorbitan monolaureate (Tween 20), potassium dihydrogen phosphate (KH2PO4), sodium nitrite (NaNO2) and aluminium chloride (AlCl3) were supplied by Merck (Darmstadt, Germany). All the other chemicals used including the solvents were of analytical grade. Nuve ST30 shaking water bath (Ankara, Turkey) was used for incubation. The solutions vortexed using Velp Scientifica T24 Digital Vortex mixer with IR Sensor (Usmate, Italy) and centrifuged by MPW-351R refrigerator Laboratory Centrifuge (MPW Med. Instruments, Warsaw, Poland). The absorbance of every antioxidant assay was measured by reading at proper wavelengths using Epoch 2 Microplate spectrophotometer (BioTek, Winooski, USA). Shimadzu Prominence Modular LC20A HPLC (Tokyo, Japan) is used for the determination of flavonoids and phenolic compounds.

Preparation of the extracts. Maceration. Ten grams of dried plant samples were each macerated with 200 ml of water, methanol, methanol/water (70:30 v/v), acetone, ethyl acetate and petroleum benzine for 24 h. After filtering, 200 ml of solvents were added into remained plant material. They were stirred for 24 h again. Filtrates coming from the same solvent were joined together. Each extract was filtered using Whatman No. 1 filter paper, and concentrated under reduced pressure to dryness below 35 °C. All extracts were stored in a refrigerator at -20 °C until use. The extracts obtained are described here with the following abbreviations: maceration with water, 70% methanol: water, methanol, ethyl acetate, acetone, and petroleum benzine are WM, 70% MM, MM, EM, AM, and PM, respectively.

Soxhlet extraction. The powdered plant material (10.0 g) was extracted in a Soxhlet extraction apparatus with 200 ml of water, 70% methanol, ethyl acetate, acetone and petroleum benzine for 6 h and twice. The pooled solutions were reduced by fully evaporating at below 35 °C, and this process was repeated after the next filtrate was received.
The extracts obtained are described here with the following abbreviations: Soxhlet extraction with water, 70% methanol: water, methanol, ethyl acetate, acetone, and petroleum benzene are WS, 70% MS, MS, ES, AS and PS, respectively.

Antioxidant assays. DPPH radical scavenging activity. The DPPH radical scavenging activity of all extracts obtained by maceration and Soxhlet extraction from *U. urens* leaves was determined according to the methodology described by Brand-Williams, Cuvelier, and Berzet [19]. A preliminary study was conducted to determine the appropriate DPPH concentration to be used in the spectrophotometric measurement. DPPH concentration was specified as 0.25 mM. The ethanolic solution of 0.25 mM of DPPH was prepared immediately before the analysis and 0.5 ml of the samples, BHT and Trolox (standards) were used. Absorbance (A) values were read using a spectrophotometer at 517 nm. Ethanol was used as the blank. The control test was made with ethanol and DPPH solution. DPPH scavenging activity is expressed as a percent and IC_{50} value was calculated by plotting of graphs of the concentrations of the extracts against values of DPPH scavenging activity (%) of the extracts. The ability to scavenge DPPH radical was calculated by the following equation:

\[
DPPH \text{ radical scavenging activity (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100
\]

Superoxide anion radical scavenging activity in PMS-NADH/O_{2} system. Measurement of the superoxide anion scavenging activity of *U. urens* samples was based on the method described by Liu, Ooi, and Chang [20]. Superoxide radicals are formed in PMS-NADH/O_{2} system by the oxidation of NADH and analyzed by the reduction of NBT. In this experiment, the absorbance at 560 nm was measured against water as a blank sample. The solution contained all the reagents except the sample which was used as a control. 0.5 ml of the samples, BHT and Trolox (standards) were used in this method. The percentage inhibition of superoxide anion generation was calculated using the following formula:

\[
\text{Superoxide scavenging effect (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100
\]

Chelating activity on ferrous ions. The chelating activity of the extracts from *U. urens* leaves on ferrous ions (Fe^{2+}) was estimated by the method of Decker and Welch [21]. Ferrozine was used as the chelating reagent. The absorbance of the solution was then measured at 562 nm against the blank performed in the same way using FeCl_{2} and water. 1 ml of the samples, EDTA and Trolox (standards) were used. All tests were run in triplicate and averaged. The metal chelating activity was calculated using the following formula:

\[
\text{Metal chelating activity (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100
\]

Reducing power. The reducing power was determined according to the method of Oyaizu [22]. The absorbance of this resulting solution was measured at 700 nm. Control was prepared similarly excluding samples. The blank sample consisted of TCA, FeCl_{3}, and water. 1.5 ml of the samples, EDTA and gallic acid (standards) were used.

Ferric thiocyanate method (FTC). The antioxidant activities of plant extracts were determined according to the FTC method [23]. The peroxide level was determined by reading the absorbance of the resulting red color at 500 nm. The last steps were repeated every 6 h until a day after the absorbance of the control reached its maximum value. 0.2 ml of the samples, BHT, resorcinol and ascorbic acid (standards) were used. The control was subjected to the same procedure as the sample. Solution without samples was used as a blank. The percentage inhibition of lipid peroxidation in the linoleic acid emulsion has calculated as follows:

\[
\text{Inhibition (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100
\]

Phytochemical assays. HPLC analysis. Determination of flavonoid and phenolic compounds in 12 different *U. urens* extracts were analyzed by HPLC method which is used for qualitative and quantitative results. The reversed phase-high performance liquid chromatography (RP-HPLC) system consisted of Shimadzu-LC20A prominence modular chromatography system with a photodiode array detector. HPLC methods were optimized to obtain the reliability of peaks. The 250 x 4.6 mm i.d., 5 μm C18 column was used and the flow rate was 0.7 ml/min, and the injection volume was 50 μl and the column temperature was set at 25 °C. Eventually, the mixture of acetic acid-water (0.1%) and acetonitrile-B in different combinations were applied as the mobile phase to enhance the resolution. The eluent started at 90% A in 26 min, at 60% A in 11 min, at 40% A in 11 min, at 90% A in 5 min, at 10% A in 5 min, and then held for 10 min with 90% A and 10% B. The quantification was made by UV-Vis absorbance photodiode-array detector (DAD) at 280 nm.

Each compound was identified by comparison with related standards. The retention time of gallic acid, caffeic acid, myricetin, p-coumaric acid, trans-ferulic acid, quercitin, kaempferol and formononetin were measured at 280 nm, which were 8.98 ± 0.40; 17.19 ± 0.50; 20.61 ± 0.50; 22.13 ± 0.60; 23.21 ± 0.50; 32.65 ± 0.60; 37.25 ± 0.50; 39.94 ± 0.70 min, respectively. Each compound was identified by their retention time and their spectral characteristics. The measurement of the peak area of each sample was determined and contents were
calculated via calibration curves of the standards. The sample and standard were filtered with 0.45 μm polитетrafluорэтилен (PTFE) before HPLC. Standards were prepared in six different concentrations, which were 3.12, 6.25, 12.50, 25.0, 50.0, and 100.0 ppm to prepare their calibration curves.

**Determination of total phenolic content (TPC).** The amount of total phenolics in extracts was determined with the method of Slinkard and Singleton using Folin-Ciocalteu reagent [24]. Gallic acid was used as standard and determination of TPC was verified by comparing to the calibration curve of gallic acid was drawn (y = 0.0226x + 0.0119; r^2=0.9967). 1 mg/ml of aliquots of the extracts were used. The absorbance of the resultant blue colored supernatant was recorded after 2 h at 760 nm using a solution without plant extract as control. The results were compared to the gallic acid calibration curve. The amount of TPC in each extract was calculated as mg of gallic acid equivalents per g of extract from the calibration curve of gallic acid standard solution.

**Determination of total flavonoid content (TFC).** The TFC of *U. urens* was determined with quercetin standard solution using the method of Park et al [25]. Quercetin was used as standard and determination of TFC was verified by comparing to the calibration curve of quercetin (y = 0.022x + 0.0176; r^2=0.9967). 1 mg/ml of aliquots of the extracts were used. The blank was performed using solution excluded plant extract. The absorbance was measured at 510 nm with a spectrophotometer and compared to the quercetin calibration curve. The total flavonoids were described as mg quercetin/g extract.

**Statistical analysis.** All data were expressed in the form of mean ± standard deviation (SD), determined for three replicates. Estimation of the significant differences between groups was determined by One Way analysis of variance (ANOVA) at the significance level (α) of 0.05 followed by Tamhane’s T2 test (the test of homogeneity of variance significance level result: p < 0.05) using SPSS version 18.0 (SPSS Inc., USA).

**RESULTS AND DISCUSSION**

To carry out the antioxidant activity tests and phytochemical analyses of the nettle plant of the species (*U. urens*) from Giresun, Turkey, we prepared the samples by extracting the dried and ground plant material. The aerial part of the plant was macerated at room temperature or refluxed in a Soxhlet extractor with different solvents (water, methanol, methanol/water (70:30 v/v), acetone, ethyl acetate, and petroleum benzine).

**Antioxidant assays.** Antioxidant activity determination can be achieved through various in-vitro methods which have been applied with different names in the literature. It is essential to carry out more than several assays of the samples to get accurate and reliable results. In this work, five antioxidant activity determination tests: DPPH free radical scavenging, ferrous ion chelating, total reducing power, superoxide anion (O2−) radical scavenging, and total antioxidant capacity determination with ferric thiocyanate procedures were applied to the maceration and Soxhlet extraction samples of the plant material in a dose dependent manner: (25, 50, 100, 200 and 400 μg/ml).

**DPPH radical scavenging activity.** Among the free radical scavenging activity methods, the DPPH assay comes to the forefront with its fast, easy to apply, reliable, economical and feasible properties [26]. This method is based on the principle that the DPPH free radical is neutralized by catching hydrogen radical or coupling an electron on its nitrogen atom by transferring an electron from an antioxidant molecule. In the presence of antioxidant structures, the deep purple color originating from the DPPH radical is lightened and the concentration of the reduced DPPH is detected spectrophotometrically by measuring its absorption at 517 nm [27]. According to the results, it has been concluded that all the extracts have from moderate to high free radical scavenging capacity when compared with known antioxidant standards: BHT and Trolox (Figure 1). The maceration extracts have a higher antioxidative effect than the Soxhlet extraction samples and among the maceration samples, 70% MM and MM samples (69.41 ± 1.51%, 72.55 ± 0.93% at 50 μg/ml, respectively) have the highest activity. This result is in agreement with the reports that include the antioxidant work on North African [28] and Indian [11] plant materials in different solvents. Comparatively, 70% MS and MS (66.94 ± 1.03%, 54.28 ± 3.0% at 50 μg/ml, respectively) extracts appeared with an activity above the average. Further, the IC50 values which point out the concentration of sample required to scavenge 50% DPPH free radicals were calculated. The lower the IC50 values, the greater the antioxidant activity. When we consider a comparison between *U. urens* extracts obtained in different solvents using various extraction methods, the best results were obtained for 70% MM and MM samples (11.49 ± 1.51 μg/ml and 12.95 ± 0.92 μg/ml, respectively). Comparatively, 70% MS and MS preparations have IC50 values of 32.65 ± 1.03 μg/ml and 31.63 ± 3.0 μg/ml, respectively.

In this study, it is possible to make a correlation between DPPH radical scavenging activities.
with each of the following methods: total antioxidant capacity determined by ferric thiocyanate method, superoxide radical scavenging activity, and total flavonoid content determination tests. The correlation coefficients ($r^2$) are 0.8612, 0.8153 and 0.8893, respectively, an indication that the antioxidant activity determined in these systems was similar to each other and that the flavonoid compounds present in the samples are responsible for the antioxidant activity.

**Superoxide anion radical scavenging activity in PMS-NADH/O$_2$ system.** The reduction of molecular oxygen by taking one electron forms the radical form of superoxide (O$_2^-$). The superoxide produced in the organism in cellular conditions can act both as an oxidant and as a reducing agent. The importance of this radical is because it is a source of hydrogen peroxide (H$_2$O$_2$) and can reduce transition metal ions. H$_2$O$_2$ is considered to be a reactive oxygen species even though it is not a radical. It can turn into the most reactive and more damaging hydroxyl radical in the presence of transition metal ions or superoxide radicals through Fenton and Haber-Weiss reactions [29]. The principle involved in superoxide radical scavenging assay is the conversion of NBT into NBT-diformazan via superoxide radical which is generated by the PMS-NADH/O$_2$ system. It is expected that the reduction of NBT via superoxide will be inhibited in the presence of compounds with superoxide radical scavenging activity. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity [30]. In this study, superoxide anion radical scavenging activity of herbal extracts was determined in a dose dependent manner (25, 50, 100, 200 and 400 µg/ml) and compared with BHT and Trolox as positive controls. The results indicate that PM, 70% MS and ES (84.77 ± 3.94%, 73.83 ± 3.0%, and 73.21 ± 2.27%, respectively at 50 µg/ml) are the most active extracts, suggesting that they could be destructive against active oxygen species. Comparatively, AM, MS, PS and AS extracts (77.25 ± 1.93%, 74.30 ± 1.26%, 66.08 ± 1.98% and 61.25 ± 1.23%, respectively at 50 µg/ml) are highly active in scavenging superoxide radicals and their activity decreased with increasing concentrations (Figure 1). In the literature, no studies have been found using this method to determine the antioxidant activity of *U. urens*. However, Mandal, Misra, Singh, Das, and Bhunia [31] found an NBT-NADH-PMS based superoxide radical scavenging activity of 53.3% in the acetone: ethanol (1:1) fraction for the *U. dioica* species.

There is a high correlation ($r^2$: 0.8153) between the superoxide and DPPH radical scavenging methods. This correlation implies that the scavenging abilities of the extract solutions of the superoxide and DPPH radicals have a commonality in exhibiting the high antioxidative property of the plant. Following superoxide radical scavenging assay, regression analysis shows that flavonoid compounds contribute to about 72% ($r^2$: 0.7209) of superoxide radical scavenging properties in the nettle.

**Chelating activity on ferrous ions.** Iron is another factor that is responsible for oxidative damage via free radical formation in the organism. Therefore, ferrous ion chelating activity assay is a complementary procedure to detect the antioxidant activity of the samples. Here, the capacity of the samples to compete with ferrozine, which is a ferrous ion-chelating agent, is determined. Percent formation inhibition of ferrozine-Fe$^{2+}$ complex was measured at 562 nm wavelength across positive controls: EDTA and Trolox. A lower absorbance indicates a higher chelating power. Metal chelating ability for the samples of PS, MS, WS and 70% MS (87.37 ± 0.76%, 77.39 ± 1.11%, 77.19 ± 1.05% and 70.46 ± 9.22% at 100 µg/ml, respectively) was determined to be almost as high as the standard antioxidant molecules, suggesting that they inhibited the formation of the ferrozine-Fe$^{2+}$ complex. Surprisingly, the petroleum benzine extract, which is the most apolar solvent used for extraction, has a high metal chelating ability. There is no significant difference ($p > 0.05$) between the preparations of
Soxhlet extraction and the positive controls: EDTA and Trolox (79.44 ± 0.06% and 74.85 ± 1.97%, respectively), (Figure 2). To the best of our knowledge, there is no publication in the literature covering the determination of the antioxidant power of U. urens using this method. However, similar to this work, Gulcin, Kufreviouglu, Oktay, and Buyukkokuroglu [16] reported high ferrous ion capturing activity for the water extract of the U. dioica species of the nettle.

Reducing power. The total reduction power of the herbal extracts is due to some of the reducing compounds they contain. The reducing power method complements other antioxidant activity determination tests and gives information mainly about the antioxidative capacity of phenols [32]. These structures terminate chain reactions caused by free radicals by giving hydrogen radical. The antioxidant substances with this ability react with K3Fe(CN)6 to form K4Fe(CN)6, which forms Fe(CN)6 3- (ferric ferrocyanide) in the presence of FeCl3. The compound with Prussian blue color gives maximum absorbance at a wavelength of 700 nm. Therefore, the concentration of the substance is determined by measuring the absorbance. Increased absorbance of the test mixtures shows an increase in reducing power. Figure 2 indicates the dose-response bars for the reducing power of the suppressions with the standard antioxidant compounds: Gallic acid and EDTA as positive controls. Almost all the extracts have moderate activity when compared with the controls. Among the extracts, WS and PM have slightly higher activity (1.76 ± 0.06, 1.67 ± 0.01 at 50 μg/ml, respectively) whereas EDTA and gallic acid have an activity of 1.85 ± 0.04, 1.12 ± 0.21 at 50 μg/ml, respectively compared with the other preparations. The samples of MM, 70% MM and AM (2.21 ± 0.20, 1.99 ± 0.02 and 1.98 ± 0.01 at 50 μg/ml, respectively) have similar reducing power to the Fe3+ ion (p > 0.05). There are only a few reports on ferric reducing ability assay for U. urens but there are many for the U. dioica species in the literature. Manu Kumar et al. [11] reported the highest ferrous reducing antioxidant power for the most apolar solvent among the others, namely: acetone, whereas the other extracts had antioxidant activity in the following order: water < ethanol < methanol. Kukric et al. [33] found weak antioxidant activity compared to the control antioxidants where the ethanolic extract showed an activity of about half that of BHT.

![Figure 2](image2.png)

**FIGURE 2**
(a) Reducing and (b) Metal chelating activity tests results of the samples, p < 0.05 (n: 3)

![Figure 3](image3.png)

**FIGURE 3**
Absorbance of the peroxide value against time (hour), p < 0.05 (n: 3)
TABLE 1

Inhibition power (% of samples against lipid peroxidation at 20th hour during incubation

<table>
<thead>
<tr>
<th>Exports</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
</tr>
<tr>
<td></td>
<td>400.0</td>
</tr>
<tr>
<td>WM</td>
<td>76.94 ± 0.048</td>
</tr>
<tr>
<td>70% MM</td>
<td>56.14 ± 0.053</td>
</tr>
<tr>
<td>MM</td>
<td>23.30 ± 0.099</td>
</tr>
<tr>
<td>EM</td>
<td>17.54 ± 0.017</td>
</tr>
<tr>
<td>AM</td>
<td>12.03 ± 0.105</td>
</tr>
<tr>
<td>PM</td>
<td>43.10 ± 0.099</td>
</tr>
<tr>
<td>WS</td>
<td>77.94 ± 0.036</td>
</tr>
<tr>
<td>70% MS</td>
<td>63.65 ± 0.065</td>
</tr>
<tr>
<td>MS</td>
<td>80.20 ± 0.028</td>
</tr>
<tr>
<td>ES</td>
<td>62.15 ± 0.041</td>
</tr>
<tr>
<td>AS</td>
<td>53.38 ± 0.109</td>
</tr>
<tr>
<td>PS</td>
<td>67.41 ± 0.003*a</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>37.59 ± 0.0b</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>33.08 ± 0.0b</td>
</tr>
<tr>
<td>BHT</td>
<td>24.56 ± 0.0b</td>
</tr>
</tbody>
</table>

(6.0, 6) Values are means ± SD (n: 3); the results with different letters in the same column are significantly different.

A good correlation coefficient (r²) between the reducing power and total phenolic content determination methods is observed as 0.7219. In this correlation, one parameter decreases while the other increases and implies with high probability that the compounds present in the extracts have a strong ability to reduce heavy metals suggesting that they could also prevent some of the metal catalyzed oxidation pathways such as Fenton and Haber-Weiss reactions. This result is in agreement with some reports [34, 35].

Total antioxidant capacity determination by ferric thiocyanate method (FTC). The total antioxidant activity determination test carried out by the FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The lipid peroxidation that occurs in an organism is an important matter, which can affect cell structure and thus its permeability. Here, the inhibitory effect of the extracts on linoleic acid peroxidation was examined. The resulting peroxides oxidize the ferrous ions (Fe²⁺) to the ferric ions (Fe³⁺), which form the ferric thiocyanate complex. This complex gives maximum absorbance at a wavelength of 500 nm. The high absorbance values observed correspond to the high peroxide amount.

In the graph below (Figure 3), it is seen that the 20th hour is the maximum of lipid peroxidation during incubation for the control solution of the assay with some U. urense extracts and positive controls (BHT, resorcinol and ascorbic acid). In Table 1, the values for inhibiting the lipid peroxidation are shown for all concentrations of the herbal extracts run at the 20th hour. The results indicate the higher peroxide inhibition potential of the Soxhlet preparations compared with the maceration preparations and positive controls. Among the extracts MS and 70% MS have the highest activity (80.20 ± 0.02% and 63.65 ± 0.06%, respectively at 50 µg/ml) while AM has the least activity (12.03 ± 0.10% at 50 µg/ml) against lipid peroxidation. PS and ES preparations have an activity of 67.41 ± 0.0% and 62.15 ± 0.04%, respectively at 50 µg/ml which are near to the highest values. As far as we know, no publications are covering the determination of the antioxidant activity of the U. urense species of nettle in this way. Some studies have found significant values for the inhibition of lipid peroxidation in linoleic acid emulsion with 86.72%, and 76% for U. pilulifera and U. dioica species, respectively [36, 37]. As in our study, the values found for the extracts were higher than for the standard antioxidants.

Phytochemical analyses. Total phenolic and flavonoid content determination as gallic acid and quercetin equivalents. The total phenolic content determination method is based on the electron transfer of the phenolic and other reducing compounds in the sample to molybdenum in the Folin–Ciocalteu reagent. The corresponding blue colored complex is identified at a wavelength of 760 nm. The results were calculated according to the regression equation of the calibration curve (y=0.0226x+ 0.0119; r²=0.9967) and are given as mg gallic acid equivalent in 1 gram extract. It was observed that extracts prepared from polar solvents by the Soxhlet extraction method have richer phenolic content. 70% MS, WM, and WS samples are in the first three places, respectively with a phenolic compound content of 32.41 ± 0.07 mg/g, 31.75 ± 0.20 mg/g and 31.51 ± 0.01 mg/g, respectively whereas Soxhlet extraction samples of methanol (MS) and ethyl acetate (ES) samples have similarly high values of 30.77 ± 0.14 and 30.32 ± 0.48 mg/g, respectively as shown in Table 2.

Total flavonoid content of the herbal extracts was determined by using AlCl₃ colorimetric method which is based on the principle of acid stable com-
plexation between chloride and the C₄ keto-,
with the C₅ or C₆ hydroxyl- groups of flavones and
flavonoids [38]. Quercetin is a suitable standard for
the determination of the total flavonoid compounds
in the plants. The content of flavonoids was deter-
mined by the regression equation of the calibration
curve of quercetin (y= 0.022x+0.0176, r²=0.9967)
and expressed in quercetin equivalents. As can be
seen from Table 2, the results vary from 263.62 ±
1.27 to 616.12 ± 5.74 (mg quercetin/g extract). The
majority of the extracts which were prepared with
polar solvents have a higher amount of flavonoid
content. Maceration extract with ethyl acetate (EM)
of the nettle has the highest flavonoid content
(616.12 ± 5.74 mg quercetin equivalent/g extract),
which is a significantly different value among the
extracts (p < 0.05). The following samples have a
value of more than 300.0 mg quercetin equivalent/g
extract in descending order (Table 2): MS > ES >
WM > AM > MM.

The results are in agreement with the work of
Maaroufi et al. [28] in which they reported that
among the different extracts, methanol has the
highest phenolic compounds. Further, the report of
Manu Kumar et al. [11] showed the high polyphen-
olic and flavonoid content for U. urens.

Total phenolic and flavonoid content
determination by comparison with the standards
of HPLC. The structures of polyphenols are classified
two classes as phenolic and flavonoid types for
plants. HPLC analysis was carried out to quantify
the selected groups of hydroxycinnamic acid, hydro-
xybenzoic acid, flavonol, and isoflavone. The
retention time determination of quercetin, gallic
acid, formononetin, kaempferol, myricetin, caffeic
acid, p-coumaric acid, and trans-ferulic acid were
found with the reverse phase-HPLC by density
gradient method via DAD detector. Phenolic acid
derivatives were located at 9-23 min and flavonoids
at 30-41 min (Figure 4).

<table>
<thead>
<tr>
<th>Extract</th>
<th>EY (%)</th>
<th>3TFC (mg/g)</th>
<th>3TPC (mg/g)</th>
<th>3IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>20.40</td>
<td>348.25 ± 0.655e</td>
<td>31.75 ± 0.201b</td>
<td>102.0 ± 0.973b</td>
</tr>
<tr>
<td>70% MM</td>
<td>20.35</td>
<td>298.09 ± 0.904c</td>
<td>29.51 ± 0.012a</td>
<td>11.49 ± 1.511c</td>
</tr>
<tr>
<td>MM</td>
<td>14.79</td>
<td>399.79 ± 3.544bc</td>
<td>27.33 ± 0.024b</td>
<td>12.95 ± 0.927c</td>
</tr>
<tr>
<td>EM</td>
<td>4.54</td>
<td>616.12 ± 5.746a</td>
<td>27.37 ± 0.009b, i</td>
<td>48.91 ± 0.862b</td>
</tr>
<tr>
<td>AM</td>
<td>4.39</td>
<td>372.12 ± 1.637</td>
<td>27.52 ± 0.026</td>
<td>150.34 ± 1.201c</td>
</tr>
<tr>
<td>PM</td>
<td>2.99</td>
<td>266.99 ± 1.518b</td>
<td>25.79 ± 0.196b, t, i</td>
<td>57.29 ± 7.492b</td>
</tr>
<tr>
<td>WS</td>
<td>13.16</td>
<td>272.57 ± 0.266</td>
<td>31.51 ± 0.012b, c, i</td>
<td>255.86 ± 4.115f</td>
</tr>
<tr>
<td>70% MS</td>
<td>15.55</td>
<td>272.31 ± 0.718c</td>
<td>32.41 ± 0.076</td>
<td>32.65 ± 1.037c</td>
</tr>
<tr>
<td>MS</td>
<td>15.74</td>
<td>308.38 ± 0.636c</td>
<td>30.77 ± 0.146c</td>
<td>31.63 ± 3.001c</td>
</tr>
<tr>
<td>ES</td>
<td>3.93</td>
<td>315.39 ± 0.300c</td>
<td>30.32 ± 0.486c, t, i</td>
<td>252.35 ± 4.535c</td>
</tr>
<tr>
<td>AS</td>
<td>2.89</td>
<td>283.70 ± 0.332c</td>
<td>29.75 ± 0.287b, t, i</td>
<td>205.02 ± 4.275b</td>
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<tr>
<td>PS</td>
<td>2.77</td>
<td>263.82 ± 1.276c</td>
<td>27.33 ± 0.024b, t, i</td>
<td>303.99 ± 2.992c</td>
</tr>
</tbody>
</table>

(1) mg quercetin/g extract; (2) mg gallic acid/g extract; (3) calculated from DPPH scavenging assay; values are
means ± SD (n: 3); the results with different letters in the same column are significantly different: p < 0.05

FIGURE 4
Chromatogram of the external standards (λ = 280 nm). (1) Gallic acid, (2) Caffeic acid, (3) Myricetin,
(4) Kaempferol, (5) Formononetin, (6) Quercetin, (7) p-Coumaric acid and (8) trans-Ferulic acid

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Chromatograms ($\lambda = 280$ nm) of the a) EM and b) MS samples of *U. urens*, top to bottom respectively.

All the extracts of *U. urens* were evaluated and, as a result, they generated qualitative and quantitative differences according to the extraction methods used (Table 3). The chromatograms of EM and MS samples can be seen below as representative examples (Figure 5). HPLC results showed that the extracts of *U. urens* contained a significant amount of quercetin, gallic acid, caffeic acid, myricetin, formononetin and kaempferol among the standards. The highest amount of the phenolic and flavonoid content was detected in Soxhlet extracts showing high similarity with the literature [39]. As an example, the kaempferol amount was 19.04 ± 0.39 in MS extract but indicated a tendency to decrease for the MM extract (12.76 ± 0.14). Although both *U. dioica* and *U. urens* species are members of the Urticaceae family, the polyphenolic contents of the plants are not related. For instance, gallic acid and kaempferol are the main phenolics and flavonoid compounds, respectively in *U. dioica*, while caffeic acid and myricetin are the main phenolic and flavonoid compounds, respectively in *U. urens* with the considerable amount [40]. Some works have shown the lower polyphenolic content of *U. urens* than *U. dioica* [41]. Furthermore, in a research study [10], the amount of myricetin in *U. urens* methanolic extract (19.72%) showed high similarity with the present work (21.98% ± 0.73). As expected, the flavonol amount found was higher than the isoflavone and phenolic content. The solvent variety also affected the amount of polyphenol content for the nettle extracts. The best solvents to extract the polyphenol content were WM, WS, MS, AS and 70% MS. As for the amount of gallic acid as the selected phenolic compound in the samples,
### TABLE 3

Total phenolic and flavonoid content of *U. uren*

<table>
<thead>
<tr>
<th>Standards*</th>
<th>WM 70% MM</th>
<th>MM</th>
<th>EM</th>
<th>AM</th>
<th>PM</th>
<th>WS 70% MS</th>
<th>MS</th>
<th>ES</th>
<th>AS</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>± 0.127</td>
<td>± 0.089</td>
<td>± 0.09</td>
<td>± 0.265</td>
<td>± 0.278</td>
<td>± 0.517</td>
<td>± 0.682</td>
<td>± 0.104</td>
<td>± 0.315</td>
<td>± 0.247</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>± 0.358</td>
<td>± 6.00</td>
<td>± 12.65</td>
<td>± 0.065</td>
<td>± BDL</td>
<td>BDL</td>
<td>± BDL</td>
<td>± 18.48</td>
<td>± 18.81</td>
<td>± 18.652</td>
</tr>
<tr>
<td></td>
<td>31.66</td>
<td>3.27</td>
<td>3.85</td>
<td>2.55</td>
<td>2.75</td>
<td>2.79</td>
<td>15.98</td>
<td>6.63</td>
<td>24.98</td>
<td>3.80</td>
</tr>
<tr>
<td>Myricetin</td>
<td>± 0.1125</td>
<td>0.451</td>
<td>0.0365</td>
<td>± 0.188</td>
<td>± 0.505</td>
<td>± 0.978</td>
<td>± 0.449</td>
<td>± 0.728</td>
<td>± 0.407</td>
<td>± 0.401</td>
</tr>
<tr>
<td></td>
<td>9.39</td>
<td>12.76</td>
<td>2.97</td>
<td>± 3.32</td>
<td>± 11.36</td>
<td>± 2.75</td>
<td>19.04</td>
<td>± 0.223</td>
<td>± 0.393</td>
<td>± 0.281</td>
</tr>
<tr>
<td>Kaemperol</td>
<td>± 0.672</td>
<td>0.281</td>
<td>0.145</td>
<td>0.446</td>
<td>± 0.054</td>
<td>± 0.142</td>
<td>± 0.608</td>
<td>± 0.223</td>
<td>± 0.393</td>
<td>± 0.281</td>
</tr>
<tr>
<td>Formononetin</td>
<td>± 0.3384</td>
<td>± 7.38</td>
<td>± BDL</td>
<td>5.49</td>
<td>± BDL</td>
<td>4.59</td>
<td>± 14.84</td>
<td>± 18.65</td>
<td>± 7.56</td>
<td>± 12.11</td>
</tr>
<tr>
<td></td>
<td>1.039</td>
<td>± 0.401</td>
<td>± BDL</td>
<td>0.423</td>
<td>± BDL</td>
<td>0.392</td>
<td>± 0.657</td>
<td>± 0.496</td>
<td>± 0.413</td>
<td>± 0.609</td>
</tr>
<tr>
<td>Quercetin</td>
<td>± 1.278</td>
<td>13.23</td>
<td>19.12</td>
<td>8.46</td>
<td>± 13.57</td>
<td>± 10.96</td>
<td>58.08</td>
<td>± 38.48</td>
<td>28.46</td>
<td>8.66</td>
</tr>
<tr>
<td></td>
<td>± 0.522</td>
<td>± 1.92</td>
<td>± 1.459</td>
<td>± 0.894</td>
<td>± 1.447</td>
<td>± 1.105</td>
<td>± 1.381</td>
<td>± 1.553</td>
<td>± 0.220</td>
<td>± 1.734</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>ND</td>
<td>ND</td>
<td>0.902</td>
<td>± 0.00</td>
<td>± 0.018</td>
<td>± 0.021</td>
<td>± 0.33</td>
<td>± 0.225</td>
<td>± 0.027</td>
<td>ND</td>
</tr>
<tr>
<td>trans-Ferulic acid</td>
<td>ND</td>
<td>ND</td>
<td>2.75</td>
<td>3.88</td>
<td>3.83</td>
<td>0.060</td>
<td>0.050</td>
<td>4.05</td>
<td>3.84</td>
<td>0.028</td>
</tr>
<tr>
<td>Total Polyphenol</td>
<td>150.56</td>
<td>44.19</td>
<td>56.58</td>
<td>55.81</td>
<td>41.62</td>
<td>22.60</td>
<td>125.51</td>
<td>95.31</td>
<td>124.63</td>
<td>45.43</td>
</tr>
</tbody>
</table>

*The results are mg standard/g extract; values are means ± SD (n: 3), p < 0.05 (please see supplementary file for significant differences); ND: not detected; BDL: found below the method’s detection limit.*

EM extract showed the highest value of 24.63 ± 0.27. Furthermore, the amount of gallic acid content for the most apolar solvent extraction preparations of PS and PM were 8.25 ± 0.06 and 5.53 ± 0.52, respectively. The detected flavonol groups yielded from 2.52 ± 0.28 to 58.08 ± 1.11 (kaempferol in 70% MM extract, quercetin in WS extract, respectively). In all of these, the HPLC results of the detected quercetin amount showed that the WM and WS extracts outperformed the other extracts, but the values for the 70% MS and AS extracts were close to the WM and WS extracts. The highest extraction yield of phenolic and flavonoid content as gallic acid equivalent and quercetin equivalent were obtained for the EM and WS extract as 24.63 ± 0.27 and 58.08 ± 1.11, respectively.

**CONCLUSION**

Antioxidant activity for a particular type of nettle plant *U. uren*, which is consumed for therapeutic purposes and is an easily accessible nutrient in both Turkey and the world, has been evaluated by various tests. Besides, the phenolic and flavonoid contents of the plant, as the active phytochemical components, have been identified and these are important in terms of the nutritional value of the plant along with their therapeutic properties. For a better understanding of solvent polarity and extraction methods used in the antioxidant activity and total phenolic and flavonoid content, we selected six solvents with different polarity values and two different extraction methods including maceration and Soxhlet extraction.

From the antioxidant activity tests conducted, it was observed that the extracts obtained with maceration were more active than those obtained with Soxhlet extraction procedures according to DPPH radical scavenging method. However, Soxhlet extracts had in higher antioxidant activity than maceration ones, according to the results of metal chelating, superoxide anion scavenging and total antioxidant activity determination with ferric thiocyanate methods. The superoxide anion scavenging test could be correlated with the DPPH method because of their similar action mechanisms revealed that one of the most active extracts is the same as that determined by the DPPH test, which is the 70% MS sample prepared with 70% methanol: water by using Soxhlet apparatus. But, in general, it is observed that the activity of the samples increases with the increasing polarity of the solvents prepared and by using the Soxhlet extraction method.

Apart from the extraction samples of water (WM, WS, 70% MM and 70% MS), the total phenolic and flavonoid content amount of the extracts which were obtained by the Soxhlet apparatus were higher than the maceration preparations. When we evaluated the total amount of polyphenols, WM has the first, and WS has the second, highest amount. As for the effect of solvent polarity on total polyphenolic content, PM and PS samples remained behind AS, AM and also ES and EM extracts.
These results are in agreement with the results of total phenolic and flavonoid content determination assays of this research.

Based on the background above, the results indicate that the edible part of *U. urens* of the Black Sea region, Turkey is a rich source of phenolic and flavonoid compounds. As a result, it has been revealed that the nutrients of this plant can be consumed in different forms, and with its alternative medicinal purposes, is an important antioxidant that can be effective in preventing diseases caused by free radicals. However, further scientific work is required to justify traditional uses of the plant precisely, and this work could stimulate new research in this area.

**SUPPLEMENTARY FILE**

Plant material. Herbarium record.

*Collection location of *Urtica urens* L. according to ‘Google Earth’ application is 40°35’7.00N (Latitude); 38°8’5.20E (Longitude).*

**FIGURE S1**

Herbarium record of the plant*
Nettle as a food source.

**FIGURE S2**
Nettle soup, nettle tea and spicy nettle paste, left to right respectively
*The photos were taken by E. Bagdallı in August, 2017 at Ordu-Turkey

The Polyphenol standards.

**FIGURE S3**
The polyphenols used as standards
Calibration curves of the polyphenol standards.

**FIGURE S4**
The polyphenol's standards calibration curves (a) Gallic acid, (b) Caffeic acid, (c) Myricetin, (d) p-Coumaric acid, (e) trans-Ferulic acid, (f) Quercetin, (g) Kaempferol, (h) Formononetin
HPLC Chromatograms of the single phenolic and flavonoid standards.

![HPLC Chromatograms](image)

**FIGURE S5**
HPLC chromatograms of the single phenolic and flavonoid standards (λ: 280 nm). (1) Gallic acid, (2) Caffeic acid, (3) Myricetin, (4) Kaempferol, (5) Formononetin, (6) Quercetin, (7) p-Coumaric acid and (8) trans-Ferulic acid

Linear equations of the standards.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Myricetin (y)</th>
<th>Kaempferol (y)</th>
<th>Formononetin (y)</th>
<th>Quercetin (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Equations</td>
<td>y=18841x + 275.05</td>
<td>y=36942x + 1016</td>
<td>y=52749x + 36532</td>
<td>y=31397x + 35828</td>
</tr>
<tr>
<td>r²</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.99</td>
<td>0.9999</td>
</tr>
<tr>
<td>tₙ</td>
<td>20.612 ± 0.5</td>
<td>37.250 ± 0.5</td>
<td>39.538 ± 0.7</td>
<td>32.649 ± 0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Entry</th>
<th>Gallic acid (y)</th>
<th>Caffeic acid (y)</th>
<th>p-Coumaric acid (y)</th>
<th>trans-Ferulic acid (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Equations</td>
<td>y=58509x – 870.36</td>
<td>y=179217x + 69026</td>
<td>y=112740x + 68545</td>
<td>y=69324x + 41935</td>
</tr>
<tr>
<td>r²</td>
<td>1.0000</td>
<td>0.9979</td>
<td>0.9998</td>
<td>0.9998</td>
</tr>
<tr>
<td>tₙ</td>
<td>8.975 ± 0.4</td>
<td>17.193 ± 0.5</td>
<td>22.125 ± 0.6</td>
<td>23.211 ± 0.5</td>
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</tbody>
</table>
Correlation graphs between antioxidant activity determination methods at 50 μg/ml concentration of the extracts. The correlation between total antioxidant activity determinations with ferric thiocyanate and DPPH scavenging activity methods.

![Correlation graph between total antioxidant activity determination – DPPH scavenging activity methods](image)

The correlation between total flavonoid content determinations and DPPH scavenging activity methods.

![Correlation graph between Total flavonoid content determination – DPPH scavenging activity methods](image)

The correlation between superoxide radical scavenging activity and DPPH scavenging activity methods.

![Correlation graph between Superoxide radical scavenging activity – DPPH scavenging activity methods](image)
The correlation between superoxide radical scavenging activity and total flavonoid content determination methods.

![Graph](image1)

**FIGURE S9**
The correlation graph between Superoxide radical scavenging activity – Total flavonoid content determination methods

The correlation between total phenolic content and reducing activity methods

![Graph](image2)

**FIGURE S10**
The correlation graph between Total phenolic content – Reducing activity methods

Statistical correlations for ‘Total phenolic and total flavonoid content determination by comparison with the standards of HPLC’.

<table>
<thead>
<tr>
<th>Standards*</th>
<th>WM (a)</th>
<th>70% MM (b)</th>
<th>MM (c)</th>
<th>EM (d)</th>
<th>AM (e)</th>
<th>PM (f)</th>
<th>WS (g)</th>
<th>70% MS (b)</th>
<th>MS (i)</th>
<th>ES (j)</th>
<th>AS (k)</th>
<th>PS (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td></td>
<td>f, g, i</td>
<td>f</td>
<td></td>
<td></td>
<td>b, c, l</td>
<td>a-c, h, k</td>
<td>j, k</td>
<td>g, h, k</td>
<td>g, h, j</td>
<td>f, i</td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>h</td>
<td>d</td>
<td>i, j, l</td>
<td>b</td>
<td>-</td>
<td>-</td>
<td>h, k</td>
<td>a, g, k</td>
<td>c, j, l</td>
<td>c, i, l</td>
<td>g, h</td>
<td>c, i, j</td>
</tr>
<tr>
<td>Myricetin</td>
<td>h</td>
<td>d</td>
<td>i, j, l</td>
<td>b</td>
<td>-</td>
<td>-</td>
<td>a, k</td>
<td>a, g, k</td>
<td>c, j, l</td>
<td>c, i, l</td>
<td>g, h</td>
<td>c, i, j</td>
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<tr>
<td>Kaempferol</td>
<td>g, i</td>
<td>j</td>
<td>c-f, h</td>
<td>b, d, e, f, h</td>
<td>b, c, e, f,h</td>
<td>b-d, f, h</td>
<td>b-e, h</td>
<td>a, h, k</td>
<td>b-f</td>
<td>a, g, j</td>
<td>a, i</td>
<td>g</td>
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<td>b, g, j</td>
<td>-</td>
<td>d</td>
<td>k</td>
<td>b, j</td>
<td>b, d, i</td>
<td>h</td>
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<tr>
<td>Quercetine</td>
<td>i</td>
<td>c, c, f</td>
<td>b, c, i</td>
<td>f, j</td>
<td>b, c, f</td>
<td>b, d, e, j, l</td>
<td>-</td>
<td>k</td>
<td>A</td>
<td>d-f</td>
<td>h</td>
<td>c, e, f</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td></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>trans-Ferulic acid</td>
<td></td>
<td>-</td>
<td>i, d</td>
<td>b, i</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>b, d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*An example as explanation: the difference between the results of WM (a) and WS (g) is statistically significant when compared with gallic acid, *p* < 0.05 (n: 3)
ACKNOWLEDGEMENTS

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NEMATICIDAL POTENTIAL OF INDIGO GREEN MANURING ON THE INCIDENCE OF ROOT-KNOT DISEASE OF JUTE CAUSED BY NEMATODE (MELOIDOGYNE JAVANICA)

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ABSTRACT

A pot experiment was carried out during the period of March 2016 to August 2016 in the net house at the Soil Science Field Laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh to evaluate the nematicidal potential of indigo green manuring on the root-knot disease caused by nematode (Meloidogyne javanica). In this experiment Jute (Corchorus capsularis) was used as test crop and Indigo (Indigofera tinctoria L.) as green manuring crop. The experiment was laid out with seven treatments having three replications. Green manuring with indigo was found to give significant response in the growth and corresponding reduction in the number of galls in jute inoculated with Meloidogyne javanica. The negative correlations between the galling incidence and the plant height and root length of jute revealed that the indigo green manuring was effective in increasing the growth of the jute by suppressing the nematode activity.

KEYWORDS:
Indigo, Green manuring, Root-knot, Jute

INTRODUCTION

Jute is the golden fiber of Bangladesh which is an important cash crop that belongs to the family Tiliaceae and genus Corchorus. It plays an important role in Bangladesh economy as the country earns about 12-13% of total foreign currency by exporting jute and jute goods [1]. Bangladesh, the second largest producer of jute, produces the best quality jute in the world and leads the export market. Jute is extensively used throughout the world because of its versatility, durability and eco-friendly in nature. Entire life cycle of jute from cultivation to usage, disposal it is friendly to the environment and produces no toxic materials [2]. It plays an important role in increasing soil fertility, nutrient availability and soil conservation [3]. Its fiber is mainly used in manufacturing various types of industrial products such as hessian, sacking, carpet backing, cloths, mats, blankets, fabrics, packing materials, etc. The fiber of jute is also used to prepare ropes and housing materials for domestic uses. Jute sticks are used as fuel and fence in rural areas, and its leaves are used as vegetables in many countries of the world. Its leaves also have been used as herbal medicine and its fibers have much advantage over synthetics and protect the environment and maintain the ecological balance [4]. Present world demand of using the natural fiber instead of synthetic (to save the environment) is also reaping the past glory of jute. Bangladesh government has also taken different steps for strengthening the jute sector.

Although the jute is an important crops in Bangladesh, but it covered only 6.95% of the total cultivated area occupied 0.73 million hectares, and produced 7.56 million metric tons fibers in 2015-16 [5], which is very low than the other country like India. Not only that, the production of quality jute fiber is decreasing day by day due to the various factors like social, political and environmental. Among the environmental factors, climatic condition is most important. The climatic condition of Bangladesh is more suitable for the different organisms, which causes different diseases. Root-knot is one of the major diseases caused by nematode. Due to the attack by nematode, the growth of jute plants is stunted and plants become thinner which causes the reduction of the fiber production. It is necessary to control the nematode of jute for increasing fiber production.
Root-knot diseases can be controlled through chemical, cultural and biological means. However, the use of chemicals cause environmental risks and could lead to the development of resistance target species. Moreover, chemical control is too costly for the poor farmers and dangerous to public health. Among them various extracts of plant parts [6, 7, 8, 9], plant leaves [10] and organic amendment with sun hemp and marigolds [11], organic amendment with indigo[12] were used to control the activity of plant parasitic nematodes caused the root-knot disease in the different crops. Although several biological measures were taken to control the root-knot diseases caused by *Meloidogine javanica*, but no information is available to control the root-knot diseases of jute using the indigo plants as green manuring. To avoid environmental pollution and minimizing the high production cost, the present research program was under taken to determine the effects of green manuring of indigo plants (*Indigofera tinctoria L.*) on the incidence of root-knot disease of jute caused by nematode (*Meloidogyne javanica*).

**MATERIALS AND METHODS**

**Location and duration.** The pot experiment was carried out in the net house of the Department of Soil Science, Hajee Mohammad DaneshScience and Technology University (HSTU), Dinajpur, Bangladesh during the period of March to August, 2016. The site is located in 25°37’ N latitude and 88°39’ E longitude on the eastern bank of the river Punarvhaba, Bangladesh. The altitude of the location was 37.5 m from the sea level. The Agro Ecological Zone (AEZ) of the area is the Old Himalayan Piedmont Plain (AEZ-1) [13].

**Soil properties.** Soils in the pots were collected from the arable land of Soil Science Research Field, HSTU, Dinajpur, Bangladesh from a depth of 0-15 cm. The collected soil was sun dried for a couple of days and weeds were removed. After drying and grinding, the soil samples were passed through a 20-mesh sieve. The soils of the experimental pot were sandy loam and acidic in nature (6.10). The pre-seeding total soil N (nitrogen) were 0.08%, indicating a deficiency of soil N. Soil available P (phosphorus), K (potassium) and Mg were 9.75, 0.08, and 0.29 meq 100g⁻¹ soil, respectively which indicates P, K and Mg deficit soil based on the critical levels of those plant nutrients but the level of other nutrients were high. Soil organic matter was 1.23%. The physical and chemical characteristics of the initial soil samples with their extraction methods are given in Table 1. The soil was then treated with 3% formalin solution for sterilization.

<table>
<thead>
<tr>
<th>Physical and chemical properties of initial soil used for the pot experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TABLE 1</strong></td>
</tr>
<tr>
<td><strong>Physical properties of soil</strong></td>
</tr>
<tr>
<td>Properties</td>
</tr>
<tr>
<td>Sand</td>
</tr>
<tr>
<td>Silt</td>
</tr>
<tr>
<td>Clay</td>
</tr>
<tr>
<td>Textural class</td>
</tr>
<tr>
<td><strong>Chemical properties of soil</strong></td>
</tr>
<tr>
<td>Properties</td>
</tr>
<tr>
<td>Soil pH (1:1.25, Soil:H2O)</td>
</tr>
<tr>
<td>Organic matter</td>
</tr>
<tr>
<td>N (%)</td>
</tr>
<tr>
<td>Available P (ppm)</td>
</tr>
<tr>
<td>Exchangeable K (meq %)</td>
</tr>
<tr>
<td>Exchangeable Ca (meq %)</td>
</tr>
<tr>
<td>Exchangeable Mg (meq %)</td>
</tr>
<tr>
<td>Available S (ppm)</td>
</tr>
<tr>
<td>Available B (ppm)</td>
</tr>
<tr>
<td>Available Zn (ppm)</td>
</tr>
<tr>
<td>Available Fe (ppm)</td>
</tr>
<tr>
<td>Available Mn (ppm)</td>
</tr>
<tr>
<td>CEC(me/100 soil)</td>
</tr>
</tbody>
</table>
Climatic conditions. Weather data on the daily average temperature, humidity and rainfall during experimental period at the experimental area were recorded regularly by the HOBO U12 Family of Data Loggers (MicroDAQ.com) at the Meteorological Station at HSTU, Dinajpur, Bangladesh. The means of meteorological information, like temperature (maximum, minimum, and average temperature- °C), rainfall (mm), and relative humidity (%) of the experimental site during the crop growing period are exposed in Table 2. The average temperature, rainfall and relative were 27.27°C, 192 mm and 77%, respectively.

Pot preparation. Ten kilograms sterilized soils were taken into each pot of 25 cm depth and 20 cm diameter. Earthen plates (25 cm) were placed below the each pot to retain excess fluid. Thus the pots were ready for seed sowing.

Experimental treatments and design. The experiment comprised seven treatments viz. T1=Control (no nematode + no Indigo manuring), T2=200 egg mass, T3=200 egg mass + Indigo manuring, T4=400 egg mass, T5=400 egg mass + Indigo manuring, T6=200 egg mass + Furadan and T7=400 egg mass + Furadan. The experiment was arranged in complete randomized design with three replications. So, the total number of the pots was 21.

Plant material. The crop used in this study was Toshajute (Corchorus olitorius L.) and the variety was O-9897. The seed was collected from Nashipur Jute Farm, Bangladesh Jute Research Institute (BJRI), Dinajpur, Bangladesh.

Experimentation. At first indigo seeds were sterilized and sown in each pot. The pots were watered with boiled cold water. After 2 months, the indigo plants were cut into pieces and mixed with the soil. Due to deficiency of N, P, and K (Table 1), the pot soil was fertilized according to the recommendation of BJRI with N, P and K @ 40, 25 and 38 Kg ha⁻¹ as urea, triple super phosphate and muriate of potash, respectively.

The jute seeds were sown in all the pots to mix with sand. It was sown 2-3 cm depth of the soil to ensure better germination. First weeding was done at 15 days after sowing (DAS). The 2nd and 3rd weeding were done 30 and 45 DAS, respectively. Thinning operations were done gradually for avoiding branching of jute. The first thinning was done at 15 DAS with maintaining 8 plants per plot. The second thinning was done 30 DAS, and 4 plants were kept in each plot. Final (3rd) thinning was done at 45 DAS, and 2 plants were kept for conducting the experiment. Furadan was applied as per treatment combination. The pots were watered uniformly for proper establishment of their roots within the soil. Every morning, seedlings were watered uniformly. When the plants were well established, the soils of the pot around the base of the plant were loosen with the help of a hand weeder (Khurpi) from time to time up to the 3rd month for better growth and development. For the inoculation of jute plants egg masses were collected from the roots of brinjal plants (Solanum melongena), which were previously inoculated with a single egg mass of Meliodogynje javanica. Surface sterilization of the egg masses were done with 0.1 per cent mercuric chloride solution for about one minute and then placed in a small nylon sieves. The sieves were placed in watch glasses containing distilled water with the water level just touching the mesh. The second stage juveniles were collected from the total quantity of the suspension of the larvae and diluted in such a way that each mL suspension contained approximately 100 larvae as counted with the help of a stereo-binocular microscope. Two mL of the larval suspension was applied in 9 pots and 4 mL in 9 pots at 20 days after germinating of jute seed. Jute plants were uprooted from pots after 90 days of inoculation.

Data collection. At first, the soil of the pot was watered to make it moist for easy uprooting of the plants. Then the whole plant along with the soil attached to the roots was lifted out from the pot and dipped into a bucket of water. The roots were separated from the soil by slow movement of the roots in water. It was further cleaned under gradually running tap water. The root portion was separated. The length of the shoot and the root was measured. The shoots and the roots were cut into small pieces and sun dried for 2 days. After drying, the weight of shoots and roots was taken. The cut pieces of the shoots and the roots were put into brown envelope

**TABLE 2**

<table>
<thead>
<tr>
<th>Months</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Rainfall (mm)</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>16.9</td>
<td>30.7</td>
<td>23.8</td>
<td>25.5</td>
<td>74</td>
</tr>
<tr>
<td>April</td>
<td>20.1</td>
<td>31.0</td>
<td>25.6</td>
<td>66.0</td>
<td>70</td>
</tr>
<tr>
<td>May</td>
<td>22.6</td>
<td>32.7</td>
<td>27.7</td>
<td>331.5</td>
<td>76</td>
</tr>
<tr>
<td>June</td>
<td>24.7</td>
<td>32.1</td>
<td>28.4</td>
<td>282.0</td>
<td>85</td>
</tr>
<tr>
<td>July</td>
<td>25.0</td>
<td>32.8</td>
<td>28.9</td>
<td>255.0</td>
<td>80</td>
</tr>
<tr>
<td>August</td>
<td>25.4</td>
<td>33.1</td>
<td>29.2</td>
<td>192.0</td>
<td>77</td>
</tr>
</tbody>
</table>
and oven dried at 80°C for 3 days. The shoots and the roots of jute plants of all the treatments were powdered to 60 meshes separately using a Wiley mill and analyzed for determining N content in the plants sample, determined by micro-kjeldahl method [24]. The roots were stained in phloxin “B” and preserved in 5% formalin solution for counting the number of galls formed on roots. The roots were cut into small pieces and 1 g of root was taken randomly from the bulk to count the number of galls formed.

Statistical analysis. All the collected data were analyzed using ANOVA and the differences among the treatment means were evaluated by the Duncan’s New Multiple Range Test as outlined by Gomez and Gomez [25].

RESULTS

Height of jute plants. Significant variation was observed on the height of jute plants due to indigo green manuring, inoculation with eggs of root-knot nematode and Furadan application (Table 3). The use of 200 eggs pot⁻¹ significantly reduced the heights of jute plants and the use of 400 eggs pot⁻¹ caused further reduction. Indigo green manuring significantly increased the plant height even in presence of 200 or 400 eggs pot⁻¹. The application of Furadan to jute plants inoculated with 200 eggs or 400 eggs pot⁻¹ also increased the plant height, which was comparable to the heights of the plants of the control treatment. The highest and significantly superior plant height (78.00 cm plant⁻¹) was observed for the control followed by 200 eggs + Furadan treatment (56.67 cm plant⁻¹). The shortest and statistically inferior plant height was found for the treatment 400 eggs pot⁻¹ (23.17 cm plant⁻¹).

Length of roots of jute plants. The inoculation with eggs of root-knot nematode and Furadan application had significant effects on the root length of jute plants (Table 3). Significantly lower root length was observed with 200 and 400 eggs pot⁻¹, compared to those receiving 200 and 400 eggs pot⁻¹ and indigo green manuring. The longest root length (50.00 cm plant⁻¹) was observed for the control followed by 200 eggs + Furadan treatment (44.83 cm plant⁻¹). The shortest and statistically inferior root length was found for the treatment 400 eggs pot⁻¹ (20.50 cm plant⁻¹).

Shoot weight of jute. Statistically significant differences were found in shoot weights among the different treatments (Table 3). Significantly reduced weight of shoots was observed in 200 and 400 eggs pot⁻¹ used treatments compared to the control. Inoculation of 200 or 400 eggs pot⁻¹ with indigo green manuring significantly increased the weights of shoots of jute. Furadan used treatment in presence of 200 or 400 eggs pot⁻¹ increased the weight of shoots, which was statistically at par with the control treatment.

Root weight of jute. The shoot weight of jute plants was significantly influenced by different treatments (Table 3). The control plants produced the highest weight of roots, which was statistically superior to the root weights obtained with the rest of the treatments. Eggs-inoculated treatments recorded the lower root weight compared to that of the control. Eggs-inoculated treatments having indigo green manuring showed the higher root weights compared to only egg inoculation treatments. The application of Furadan in the presence of 200 or 400 eggs pot⁻¹ recorded lower root weights compared to that of control but stayed higher over the rest treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Root length (cm)</th>
<th>Shoot weight (g)</th>
<th>Root weight (g)</th>
<th>No. of gall g⁻¹ root</th>
<th>Nuptake by shoot (mg plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁: control</td>
<td>78.00 a</td>
<td>50.00 a</td>
<td>39.17 a</td>
<td>12.00 a</td>
<td>0.0</td>
<td>767.7 a</td>
</tr>
<tr>
<td>T₂: 200 eggs</td>
<td>38.00 cd</td>
<td>30.83 b</td>
<td>29.67 c</td>
<td>8.50 cd</td>
<td>77.00 b</td>
<td>311.5 cd</td>
</tr>
<tr>
<td>T₃: 200 eggs+InM*</td>
<td>45.33 c</td>
<td>34.00 b</td>
<td>32.00 c</td>
<td>9.17 c</td>
<td>21.00 f</td>
<td>384.0 c</td>
</tr>
<tr>
<td>T₄: 400 eggs</td>
<td>23.17 f</td>
<td>20.50 c</td>
<td>22.33 d</td>
<td>6.17 e</td>
<td>89.83 a</td>
<td>149.6 e</td>
</tr>
<tr>
<td>T₅: 400 eggs+InM</td>
<td>27.83 ef</td>
<td>26.33 bc</td>
<td>24.67 d</td>
<td>7.50 d</td>
<td>36.50 d</td>
<td>234.4 de</td>
</tr>
<tr>
<td>T₆: 200 eggs+Fura,**</td>
<td>56.67 b</td>
<td>44.83 a</td>
<td>35.00 b</td>
<td>10.83 b</td>
<td>28.50 c</td>
<td>637.0b</td>
</tr>
<tr>
<td>T₇: 400 eggs+Fura,**</td>
<td>33.57 de</td>
<td>27.67 bc</td>
<td>29.33 c</td>
<td>8.00 d</td>
<td>66.33 c</td>
<td>305.0cd</td>
</tr>
<tr>
<td>SD</td>
<td>7.928</td>
<td>7.805</td>
<td>2.609</td>
<td>0.958</td>
<td>11.22</td>
<td>8.929</td>
</tr>
<tr>
<td>CV(%)</td>
<td>10.31</td>
<td>13.12</td>
<td>4.84</td>
<td>6.06</td>
<td>13.83</td>
<td>12.60</td>
</tr>
</tbody>
</table>

In the column, figures having similar letter(s) do not differ significantly at 5% level of probability.

*InM=Indigo green manuring, **Fura=Furadan

TABLE 3

Effect of indigo (Indigofera tinctoria L) green manuring in controlling gall formation in tobacco by root-knot nematode (Meloidogyne javanica) and its reflection on the yield of jute

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**Gall number.** The gall number g⁻¹ of root was significantly affected due to inoculation with eggs of root-knot nematodes, indigo green manuring and Furadan application (Table 3). There was no gall formation in roots of jute plants in control treatment. The inoculation of 200 eggs pot⁻¹ had 77.00 galls g⁻¹ root, which increased to 89.83 galls g⁻¹ root due to inoculation with 400 eggs pot⁻¹. Indigo green manuring significantly decreased the galls g⁻¹ of root compared to those without indigo green manuring. The use of Furadan also reduced the number of galls significantly.

**Nitrogen uptake by the shoots of jute.** There was a marked influence of the different treatments on the uptake of N by the roots of jute plants (Table 3). The nitrogen uptake by the shoots ranged from 14.96 to 76.77 mg plant⁻¹ (Table 3). The highest uptake (76.7 mg plant⁻¹) was found for control treatment, and it was statistically superior to that of all other treatments, and the lowest uptake was recorded to 400 egg mass inoculated treatment. Inoculation of nematode eggs induced significantly lower uptake of N by the shoots of jute plants compared to that of control. Application of Furadan also increased the uptake of N over its no application.

**Increase or decrease (%) of gall number (g⁻¹ root) and N uptake by shoot.** The per cent decrease in the number of gall (g⁻¹ root) due to application of Indigo and Furadan over nematode infected plants ranged from 59.37 to 72.73% and 26.16 to 62.99%, respectively (Table 4). However, in case of lower nematode infested plot (T2: 200 eggs), the treatment InM reduced higher nematode gall (72.73%) than Furadan (62.99%). On the other hand, manuring with Indigo successfully reduced (59.37%) the (g⁻¹ root) as compared to Furadan application (26.16%) under higher nematode infected plots (T3: 400 eggs). Incidence of nematode in plots significantly reduced the N uptake by shoot over control plots, and the values were 59.42 and 80.52% under 200 and 400 eggs infected plots, respectively. Nevertheless, under the same conditions (200 & 400 eggs infected plots), the reduction was only 49.98 & 69.47% due to application of Indigo manuring, and only 17.02 & 60.27% in Furadan application, respectively.

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Gall number (g⁻¹ root)</th>
<th>Increase/decrease over control (%)</th>
<th>N uptake by shoot (mg plant⁻¹)</th>
<th>Increase/decrease over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1: control</td>
<td>0.0 g</td>
<td>-</td>
<td>767.7 a</td>
<td>-</td>
</tr>
<tr>
<td>T2: 200 eggs</td>
<td>77.00 b</td>
<td>-</td>
<td>311.5 cd</td>
<td>59.42</td>
</tr>
<tr>
<td>T3: 200 eggs+InM*</td>
<td>21.00 f</td>
<td>72.73</td>
<td>384.0 c</td>
<td>49.98</td>
</tr>
<tr>
<td>T4: 400 eggs</td>
<td>89.83 a</td>
<td>-</td>
<td>149.6 e</td>
<td>80.52</td>
</tr>
<tr>
<td>T5: 400 eggs+InM</td>
<td>36.50 d</td>
<td>59.37</td>
<td>234.4 de</td>
<td>69.47</td>
</tr>
<tr>
<td>T6:200eggs+Fura,**</td>
<td>28.50 e</td>
<td>62.99</td>
<td>637.0b</td>
<td>17.02</td>
</tr>
<tr>
<td>T7: 400eggs+Fura,**</td>
<td>66.33 c</td>
<td>26.16</td>
<td>305.0cd</td>
<td>60.27</td>
</tr>
</tbody>
</table>

*InM=Indigo green manuring, **Fura=Furadan

**FIGURE 1**

Relationships between (a) plant height and shoot weight, (b) plant height and gall number and (c) shoot weight and root weight by the jute plants inoculated with *Meloidogyne javanica*
Correlation results. The correlation results among the different parameters are shown in the Fig. 1. The plant height was positively correlated with the shoot weight and strong positive correlation ($R^2=0.933$) was recorded (Fig. 1a). On the other hand, a moderate negative correlation ($R^2=0.640$) was observed between the plant height and the number of galls g$^3$ of root (Fig. 1b). The shoot weight was positively correlated with the root weight indicating $R^2=0.965$ (Fig. 1c) and the root length was negatively correlated with the galls g$^3$ of root providing the value of $R^2=0.656$ (Fig. 2a). In addition, the shoot weight was positively correlated with the N uptake by the plants, indicating the value of $R^2=0.922$ (Fig. 2b).

DISCUSSION

Indigo green manuring was tested against the root-knot of jute caused by nematode (Meloidogyne javanica). Treatments 200 eggs + InM, and 400 eggs + InM, were found to give significantly higher response in respect to shoot heights and roots length and fresh weight of shoots and roots, and nutrient uptake by the plants compared to the treatments of only 200 eggs and 400 eggs. In respect to the number of galls g$^3$ of root in the 200 eggs + InM, and 400 eggs + InM treatments, significantly lower response was observed compared to the treatments 200 eggs and 400 eggs (Table 2). This indicates the suppressing effect of the indigo green manuring on the nematode activity on jute. Among the four treatments viz. 200 eggs, 200 eggs + InM, 400 eggs, and 400 eggs + InM, the highest plant height and root lengths was recorded in 200 eggs + InM followed by 400 eggs + InM, and the highest number of galls were recorded in 400 eggs treatment followed by 200 eggs treatment. But statistically significant differences were found at all stages of the above four treatments. Moreover, the N uptake by the shoots and the roots were more or less higher in the indigo incorporated treatments (T$_3$ and T$_5$). It indicates that indigo green manuring had nematocidal effects on jute root-knot nematodes. The results are in agreement with those of Kumar and Nair [26], Alam [27] and Hossain et al. [28].

Egg inoculation treatments of T$_2$ and T$_4$ increased the number of gall from 0 to 77.00 and 89.93 (g root$^{-1}$), respectively. Using of Indigo and Furadan remarkably reduced the gall number and the diminution of nematode gall number under Indigo manuring was 2.27 times than Furadan. Nematode infested plants reduced the N uptake over non-infested control plants but the uptake of N by shoots increased through the practicing of Indigo manuring as well as Furadan treatment. The results showed that N uptake increased in nematode infected jute plants due to application of Indigo manuring and Furadan by reducing the number of nematode galls in roots, and N uptake was higher in Furadan applied plants than in Indigo manuring plants.

The negative correlations between galling incidence and plant height, and galling incidence and root length of jute (Fig. 1) revealed that the treatments with indigo amendment were effective in increasing the growth characteristics of the crop by suppressing the nematode activity. Between the soils amended with organic (200 eggs + InM, 400 eggs + InM), and chemicals (200 eggs + Furadan,
400 eggs + Furadan), the lower lengths and dry weights of shoots and roots were observed in organic amended treatments (200 eggs + InM, 400 eggs + InM) but the higher number of galls were found in chemical amendment treatments (200 eggs + Furadan, 400 + Furadan). Nevertheless, in the case of gall number significant differences were obtained between these two treatments. As the chemicals are costly, particularly for poor farmers of Bangladesh as well as its harmful effect on air, soil and water pollution as stated by Alam [27]. The use of indigo amendment has been found encouraging trend in this study to control the root-knot of jute caused by Meloidogyne javanica. A strong positive correlation between plant height and shoot weight, shoot weight and root weight, and shoot weight and N uptake were observed by Hossain et al. [15] in tobacco crop.

CONCLUSIONS

Indigo green manuring that was done before sowing seeds of jute in the pot, helped to reduce the incidence of root knot disease in jute, and thereby increased the yield. Further research in the field level at the different AEZs (Agro-ecological Zones) of Bangladesh should be carried out before making any recommendation.

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REFERENCES


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STUDY ON RAPID RESTORATION OF ROADBED SLOPE VIA AIR-FOAM TREATED LIGHTWEIGHT SOIL AND ROOT PILES

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2Key Laboratory of New Technology for Construction of Cities in Mountain Area, Chongqing University, Ministry of Education, Chongqing 400045, China
3Department of Engineering Management, Logistical Engineering University of PLA, Chongqing 401311, China

ABSTRACT

Landslide of roadbed slope easily leads to vegetation deterioration and bring a lot of waste soil which cause a series of secondary disasters. Due to the low efficiency and helpless for utilization of the waste soil of traditional methods, this study comes up with a new approach via air-foam treated lightweight soil and root piles to restore the roadbed slope rapidly and to prevent secondary disasters. To obtain fast foundation construction, simple supporting-retaining structure, and quick backfill of roadbed materials, the authors put forward a roadbed slope support system based on the air-foam treated lightweight soil and root piles.

Taking root piles made from the fast-setting concrete as the foundation, the study achieves quick backfill, as air-foam treated lightweight soil is light-weighted, mobile and self-compacting with fast construction speed and requiring no permanent supporting structure. In addition, the proposed method has verified through numerical simulation. Suffice it to say, that this research sheds new light on rapid restoration of roadbed slope and achieve the goal of harmony between support technology and environmental protection.

SAMPLE PREPARATION AND EXPERIMENT ON TRIAXIAL COMPRESSION

Material and testing equipment. Experiment materials: 42.5R grade ordinary Portland cement is used as cementitious materials, engineering waste is used as raw material soil, self-made efficient foaming agent, and tap water. Test equipment: JJ-5 planetary cement mortar mixer, 39.1×80 cylinder die trials, HBY-40B standard constant temperature and humidity curing box, WE-600 universal testing machine, strain control three-axis compression device, WGD702 high and low temperature test chamber, electronic balance, and so on.

Mixing ratio of design and specimen fabrication. The mechanical properties of Foamed Cement Banking are changeable with bubble content, cement content and water content. Considering this, we have determined the mixing ratio of design materials in Table 1. Under the mixing scheme, we...
have weighted a certain amount of raw soil, poured it into the mixer together with a quota of cement, and set the rotation speed at 100r / min. Then, with some water and foaming agent that had diluted according to a specific proportion, we stirred the mixture well using a blender. Finally, the mixture has encased into the 39.1×80 cylinder mold before being cured for 24h in the standard curing box. The cured specimen would be demoulded and cured for an extra time until reaching the design age to undergo the performance test [3].

**TABLE 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Bubble content</th>
<th>Cement content</th>
<th>Water content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8%</td>
<td>20%</td>
<td>55%</td>
</tr>
</tbody>
</table>

**FIGURE 1**

The results of triaxial compression test

**FIGURE 2**

Non-consolidated unstrained shear strength envelope curve

Experiment on triaxial compression. In order to study the mechanical properties of the Foamed Cement Banking under the condition of triaxial stress, the above specimens are selected to carry out the non-consolidated undrained shear test at the confining pressure of 50,100, and 150 kPa, respectively, with the strain control method. The test results are shown in Figure 1.

With the static-force triaxial compression test, we have plotted the non-consolidated unstrained shear strength envelop curve in Figure 2, under the cohesion c of 130.1 kPa, and the internal friction angle of 6.03°.

**PROGRAM DESIGN**

**General train of thought.** Root piles made from the fast setting concrete not only provide quick support for roadbed slope, but also serve as roadbed and play an anti-skid role. Root piles can transfer the weight of backfill soil and vehicle load to the lower stable rock base, keeping the roadbed stable. Meanwhile, root piles can prevent the roadbed slope from sliding along the contact surface between the old and new materials. Root piles are quick and easy to construct, which can meet the fast restoration requirement of mountain roads.

Being a kind of lightweight geotechnical material, the air-foam treated lightweight soil is obtained through fully mixing and stirring the raw soil with proportional curing agents, water, and pre-produced foam cluster. It possesses the excellent properties of lightweight, adjustable intensity, high mobility, self-reliance and good constructability [4-6]. Besides, ATLS can achieve rapid backfill as it can be obtained in local raw materials, and it is self-compacting with fast construction speed and requiring no permanent supporting structure.

Based on the engineering characteristics of root piles and ATLS, the study proposes the rapid restoration approach via root pile and ATLS (Figure 3) to restore the roadbed slope of mountain roads quickly.

**FIGURE 3**

Design of rapid restoration of roadbed slope via ATLS and root piles

**Design requirements.** The plane position and spacing of root piles should have determined after comprehensively considering the stratigraphic nature of the landslide, thrust force, sliding surface slope, landslide thickness, construction conditions, pile section size, and anchorage depth [7-8]. The relative anchorage depth of root piles in the stable base below the sliding surface has related to the
strength of the base, the landslide thrust, the relative stiffness of the piles and the counter force of the sliding body against the piles above the sliding surface. The lateral pressure passed by the piles at the anchorage depth below the sliding surface should not be greater than the allowable lateral pressure of base. The maximum pressure at the bottom of the piles shall not be greater than the allowable bearing capacity of the base.

**TABLE 2**

<table>
<thead>
<tr>
<th>Rock category</th>
<th>E/GPa</th>
<th>Poisson’s ratio (µ)</th>
<th>Cohesion (C/Pa)</th>
<th>Internal friction angle (°)</th>
<th>Density (/kN.m⁻³)</th>
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<tr>
<td>ATLS</td>
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<td>35</td>
<td>1.2</td>
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**TABLE 3**

<table>
<thead>
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<th>Slope (°)</th>
<th>Backfill height (m)</th>
<th>Collapse surface horizontal angel (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
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<td></td>
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<td>10</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

**FIGURE 4**

Vertical displacement contour map

**NUMERICAL SIMULATION CALCULATION AND CHECKING**

**Calculation.** According to the field investigation, the study takes the common sandstone as the bedrock for calculation, and the physical and mechanical indicators are tested through lab rock mechanics experiment [9-11]. The load of first grade lane is the uniform load plus concentrated load. The standard uniform load is 10.5kN/m². The concentrated load is decided according to the following criteria: if the calculation span of L₀ is equal to or
less than 5m, then the $P_x$ is 180kN; if it is equal to or larger than 50m, then the $P_x$ is 360kN; if it is larger than 5m and less than 50m, the $P_x$ is calculated through linear interpolation. The mechanical parameters of the original rock and ATLS are shown in Table 2.

According to the different backfill height, sliding surface and horizontal angle, this study adopts the FLAC V5.0 to carry out the calculation and checking (as shown in Table 3). As for numerical simulation calculation, the study takes into consideration the following three conditions: (1) the backfilling ATLS calculation. The study analyzes the overall force born by the roadbed and deformation under all kinds of intercepted roads backfilled with ATLS. (2) On the basis of the first condition, taking the vehicle load into consideration, the study then analyzes the pressure and deformation of the roadbeds backfilled with ATLS. (3) On the basis of the first and second conditions, with root piles deployed between ATLS and the base rock, the study analyzes the pressure and deformation of the roadbed, the sheared situation and deformation of root piles, as well as the effect of root piles in keeping the overall stability of the roadbed.

FIGURE 5
Horizontal displacement contour map

FIGURE 6
Pile axial bending moment diagram
FIGURE 7
Pile axial shear force diagram

TABLE 4
Horizontal and vertical displacement of back fillingbody

<table>
<thead>
<tr>
<th>Backfill height (m)</th>
<th>Collapse surface horizontal angle (°)</th>
<th>ATLS Vertical displacement (cm)</th>
<th>ATLS + load Vertical displacement (cm)</th>
<th>ATLS + root piles + load Vertical displacement (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Horizontal displacement (cm)</td>
<td>Horizontal displacement (cm)</td>
<td>Horizontal displacement (cm)</td>
</tr>
<tr>
<td>10</td>
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<td>3.5</td>
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<td>30</td>
<td></td>
<td>17.5</td>
<td>4</td>
<td>17.5</td>
</tr>
</tbody>
</table>

RESULTS

With the slope angle of 60°, backfill height of 15m and collapse surface horizontal angle of 30°, Figure 4 to Figure7show the vertical displacement, horizontal displacement and the bending moment and shearing force of root piles under the conditions of ATLS, root piles and roadbed load.

Table 4 and Table Sshow the deformation value of ATLS and resistance of root piles under various conditions. It can be seen from the tables that: (1) the horizontal displacement is little after backfilling with ATLS under all kinds of intercepted roads; the displacement increases with vehicle load; the displacement reduces after inserting root piles.

(2) The maximum shearing force and bending moment of root piles are small. Therefore, root piles with small diameter can meet the requirements, which is also conducive to the rapid construction.

(3) The vertical displacement is not obvious with the ATLS and root pile supporting system after exerting vehicle load, which indicates that the system can effectively bear the vehicle load and its own weight. (4) The vertical displacement of ATLS is relatively large, which has related to the proportion of foam, cement, and raw soil in ATLS. According to the experience of applying ATLS in soft soil base, the vertical deformation of the backfill material can be controlled through adjusting the mixing proportion of ATLS.
<table>
<thead>
<tr>
<th>Backfill height (m)</th>
<th>Collapse surface horizontal angel (°)</th>
<th>ATLS + root piles + load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shearing force (N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bending moment (Pa)</td>
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<tr>
<td>10</td>
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<td>-4,847</td>
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<td>2,036</td>
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<td></td>
<td>10</td>
<td>3,081</td>
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<tr>
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<td>20</td>
<td>3,963</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-4,403</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Aiming at the collapse of high and steep rocky roadbed in mountainous area, this study studied the air-fom treated lightweight soil, and designed a quick restore method via backfilling ATLS and rooted piles. The study prepared specimens, carried out static triaxial compression test, measured the mechanical properties of the air-fom treated lightweight soil, calculated, and checked the numerical simulation under different working conditions. The main conclusions are as follows.

1. ATLS is light weighted and easy to pump with high intensity and good mobility. Therefore, it is the ideal backfill material for mountainous roadbed slope restoration.

2. The root piles suffer small maximum shear force and bending moment. Therefore, root piles with small diameter can meet the requirements and is conducive to rapid construction.

3. The horizontal displacement is small and the increase of vertical displacement is slow, which can meet the requirements of rapid restoration of roadbed slope.

The intensity of air-fom treated lightweight soil and the vertical deformation have related to the mixing ratio. In order to meet the strength and vertical deformation of different engineering requirements, it is necessary to carry out experiments with a variety of mixing ratios. Besides, the interaction mechanism between ATLS and root piles remains to be further studied.

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ANALYSIS OF LAWN SOILS OF SOME IMPORTANT PARKS IN ESENLER DISTRICT OF ISTANBUL

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2Caglayan Agriculture Products Company, 34480 Basaksehir, Istanbul, Turkey

ABSTRACT

In this study, the soil samples of lawns from 20 parks of significant scale in Istanbul city, district of Esenler, were analysed and evaluated. According to the results, the texture of lawn soils is mostly clay loam, pH was 6.86-8.57, salt contents were 0.02-0.046%, and lime contents were 0.00-11.53%. The organic content of the soils was 2.16% in average and belonged to class of “soil containing medium amount of organic matter”. The relationship between all parameters of the soils analysed were statistically significant (P < 0.01). Grass plants are the most important part of the green fields of the parks. Soil analysis and fertilization programs according to these analyses are important for the grass to be long-lived and of good quality. Istanbul is a megacity famous of its parks. Majority of these important parks belong to the Esenler district. Taking care of the grass in an environmentally sensitive point of view may have a greater effect than we think.

KEYWORDS:
Soil, park, lawn, Esenler

INTRODUCTION

The positive effects of lawns to the environment and the human life have been scientifically proved. It should not be forgotten that no life is possible without the plants. However, the soil structure of the sites where plants will reside should be available for them to live healthy and in a sustainable way. Particularly the soil characteristics such as the organic matter, other macro and micro nutritional elements and lime contents, pH, salinity, and texture of the soil should satisfy the needs of grass. Sustaining the natural productivity of the soil is possible only with keeping the organic matter content in a certain level.

Recently, green fields, lawns and parks are of increasing need in metropolis due to fast and unconscious urbanization, rapid increase of population, developments in industry, technology and computerization, and arbitrarily destroy the natural vegetation [1]. Grass is the most common plant material used in horizontal design by landscape architectures worldwide in terms of aesthetic value and biological comfort [2]. In recent years solid and liquid vermicompost have been used intensely used in agriculture and landscaping worldwide [3]. According to Güneylioğlu [4], grass can adapt various soil types as long as the groundwater level is not high. They develop well in soils which are rich in macro and micro nutritional elements, well hosed and with good drainage. However, they cannot grow well in sandy soil, soils with low pH and arid. Tosun [5] reported that perennial grasses can develop well in clay loam, which is rich in organic matter. Aşık and Kütük [6] stated that soil structure and suitable fertilization is important for the lightweight grass seeds to moisten, swell and be ventilated. Grasses fall into two main groups as “cool climate species” and “warm climate species” [7]. According to Beard [8], even though the warm climate grasses are well adapted to fine textured soils and fertile soils, they can widely adapt any kind of soils, are salt tolerant, and their pH needs vary between 5.5-7.5. Saline soil is also well-known to limit crop production and environmental quality. More salts have adverse effects on chemical, physical and biological properties of soil and plant growth [9].

Lawns need to have soils of not too sandy and they should have enough amount of organic matter, macro and micro nutritious elements [10]. If the texture of lawns is not suitable, rehabilitation should be carried out. Factors affecting grass root development should be in the foreground in rehabilitation works. Karaöz [11] reported that the fertilizations for the landscapes as an important cultivation maintenance application that provides the plants to grow fast and healthy, and maintains the sustainability of the expected form, and decorative characteristics such as leaf, flower, plant colour and fruit formation.

In a study carried out in experimental fields of Ege University Bandirma Vocational High School, the green field performances of cool climate grass plants by different fertilizer doses were determined. The results revealed that intense fertilization was of need as the texture of soil was clay loam, and 50 kg/da/year fertilizer gave a good result in mixes that
contain plain or intense *Festuca arundinacea* [12]. Aşk and Kütük [6] found that the effects of tea waste compost and some other organic materials were significant in forming lawns. Moreover, it was reported that 2-3 tons/da fertilizer or similar organic materials should be applied as cover fertilizer for lawn formation. As it is well known, soil analysis is the first step for a right fertilization.

According to Özcan [13], fertilization to the corresponding area should be applied after the soil analysis for the lawn formation if a homogenous germination, a good root structure and development are desired. In this study, some soil analysis results of 20 lawn having parks of Istanbul, Esenler district were statistically evaluated.

**MATERIALS AND METHODS**

This study was carried out with soil samples from 20 parks of Istanbul, Esenler district. The average climate data of Istanbul for long years including 2018 in which this study was done is given in Table 1 [14].

<table>
<thead>
<tr>
<th>The average of years 1937-2018</th>
<th>Aver. Temp. (°C)</th>
<th>Max. Temp. (°C)</th>
<th>Min. Temp. (°C)</th>
<th>Relative Humidity (%)</th>
<th>Air Pressure (hPa)</th>
<th>Aver. Wind Speed (m-s⁻¹)</th>
<th>Aver. Sunshine Duration (hour)</th>
</tr>
</thead>
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<tr>
<td>14.3</td>
<td>39.5</td>
<td>-12.6</td>
<td>74.4</td>
<td>1011.4</td>
<td>2.8</td>
<td>6.6</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

Names and coordinates of points where soil samples were taken.

<table>
<thead>
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<th>Sample No</th>
<th>X</th>
<th>Y</th>
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</thead>
<tbody>
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<td>28.877291</td>
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</tbody>
</table>

**FIGURE 1**

The position of the sampled Esenler district in the map.
The study was done by analysing the soil samples (0-20 cm depth) taken from 20 parks having significant size of lawn residing in Esenler district, Istanbul and evaluating the data statistically. The names and coordinates of the sampled parks are given in Table 2, spatial distribution of the areas is altogether given in Figures 1 and 2.

Some pictures regarding soil sampling of this study is given in Figure 3.

Soil samples were prepared by air drying and filtering with 2mm mesh after brought to the analysis lab [15]. Soil analyses were carried out in Hayrabolu Trade Exchange, Soil-Plant Analysis Lab, Hayrabolu, Tekirdağ, Turkey. Some physical and chemical analysis methods for soil samples are given below.
Texture class was determined by saturation with water; soil reaction was determined by dilution of the soil by 1:2.5 as recommended by International Soil Science Society. Soil pH and salinity were measured by direct reading of digital pocket instruments from Eutech (i.e. pH+Salt Tester Respectively) after dissolving the soil in some amount of distilled water. Soil salinity was measured by percentage [16]. Lime (CaCO₃) content was measured volumetrically with Scheibler Calcimeter [17]. Soil organic matter was estimated by Walkley-Black method [18]. The results were analysed with variance analysis, the significance of difference between means were tested with LSD in MSTAT package program [19].

RESULTS AND DISCUSSION

Some physical and chemical analysis results of soil samples are given in Table 3.

Acidity or pH of soil highly affects plant growth. The average pH values of the soil samples were 7.38 which is in “neutral” class. The maximum value was measure in sample no 15, this soil belonged to “strong alkali” class. This soil should be taken into rehabilitation and negative effects of pH to grasses should be avoided [20, 21, 22, 23]. Organic fertilization is quite favourable when the pH of the soil is either too high (alkali) or too low (acidic). Therefore, organic fertilization should definitely be taken into rehabilitation programs which are important both for pH regulation and increase of water retention capacities [24]. Maintaining an optimum soil pH level is important for maximizing availability of plant nutrients and for maintaining soil conditions that will support good root growth and grass quality. Most grass can grow well within a range of soil pH from 6.0 to 7.0 [25]. In this case, it appears to be compatible with previous studies in terms of pH values of the soils. Salinity indicated the amount of salts contained in the soil. The total salt contents (salinity) of the soil samples were minimum 0.02% (saltless) and maximum 0.05% (saltless) which shows that the soils do not have a salt problem [20, 21, 22, 23]. The grasses were watered with automatic watering systems in the sampled 20 parks and this can be evaluated as positive as no salt problem occurred due to watering. According to Doğan et al. [26], the main source of salt in lawns can be due to main salt rock and main material, as well as watering, drains water and groundwater. While saltiness was not a problem in this research area, it is a common problem in arid regions.

The organic matter content of the soils was 2.16% in average and falls into “medium” class. The ratio of the soils having <1% organic matter content in average (soils with very low amount organic matter) is 85% of agricultural lands in Turkey [24], the organic content of the research soils of Esenler district was determined to be minimum 0.75% (only 1 park) and maximum 5.1%. The lime content of the soil samples was minimum “non-calcareous” and maximum 11.53% CaCO₃, therefore they were in “medium level calcareous” class. The parks no 7 and 11 were “medium” in terms of lime content, therefore no chemical fertilizers should be used in these parks as they increase the pH.

<table>
<thead>
<tr>
<th>Sample No</th>
<th>pH (1:2.5 Soil:Water)</th>
<th>Total Salt (%)</th>
<th>Organic Matter (%)</th>
<th>CaCO₃ (%)</th>
<th>Saturation (%)</th>
<th>Texture Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.860</td>
<td>0.033</td>
<td>3.110</td>
<td>0.000</td>
<td>62.700</td>
<td>Clay loam</td>
</tr>
<tr>
<td>2</td>
<td>6.910</td>
<td>0.030</td>
<td>2.540</td>
<td>0.000</td>
<td>57.200</td>
<td>Clay loam</td>
</tr>
<tr>
<td>3</td>
<td>7.480</td>
<td>0.027</td>
<td>1.280</td>
<td>1.350</td>
<td>60.500</td>
<td>Clay loam</td>
</tr>
<tr>
<td>4</td>
<td>7.260</td>
<td>0.035</td>
<td>4.520</td>
<td>0.800</td>
<td>66.000</td>
<td>Clay loam</td>
</tr>
<tr>
<td>5</td>
<td>6.880</td>
<td>0.037</td>
<td>3.230</td>
<td>0.000</td>
<td>64.900</td>
<td>Clay loam</td>
</tr>
<tr>
<td>6</td>
<td>7.660</td>
<td>0.043</td>
<td>1.580</td>
<td>1.190</td>
<td>62.700</td>
<td>Clay loam</td>
</tr>
<tr>
<td>7</td>
<td>7.460</td>
<td>0.023</td>
<td>1.350</td>
<td>1.590</td>
<td>66.000</td>
<td>Clay loam</td>
</tr>
<tr>
<td>8</td>
<td>7.580</td>
<td>0.034</td>
<td>1.280</td>
<td>4.530</td>
<td>69.300</td>
<td>Clay loam</td>
</tr>
<tr>
<td>9</td>
<td>7.370</td>
<td>0.022</td>
<td>1.120</td>
<td>7.160</td>
<td>53.900</td>
<td>Clay loam</td>
</tr>
<tr>
<td>10</td>
<td>6.880</td>
<td>0.025</td>
<td>5.100</td>
<td>0.000</td>
<td>91.300</td>
<td>Clay</td>
</tr>
<tr>
<td>11</td>
<td>7.580</td>
<td>0.046</td>
<td>0.750</td>
<td>11.530</td>
<td>69.300</td>
<td>Clay loam</td>
</tr>
<tr>
<td>12</td>
<td>7.290</td>
<td>0.020</td>
<td>1.960</td>
<td>1.750</td>
<td>59.400</td>
<td>Clay loam</td>
</tr>
<tr>
<td>13</td>
<td>7.360</td>
<td>0.026</td>
<td>1.670</td>
<td>2.620</td>
<td>60.500</td>
<td>Clay loam</td>
</tr>
<tr>
<td>14</td>
<td>7.150</td>
<td>0.034</td>
<td>1.670</td>
<td>2.390</td>
<td>66.000</td>
<td>Clay loam</td>
</tr>
<tr>
<td>15</td>
<td>8.570</td>
<td>0.029</td>
<td>1.040</td>
<td>3.100</td>
<td>60.500</td>
<td>Clay loam</td>
</tr>
<tr>
<td>16</td>
<td>7.280</td>
<td>0.024</td>
<td>3.120</td>
<td>4.370</td>
<td>66.000</td>
<td>Clay loam</td>
</tr>
<tr>
<td>17</td>
<td>7.530</td>
<td>0.036</td>
<td>2.560</td>
<td>1.190</td>
<td>61.600</td>
<td>Clay loam</td>
</tr>
<tr>
<td>18</td>
<td>7.290</td>
<td>0.030</td>
<td>1.110</td>
<td>2.390</td>
<td>61.600</td>
<td>Clay loam</td>
</tr>
<tr>
<td>19</td>
<td>7.430</td>
<td>0.023</td>
<td>2.380</td>
<td>0.000</td>
<td>58.300</td>
<td>Clay loam</td>
</tr>
<tr>
<td>20</td>
<td>7.800</td>
<td>0.030</td>
<td>1.970</td>
<td>1.590</td>
<td>62.700</td>
<td>Clay loam</td>
</tr>
</tbody>
</table>

Min. 6.860          0.020  0.750  0.000  53.900 Clay loam
Max. 8.570          0.046  5.100  11.530  91.300 Clay loam
Average 7.381      0.030  2.163  2.378  64.020 Clay loam
The primary function of lime is to raise the pH of the soil. However, over application of lime can result in raising the pH above optimum levels, and high pH levels (> 7.5) can cause poor nutrient availability and create other grass problems [25].

One of the soils (park no 10) was loamy in terms of saturation percentage values, and the other 19 were in “clay loam” texture class. The soils are accounted to be “suitable” for grass cultivation in terms of texture class [20, 21, 22, 23]. According to Doğan et al. [26], grasses grow in natural environments with clay and sandy soil mixtures which are rich in organic content. Therefore, the soils in the research area are appropriate for grass cultivation in terms of texture.

**pH of the Soils.** The statistical analysis of the pH values of research soils are given in Table 4 and Figure 4.

The pH of soil samples generally affects the obtainability of macro and micro nutritious elements directly. The reason of absence of Fe in soils with high levels of Ca and Mg and high levels of lime is this fact [22]. The absence of Fe may occur when the pH level becomes above 7.5. The pH level of the park (soil) no 15 was 8.57, therefore attention should be paid for fertilizations not to increase the pH more.

**Total Salt Contents of the Soils:** Statistical analysis results of salt contents of the soil samples are given in Table 5 and Figure 5.

### TABLE 4

**pH averages of soils from different parks and their groups according to LSD test.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample No</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10</td>
</tr>
<tr>
<td></td>
<td>6.86n 6.91 7.48f 7.26j 6.88m 7.66c 7.46f 7.58d 7.37h 6.88nm</td>
</tr>
<tr>
<td>Sample No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11  12  13  14  15  16  17  18  19  20</td>
</tr>
<tr>
<td></td>
<td>7.58d 7.29i 7.36h 7.15k 8.57a 7.28j 7.53c 7.29i 7.43g 7.80b</td>
</tr>
</tbody>
</table>

There is no difference between the means of same letter at 0.01 significance level. LSD (1%)= 2.213978

![pH Chart](image)

**FIGURE 4**

Relationships between soil pH levels.

### TABLE 5

**Salt content averages of soils from parks and their groups according to LSD test**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10</td>
</tr>
<tr>
<td></td>
<td>0.03ab 0.03ab 0.03ab 0.04ab 0.04ab 0.04a 0.02ab 0.03ab 0.02ab 0.03ab</td>
</tr>
<tr>
<td>Sample No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11  12  13  14  15  16  17  18  19  20</td>
</tr>
<tr>
<td></td>
<td>0.05 a 0.02 b 0.03ab 0.03ab 0.03ab 0.02ab 0.04ab 0.03ab 0.02 b 0.03ab</td>
</tr>
</tbody>
</table>

There is no difference between the means of same letter at 0.01 significance level. LSD (1%)= 2.213960
FIGURE 5
Relationships between soil salt contents.

TABLE 6
Organic matter content averages of soils from parks and their groups according to LSD test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter</td>
<td>3.11d</td>
<td>2.54e</td>
<td>1.28k</td>
<td>4.52b</td>
<td>3.23c</td>
<td>1.58i</td>
<td>1.35j</td>
<td>1.28k</td>
<td>1.12l</td>
<td>5.10a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter</td>
<td>0.75n</td>
<td>1.96g</td>
<td>1.67h</td>
<td>1.67h</td>
<td>1.04m</td>
<td>3.12d</td>
<td>2.56c</td>
<td>1.11l</td>
<td>2.30f</td>
<td>1.97g</td>
</tr>
</tbody>
</table>

There is no difference between the means of same letter at 0.01 significance level. LSD (1%)= 2.213978

FIGURE 6
Relationships between soil organic matter contents.

The soils in this research do not pose a problem in terms of salinity and salt contents are in allowable limits [27].

**Total Organic Matter Contents of the Soils:**
Statistical analysis results of organic matter content of the soil samples are given in Table 6 and Figure 6.

Soil organic matter is one of the characteristics that need to be taken care of in lawns. The low levels of organic matter may lead to problems such as low total N content and turning the grasses to
yellow. The ratio of the parks having organic matter content greater than 3% is determined to be 25%. Since, the organic matter contents for the parks are very variable, three quarters less than 3%, they are considered as “medium” class and they can be evaluated as “soils with adequate level organic matter” [28].

**Total Lime (CaCO₃) Contents of the Soils:** Statistical analysis results of lime content of the soil samples are given in Table 7 and Figure 7.

The lime content of the soil no 11 is the maximum with the value 11.53%. High levels of lime may negatively affect the passage of elements such as Fe and Mn to the plant [27].

**Saturation Amounts of Soils:** Statistical analysis results of saturation amounts of the soil samples are given in Table 8 and Figure 8.

The soil structure and soil texture affect air and water economy, availability of plant nutritious elements, biological activity and root development, diffusion and availability of fertilizer in lawns as it also affects root diffusion [11]. High sandy soils have low water and nutritious elements retention; therefore, soil wash out is high. However, the soils in this research are not sandy in terms of texture, but they are mostly clay loams.

**TABLE 7**

<table>
<thead>
<tr>
<th>Parameter (CaCO₃) averages of soils from different parks and their groups according to LSD test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>CaCO₃</td>
</tr>
<tr>
<td>Sample No</td>
</tr>
<tr>
<td>CaCO₃</td>
</tr>
</tbody>
</table>

There is no difference between the means of same letter at 0.01 significance level. LSD (1%)= 2.213978

**FIGURE 7**

Relationships between soil CaCO₃ contents.

**TABLE 8**

<table>
<thead>
<tr>
<th>Saturation amount averages of soils from different parks and their groups according to LSD test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Saturation</td>
</tr>
<tr>
<td>Sample No</td>
</tr>
<tr>
<td>Saturation</td>
</tr>
</tbody>
</table>

There is no difference between the means of same letter at 0.01 significance level. LSD (1%)= 2.213978
CONCLUSIONS

The results of this study revealed that there was no salinity problem in the soil samples; the organic content was in “medium” class with 2.16% in average, the lime content was in “medium” class in 90% of the samples, and the texture class was clay loam according to the saturation percentages. The results are supportive and contribute to grass studies carried out in Istanbul and to the nutrition potentials according to the analysis and evaluations of soils of lawns in Esenler district parks.

The park and garden numbers are increasing in parallel to the increasing population in Turkey, where there have been many changes both in agriculture and landscaping. Fertilization is important in agriculture, and so is for landscaping and lawns. Imperfect fertilizations may negatively affect plant growth, development and appearances, and economic losses. This study reveals that soil analysis is crucial for forming the fertilization programs for gardens and parks. It should be not be forgotten that green areas are formed once by intense work and expenses to serve for many years. Therefore, soil and leaf analyses should be carried out time to time and fertilizers and required nutritional elements should be applied accordingly to benefit from these areas for a long time. Otherwise, lawns may not be long-lived and they should be reformed, but they are considered as expensive investments. Every plant in the parks should be taken care of regularly, the soil analysis results especially in lawns should be considered in order to form fertilization programs for the parks to be sustainable.

The results of this study were prepared to guide governmental agencies such as municipalities, hotels, people who deal with lawn care, as well as academics to study on this subject today or in the future. Future studies should attempt to gather more objective measures of fertilizer application to the lawn in the parks. Finally, these results are based on a relatively medium sample size that is geospatially situated.

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REFERENCES


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ENVIRONMENTAL VIBRATION EXPERIMENT AND OPTIMIZATION OF NEW ENERGY CAR BATTERY COOLING FAN

Jinbo Ren, Yu Xie

College of Mechanical and Electrical Engineering, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

ABSTRACT

This paper is a study on improving the reliability of battery cooling fans for new energy vehicles to ensure the normal operation of batteries. In order to improve the reliability of the new energy vehicle battery cooling fan developed by a company in Fuzhou, China, it is necessary to reduce the vibration level of the fan. This paper studies the vibration effects of three different vibration damping structures on the cooling fan of the new energy vehicle battery in different combinations. The optimal damping structure was obtained through experimental comparison and analysis. Under the condition of optimal damping structure of the fan, the influence of six different damping materials on the vibration of the fan was tested experimentally. The results show that the vibration energy of the two-stage damping structure of the fan is smaller in the radial direction and the axial direction when the AB type combination is used. When the damping structure adopts the TPV material under the AB type combination, the fan is in the whole speed range. The vibration is relatively stable, which may be mainly due to the small stiffness of the damping structure, the low natural frequency, and no resonance with the fan component system. This study can provide engineering reference for the vibration reduction and noise reduction of new energy vehicle battery cooling fans, thus ensuring the normal operation of new energy vehicle batteries, extending battery life and reducing environmental pollution caused by used batteries.

KEYWORDS:
Environment, Vibration, Optimization, New Energy Car, Battery Cooling Fan

INTRODUCTION

The battery is the core component of the hybrid electric vehicle and the pure electric vehicle in the new energy vehicle. During the process of high current operation or charging, a large amount of thermal energy is generated due to the internal resistance of the battery, which causes the battery temperature to rise rapidly and the electrolyte. Vaporization causes the battery to explode and is dangerous. Therefore, it is inevitable to use a fan to perform forced cooling to suppress the temperature rise of the battery during the driving or charging of the new energy vehicle. Vibration and noise reduction is one of the key issues that fans need to solve in engineering applications. Domestic and foreign scholars have done more research in this area. The literature [1-4] adopts methods such as changing the shape and angle of the fan blade to reduce the fan noise in the automobile industry; the literature [5] achieves the purpose of reducing the connection line of the automobile air conditioner by changing the structure of the muffler and improving the muffling performance. Literature [6] proposed a method to reduce noise by air-gap magnetic-density waveform reconstruction. In [7], the method of torque ripple suppression for permanent magnet synchronous motor for electric vehicles is studied.

In the past, the research on fan vibration reduction and noise reduction is mostly used in the engine cooling system of the general automobile industry. The research on the heat dissipation of new energy vehicle batteries is mainly focused on the thermal effect direction of the battery. This paper takes the battery cooling fan designed by Chery Automobile Co., Ltd. in China for the model A21ISG hybrid electric vehicle as an example. It tests and compares the vibration level of the fan with different damping structures, and studies the different reductions. The vibration material affects the vibration of the fan, and provides technical support for the vibration research of the new energy vehicle battery cooling fan, thereby improving the service life of the new energy vehicle battery and reducing the environmental impact of heavy metals during the abandonment, disposal and recycling of the battery after use.

MATERIALS AND METHODS

Model and formula. Firstly, the structure, working principle and vibration damping structure of the new energy vehicle battery cooling fan are introduced. The structure of the fan is shown in Figure 1. It consists mainly of integral outer rotor impeller, DC brushless motor, vibration damping structure and flange base. The working principle is that the
sensor sends the collected output signal to the controller. After the pulse width modulation, the drive controls the motor to drive the impeller to rotate continuously to achieve the purpose of cooling the battery pack. The fan adopts two-stage vibration reduction. In the structural solution, the first vibration-damping structure is connected to the flange through the motor wire frame, and the second vibration-damping structure is connected to the volute through the end hole of the flange base.

To simplify the calculation, the fan can be further simplified in the radial and axial directions as a single-degree-of-freedom forced vibration consisting of mass spring damping. The differential equation of motion of the model is:

\[ M_{eq} \ddot{x}(t) + C_{eq} \dot{x}(t) + K_{eq} x(t) = -\Delta m \ddot{x}_{m0} \sin \omega t \]  

In the formula: \( \ddot{x}_{m0} \) is the acceleration amplitude of the unbalanced force, \( \omega \) is the circular frequency of the load.

The steady state solution of equation (1) is:

\[ x(t) = A \sin(\omega t - \varphi) = \frac{\Delta m \ddot{x}_{m0}}{K_{eq} \sqrt{1 - \lambda^2}} \sin(\omega t - \varphi) \]  

Among of them:

\[ \varphi = \tan^{-1} \frac{2 \xi \lambda}{1 - \lambda^2} \]

\[ \omega_n = \frac{\sqrt{K_{eq}}}{M_{eq}}, \quad \xi = \frac{C_{eq}}{2 M_{eq} \omega_n}, \quad \lambda = \frac{\omega}{\omega_n} \]  

The single-degree-of-freedom structure is excited, and the force transmitted to the base through the spring and the damper can be expressed as:

\[ f_o = K_{eq} \ddot{x}(t) + C_{eq} \dot{x}(t) \]
Substituting equation (2) (3) into (5), the force transfer coefficient is obtained:

\[ f_0(t) = A[K_{eq} \sin(\omega t - \phi) + C_{eq} \omega \cos(\omega t - \phi)] \]

\[ f_0(t) = \frac{\Delta m}{K_{eq} \sqrt{(1 - \lambda^2)^2 + (2\xi\lambda)^2}} \left[K_{eq} \sin(\omega t - \phi) + C_{eq} \omega \cos(\omega t - \phi)\right] \]

Substituting equation (2) (3) into (5), the force transfer coefficient under excitation is related to the stiffness and damping of the vibration-damping structure. Increasing the damping, i.e., increasing the energy consumption, generally reduces the force transmission, but from the perspective of vibration isolation, the change is reduced. The frequency (stiffness) of the vibrating structure is an effective way to reduce fan vibration. In general, the lower the natural frequency of the damping system, the better it is to reduce vibration. Damping system natural vibration frequency \( f_0 \) with the relationship between static compression \( X \):

\[ f_0 = \frac{5}{\sqrt{X}} \]

(7)

For general damping materials, the dynamic characteristics need to be considered.

At this time:

\[ f_0 = \frac{5}{\sqrt{X}} \sqrt{\frac{E_d}{E_s}} \]

(8)

In equations (7) and (8): \( f_0 \) means natural vibration frequency of the system; \( X \) means static compression; \( E_d, E_s \) means dynamic and static elastic modulus of the damping material.

Analysis of equations (7) and (8) shows that a larger static frequency is required to obtain a lower natural frequency. In the case where conditions permit and ensure system stability, a large amount of static compression can be obtained by increasing the base mass or selecting an elastic member having a small rigidity.

**Experimental device and method.** The new energy battery cooling fan vibration test system is shown in Figure 3. The experimental device mainly includes piezoelectric acceleration sensor, DLF-4 multi-channel charge voltage filter integrating amplifier, INV5160U intelligent signal acquisition analyzer (16 channels), DASP2005 data acquisition analysis software, etc.; acceleration sensor is used to collect the vibration signal in the radial and axial directions when the fan works. To ensure the test accuracy and the reliability of the test data, the sensor is calibrated before the experiment, and the calibration coefficient is \( \lambda = 41.8 \). The amplifier is used to convert the charge signal collected by the sensor into a voltage signal, and amplify the signal for use by the data acquisition analyzer. The amplifier is provided with high and low pass filtering links. The signal acquisition analyzer is combined with the data acquisition and analysis software to form a high-performance data acquisition and signal processing system, which can analyze waveform and data records, waveform analysis, spectrum analysis, and modal analysis in the fan dynamic and static test. The test uses random sampling to set the maximum frequency that the signal can pass through 4000 Hz. The sampling frequency is 20000 Hz, in order to reduce the measurement random error, improve the test accuracy. Each measurement point is sampled 5 times, and the resulting frequency response function
is averaged. The excitation signal is processed using a Hanning window to reduce leakage errors.

During the vibration test, the fan is placed on the vibration test bench, and the fan is started. Starting from the normal working speed of 1200 r/min, the vibration of the two channels in the radial and axial directions is collected by the sensor placed on the flange. The signal is measured every 100 r/min increment until the maximum speed is 3000 r/min, i.e., the fan is tested 19 times in the range of 1200 - 3000 r/min. After the test signal is amplified by the DLF-4 amplifier and filtered by the 3160 intelligent signal acquisition instrument, the waveform and spectrogram of the fan at each stable speed are obtained, and then the vibration energy of the corresponding frequency in the spectrogram is analyzed and its influence is analyzed. Regularity, the vibration of the fan is obtained when different damping structures are used. Through comparison analysis, the combination of damping structures with the least influence on fan vibration is found. Then change the material of the optimal damping structure to polyvinyl chloride (PVC), thermoplastic vulcanizate (TPV), nitrile rubber (NBR), polytetrafluoroethylene (PTFE), EPDM (EPDM), The same experimental method was used to analyze the influence of the vibration damping structure on the vibration energy of the fan when different damping materials were used.

RESULTS AND DISCUSSION

Literature [8] and [9] have conducted preliminary research on the vibration and vibration reduction technology of new energy battery cooling fans, and concluded that the two main factors affecting fan vibration are the unbalanced mass of the rotating body and the DC brushless motor. Commutation and cogging pulsation. Among them: the vibration frequency caused by the unbalanced mass of the rotary body is located at the 1st frequency component of the rotational speed, and the vibration frequency caused by the commutation of the motor and the pulsation of the cogging is located at the 12-frequency component of the rotational speed. In this paper, the vibration damping structure of the fan is deeply studied and optimized. The power spectrum of the 1x and 12th frequency of the rotating body is still used as the vibration energy measurement index. In the experiment, 9 sets of experiments were carried out using 3 different damping structures. The damping structures of 3 different types are shown in Fig. 4. The first and second damping structures used in the nine sets of experiments are shown in Table 1.

![Damping pad structure](image)

**FIGURE 4**  
Damping pad structure

| Experimental conditions |  
|---|---|---|---|
| No | Record short name | First damping structure type | Second damping structure type | Range of rotation (r/min) |
|---|---|---|---|
| 1 | AA | A | A | 1200-3000 |
| 2 | AB | A | B |   |
| 3 | AC | A | C |   |
| 4 | BA | B | A |   |
| 5 | BB | B | B |   |
| 6 | BC | B | C |   |
| 7 | CA | C | A |   |
| 8 | CB | C | B |   |
| 9 | CC | C | C |   |

Effect of vibration on the 1st frequency component of the rotating speed. The vibration energy at the 1st frequency of the rotating speed is mainly caused by the imbalance of the mass of the rotating body. In the experiment, the vibration signals of the 2 channels in the radial and axial directions are tested and analyzed, and the influence law of the vibration energy is obtained as shown in Fig. 5.
It can be seen from Fig. 5 that the vibration effects at the 1st frequency of the rotor in the radial and axial directions are very regular and basically similar. Under various operating conditions, the rotating body at low speed (1200-2200 r/min) the mass imbalance has little effect on the vibration of the fan, and the influence of vibration at high speed (2300-3000 r/min) increases sharply. In the radial direction: in the first group of experiments, the vibration-damping structure has the greatest influence on the vibration of the fan; in the second group of experiments, the vibration of the fan in the whole speed range is small and the vibration energy is small, and the vibration energy of the second group at maximum speed (3000 r/min) was only 7.3% of the first set of experiments. In the axial direction: the damping structure in the 9th experiment has the greatest influence on the vibration of the fan; in the second experiment, the vibration energy of the fan is the smallest, and the vibration energy in the second group is only the maximum speed (3000 r/min). 6.52% of the 9th experiment.

Comparing the values of the vibration power spectrum in the radial and axial directions, it is also found that the vibration intensity in the radial direction is significantly higher than the axial direction; in several of the experiments, it can be seen that the fan is rotating at 2000 r/min, 2600 r/min running, the curve is convex upwards, indicating that there may be resonance between the parts at the two speeds or the resonance of the parts and the fan system; when the fan is running at a speed of about 2700 r/min, it can be observed by the rotation. The vibration energy caused by the imbalance of body mass is significantly reduced. The reason for this phenomenon can be understood as the possibility that the vibration frequency of the fan parts at this speed is equal and the phase is opposite, thus generating a resonance cancellation phenomenon.
The vibration energy at the speed of 12 times is mainly caused by the commutation of the brushless DC motor and the pulsation of the cogging. The vibration and test are also performed on the radial and axial directions in the experiment. The results are shown in Fig. 6.

It can be seen from Fig. 6 that the mass imbalance of the rotating body at 1 octave has the opposite effect on the vibration of the fan. The influence of the commutation of the brushless DC motor and the pulsation of the cogging on the vibration of the fan is mainly manifested at low speed and low speed. The time (1200-2100 r/min) has a great influence on the vibration. When the rotation speed is increased (2200-3000 r/min), the vibration energy is small and relatively stable. In the whole speed range, the energy fluctuations are more frequent, and there is no obvious regularity. Among them, in the first and fourth sets of experiments, the human ear can hear the humming sound in the whole speed range of the fan, and it is judged that the electromagnetic vibration force generated by the commutation of the motor after using the two kinds of vibration damping structures is more induced. The noise generated by the vibration, so the two damping structures used in the first and fourth sets of experiments are less effective. In the second, third and fifth experiments, the 12-fold pulsating energy is weaker over the entire speed range. The reason for this result may be: when using the three damping structures in the 2, 3, and 5 sets of experiments, the system stiffness is reduced, the contact area is reduced, and the natural frequency of the fan system is reduced, no resonance occurs, and the vibration damping structure is different. The pulsation of some frequency components is absorbed.
and eliminated by the vibration damping structure, so the vibration of the high frequency pulsation is greatly reduced. When other types of vibration damping structures are used, when the fan is running at a certain speed, the high frequency ripple of the brushless motor may be exactly the same as the natural frequency of the fan, which may cause high frequency pulsation and resonance of the fan system, so the high frequency pulsation is large.

It can be seen from the comparative analysis of Figures 5 and 6 that the vibration-damping structure of the nine different experiments has different effects on the vibration of the fan. After comprehensive comparison, it is found that the fan vibration caused by the AA type vibration damping structure is the largest, and the CC type vibration damping structure is the second. The vibration of the fan is the smallest when the AB type vibration damping structure is adopted, indicating that the AB type vibration damping structure can greatly absorb and attenuate the fan. Vibration in the radial and axial directions; on the other hand, the stiffness of the AB-type damping structure makes the natural frequency of the brushless DC motor and the operating frequency do not coincide, avoiding resonance. In this paper, the AB type optimal vibration damping structure will be used to study the vibration of the fan.

**Effect of damping material on fan vibration.**
In the experiment, the fan damping structure adopts the AB type combination. The first and second damping structures are made of the same material each time, which are respectively silicone rubber (MVQ), polyvinyl chloride (PVC), thermoplastic vulcanizate (TPV), butyronitrile. Rubber (NBR), polytetrafluoroethylene (PTFE), EPDM (EPDM), test the vibration level of the damping structure using different materials, as shown in Fig. 7 and 8.

![Vibration at 1 octave of the rotational speed of different damping materials](image)
It can be seen from Fig. 7 and Fig. 8 that when the PVC material is used, the vibration of the fan at the 1st frequency of the rotating speed is severe. When the NBR, PVC, and MVQ materials are used, the vibration intensity of the fan at a speed of 12 times is high at a low speed. When the TPV material is used, the vibration level of the fan at the 1st and 12th frequency components is relatively stable, and the vibration energy is not large. When the fan is operated at 3000 r/min, the vibration at the 1st frequency component is The maximum value is reduced by 77.2%, and the vibration at the 12-fold frequency component is reduced by 70.6% compared with the maximum value, thereby improving the reliability of the fan and ensuring the normal heat dissipation of the battery. Delay the battery's environmental pollution.

(2) Comparing the amplitude and power spectrum of the vibration waveform in both the radial and axial directions, it is found that the vibration energy in the radial direction of the fan is significantly higher than the axial direction.

**CONCLUSIONS**

1. The fan damping structure adopts AB type combination and the vibration damping effect is best when using TPV material. At this time, the vibration energy of the fan at the 1st frequency component of the rotation speed is decreased by 77.2%, and the vibration at the 12th frequency component is decreased by 70.6% compared with the maximum value, thereby improving the reliability of the fan and ensuring the normal heat dissipation of the battery. Delay the battery's environmental pollution.
(3) As the rotational speed increases, the vibration energy at the 1st frequency component of the rotational speed in the radial and axial directions gradually increases, especially at high rotational speeds; the vibration energy increases sharply; as the rotational speed increases, the radial and axial directions. The vibration energy at the 12th frequency component of the upper speed gradually decreases, and the vibration energy at the low speed is larger; the vibration imbalance of the rotor is affected by the vibration of the fan at high speed, the commutation and teeth of the DC brushless motor. The effect of slot pulsation on the vibration of the fan is manifested at low speed, which also indicates that the brushless DC motor is suitable for high speed conditions.

(4) In the vibration test at 1 r/min, the energy curve of vibration at part of the speed is convex upwards, and there may be resonance phenomenon; when the speed is about 2700 r/min, the vibration energy is significantly reduced, possibly due to vibration. Energy produces a resonance cancellation phenomenon;

(5) In the vibration test at 12 times frequency, the vibration energy in the 2, 3, and 5 sets of experiments is weak in the whole speed range, indicating that there is no resonance phenomenon in the system or the pulsation of some frequency components is absorbed by the vibration damping structure.

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EFFECT OF MATRIC SUCTION ON STRENGTH CHARACTERISTICS OF UNDISTURBED UNSATURATED SILTY CLAY

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ABSTRACT

To study the influence of matric suction on the strength characteristics of soil, consolidated and drained triaxial shear tests on undisturbed silty clay were conducted using GDS unsaturated soil triaxial apparatus under different matric suction conditions. The results show that the peak strength of soil increases with the increase of matric suction, and the rate of increase increases first and then decreases. With increased matric suction, the total cohesion increases and the extent of this increase gradually decreases, while the effective internal friction angle decreases slightly and then tends to be stable. The effect of matric suction on shear strength is mainly reflected in the total cohesion. Matric suction has the most significant influence on soil strength at 50 kPa to 100 kPa. In the presence of matric suction, the area of action of water film on soil particles determines the change of total cohesion, and the pore air pressure and pore water determine the change in effective internal friction angle. The angle used to describe the increase in the shear strength of soil with an increase in matric suction is \( \varphi^b = 28.2^\circ \). According to Fredlund’s strength theory, the formula of shear strength parameters of unsaturated silty clay in the study area under consolidation and drainage conditions was given, and the relationship between strength parameters and matric suction was fitted.

KEYWORDS:
Matric suction, unsaturated soil, strength, GDS triaxial apparatus

INTRODUCTION

Due to changes in climate, temperature and other natural conditions and the effect of the alternation of dry and wet periods, natural soils are generally in an unsaturated state within a certain depth range: in practice, on underground engineering, road engineering, slope engineering projects, etc., the soil layer involved is even more so. Soil strength forms an important basis for engineering design and evaluation of rock and soil properties. For unsaturated soils, it is impossible to analyse and interpret all kinds of engineering problems caused by strength comprehensively by continuing to apply the strength theory of saturated soils to all kinds of engineering construction. Therefore, the unsaturated strength characteristics of soils are particularly important, and the strength of unsaturated soils depends directly on suction, therefore, it is necessary to study the strength characteristics of unsaturated soils with suction.

To date, some researchers have used improved triaxial apparatus [1], direct shear apparatus, and ring shear apparatus [2], which can control suction, and combined with model prediction and test comparison [3], analyse strength variations in different types of unsaturated soils, such as clay [4], silt [5], and expansive soil [6] under the action of suction. Due to suction and its being influenced by internal and external environmental factors, the properties of different types of unsaturated soils are complex and changeable. In addition, there are some differences in test equipment, sample size, operation methods and related theoretical models in existing studies, which leads to some deficiencies in current research results. Firstly, compared with saturated soils, it is time-consuming and expensive to measure shear strength parameters of unsaturated soils by direct testing, which results in existing research focusing on empirical formula estimation, model prediction, and other aspects, such as: multiple-linear regression (MLR) prediction models [7], adaptive neuro-fuzzy inference system (ANFIS) models [8], pedotransfer function (PTF) estimation methods [9], three-dimensional discrete element analyses [10], artificial neural network (ANN) and regression tree (CART) prediction technologies [11], and so on. The universality of the results needs to be verified due to the relatively small amounts of laboratory test data and the constraints of basic premises and assumptions. Secondly, compared with triaxial testing, direct shear testing has cheaper and faster, therefore, to achieve rapid, practical, results, the improved direct shear apparatus [12, 13, 14, 15], which can control suction, or the separation method of suction measurement
(mainly filter paper method) and strength test (mainly direct shear test) [16, 17, 18] are often used, so it is difficult to ensure the matric suction is constant during shearing. In addition, the shear plane is determined artificially by a direct shear test, the consolidation conditions cannot be controlled, and the imposed stress state does not reflect in situ conditions.

In view of the above problems, combined with the current “One Belt, One Road” initiative in China, a typical unsaturated silty clay in the planning and construction area was taken as a research object. GDS unsaturated soil triaxial apparatus, which integrates suction control and strength test, was used to study the influence of matric suction on soil strength during dehydration process, and the relationship formula related to the strength of silty clay in the study area was given according to the experimental results to provide reference and guidance for theoretical research and construction on such unsaturated soils.

STRENGTH THEORY OF UNSATURATED SOILS

Based on unsaturated effective stress principle and double stress state variable theory, many scholars have proposed various theoretical formulae for calculating the shear strength of unsaturated soils. Among them, the most representative is Fredlund’s strength theory of unsaturated soils [19], which uses two stress state variables, net normal stress \((\sigma - u_w)\) and matric suction \((u_a - u_w)\), to describe the stress state of unsaturated soils, so as to determine the shear strength of soils, as shown in formula (1).

\[
\tau_f = c' + (\sigma - u_a) \tan \varphi' + (u_a - u_w) \tan \varphi^b \tag{1}
\]

where \(c'\) is the effective cohesion (kPa), \(\sigma\) denotes the total normal stress (kPa) on the failure surface at the failure time, \(u_a\) is the pore air pressure (kPa) on the failure surface, \(u_w\) is the pore water pressure (kPa) on the failure surface, \(\varphi'\) is the internal friction angle (°) related to the net normal stress state variable \((\sigma - u_a)\), and \(\varphi^b\) is the rate at which shear strength increases with the matric suction \((u_a - u_w)\).

The total cohesion \(c'_t\) is the sum of the effective cohesion and the apparent cohesion caused by matric suction, as given by formula (2).

\[
c'_t = c' + (u_a - u_w) \tan \varphi^b \tag{2}
\]

The stress state variables adopted in Fredlund’s formula for shear strength of unsaturated soils are the combination of \((\sigma - u_a)\) and \((u_a - u_w)\) based on pore air pressure \(u_a\). Based on Mohr-Coulomb strength theory, this formula for the shear strength of unsaturated soils was proposed, and the transition between the two formulae can be smooth. When the soil is saturated, the pore space is completely filled with water and there is no pore air. At this time, the pore pressure is zero and there is no matric suction, and the above formula gives the shear strength of such a saturated soil.

SOIL SAMPLE PROPERTIES AND EXPERIMENTAL WORK

Basic properties of soil samples. The soil sample is silty clay, taken from the node city of the “China-Mongolia-Russia economic corridor” in China’s “One Belt, One Road” construction plan, in the Changchun Jingyue National high and new-tech development zone (Figure 1). To ensure that the soil sample is in the same state to the greatest extent possible, the distance between adjacent boreholes is 2 m and the sampling depth is 5.8 m to 6.0 m. The soil samples, in their native state, are yellowish-brown and plastic. The results of laboratory tests show that the basic physical and mechanical indices in Table 1, the mineral composition of undisturbed soil samples is summarised in Table 2, and the particle size distribution curve is as shown in Figure 2. The clay (<0.005mm) is 35.53%, and the silt (0.005-0.075mm) is 63.73%. According to the analysis, the soil in this area is a medium-compressive silty clay, and the primary minerals dominated by quartz and feldspar account for 69.3%, and the secondary minerals dominated by clay minerals account for 30.7%. Among them, the mixed layer with strong hydrophilicity accounts for 24%, and this indicates that the silty clay in this area has relatively strong hydrophilicity.

<table>
<thead>
<tr>
<th>Soil depth (m)</th>
<th>Soil sample number</th>
<th>Moisture content (\omega) (%)</th>
<th>Saturation (S_{s}) (%)</th>
<th>Natural density (P) (g/cm(^3))</th>
<th>Liquid limit (\omega_{1l}) (%)</th>
<th>Plastic limit (\omega_{pl}) (%)</th>
<th>Liquidity index (I_l)</th>
<th>Plasticity index (I_p)</th>
<th>Coefficient of compressibility (a_{c}) (MPa(^{-1}))</th>
</tr>
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<tr>
<td>5.8-6.0</td>
<td>1</td>
<td>25.9</td>
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<td>1.85</td>
<td>37.9</td>
<td>23.7</td>
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<td>14.16</td>
<td>0.33</td>
</tr>
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<td>2</td>
<td>26.8</td>
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<td>1.88</td>
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<td>26.4</td>
<td>86.5</td>
<td>1.87</td>
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<td>14.12</td>
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<tr>
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<td>88.1</td>
<td>1.89</td>
<td>39.3</td>
<td>25.0</td>
<td>0.10</td>
<td>14.31</td>
<td>0.34</td>
</tr>
</tbody>
</table>

TABLE 1
Basic physico-mechanical soil properties
FIGURE 1
Soil sample location diagram -- Changchun, the node city of the “China-Mongolia-Russia economic corridor” in China’s “One Belt, One Road” construction plan

TABLE 2
Mineral composition of undisturbed soil samples

<table>
<thead>
<tr>
<th>Mineral content of(B)/10²</th>
<th>Qtz</th>
<th>Kfs</th>
<th>Pl</th>
<th>I/S</th>
<th>Ill</th>
<th>Kln</th>
<th>Chl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48.3</td>
<td>5.3</td>
<td>15.7</td>
<td>24.0</td>
<td>4.6</td>
<td>1.2</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Note: Qtz: Quartz  Kfs: Kfeldspar  Pl: Plagioclase  I/S: Illite/Smectite mixed layer  Ill:Illite  Kln:Kaolinite  C: Chlorite
Experimental apparatus. Because of the existence of matric suction, it is impossible to analyse and interpret all kinds of engineering problems caused by mechanical properties of unsaturated soils by conventional triaxial apparatus, therefore, the strength characteristics of unsaturated silty clay in the Changchun area were studied by using GDS unsaturated soil triaxial apparatus while considering the influence of matric suction. As shown in Figure. 3, the system consists of a controller, a triaxial double pressure chamber (including a loading-reaction system), and a data acquisition system. Among them, the controller provides, and controls, the axial pressure, confining pressure, pore air pressure, pore water pressure, and temperature. Data were converted, collected, and processed by the data acquisition system through a data acquisition board, sensor, and computer. The soil sample was installed in the internal pressure chamber, and the controller and data acquisition board were connected to the triaxial double pressure chamber test equipment. The whole test process was monitored, and controlled in real-time, by computer software. The axial pressure, confining pressure, pore air pressure, and pore water pressure of the sample in the double pressure chamber could be measured and controlled. The structure of the internal and external pressure chamber is shown in Figure. 4. The equipment was mainly used to demonstrate the characteristics of soil above groundwater level. It can simulate actual working conditions according to prevailing engineering geological conditions, hydrogeological conditions, and stress states under different construction environments. It offers advantages in terms of stress control (stress path, etc.), data acquisition (measurement accuracy, etc.), loading mode (low-frequency cyclic loading, etc.) and temperature control.

FIGURE 3
GDS unsaturated soil triaxial test system

FIGURE 4
Triaxial double pressure chamber test equipment for GDS unsaturated soil
TABLE 3
Consolidation and drainage shear experiment plan of soils under different matrix suctions

<table>
<thead>
<tr>
<th>Group number</th>
<th>Soil sample number</th>
<th>Pore air pressure $\sigma_a$ (kPa)</th>
<th>Matric suction $\sigma_m - \sigma_w$ (kPa)</th>
<th>Confining pressure of constant-suction consolidation $\sigma_3$ (kPa)</th>
<th>Net confining pressure $\sigma_3 - \sigma_a$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>1-2</td>
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<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1-3</td>
<td>2-1</td>
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<td>50</td>
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<td>3-1</td>
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<td>100</td>
<td>305</td>
<td>200</td>
</tr>
<tr>
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<td>150</td>
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</tr>
<tr>
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<td>5-2</td>
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<td>200</td>
<td>405</td>
<td>300</td>
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<tr>
<td>5-3</td>
<td></td>
<td></td>
<td></td>
<td>505</td>
<td>300</td>
</tr>
</tbody>
</table>

TABLE 4
Experimental results: peak strength of soil samples under different suction and confining pressures

<table>
<thead>
<tr>
<th>Matric suction (kPa)</th>
<th>$\sigma_3$ (kPa)</th>
<th>Peak strength (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>214.168</td>
</tr>
<tr>
<td>50</td>
<td>200</td>
<td>245.734</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>364.734</td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experimental plan. Suction is an important stress variable of unsaturated soils, which directly determines the strength characteristics of most soils, it usually consists of two parts: matric suction and osmotic suction (or solute suction). When the solute concentration in the pore water remains constant, the osmotic suction corresponding to any water content is also constant [20], therefore, at room temperature, neglecting the influence of osmotic suction, the matric suction was controlled to 0 kPa, 50 kPa, 100 kPa, 150 kPa, and 200 kPa respectively, and the shear strength characteristics of soil were analysed; because unsaturated soils encountered in engineering practice, were mostly formed by dehydration processes, the soil sample should be saturated before testing. The dehydration process was simulated by GDS unsaturated soil triaxial test equipment, and the suction controlled to a specified value. Triaxial consolidation and drainage shear tests were carried out in three stages of constant-suction balance, constant-suction consolidation, and constant-suction shear.

The soil sample used in the test is a cylindrical sample measuring 38 mm x 76 mm, which is vacuum-saturated. Because the soil depth sampled is 5.8 m to 6.0 m, referring to the standard for soil test method [21], the net confining pressure is provided by the confining pressure controller and set to 100 kPa, 200 kPa, and 300 kPa, respectively. The pore air pressure is provided by the air pressure controller, and the pore water pressure is provided by the water pressure controller, and the difference between the two is the matric suction. To dissipate the pore air pressure and pore water pressure during shearing, a shear displacement rate of 0.005 mm/min was adopted in accordance with the relevant standard [21] and previous experience. The experiment plan is summarised in Table 3.

RESULTS

Effect of confining pressure on deformation and strength characteristics of soil samples. The experiment results of peak strength under different confining pressures and matrices suction are summarised in Table 4. When a certain matric suction is applied, the stress-strain curves of the soil sample under different confining pressures are as shown in Figure. 5. It can be seen (Table 4) that, with the increase of confining pressure, the peak strength of the soil increases gradually, and with the increase
of matric suction, the overall of increasing extent trend increases and then decreases.

It can be seen (Figure 5) that, when the matric suction is zero, the stress-strain curves of soils show certain strain softening characteristics with the change in confining pressure, and the smaller the confining pressure, the more pronounced the softening characteristics. When the matric suction is non-zero, the stress-strain curves with the change of confining pressure all show certain hardening characteristics, and the higher the suction, the more pronounced the strain hardening.

It can be seen from the analysis that the inter-particle bond strength arises from cohesive forces (cementing force, double-layer electric force, van der Waals force, etc.) and an interlocking force. When the matric suction is zero, the bond between soil particles is destroyed with the increase of shear stress under low confining pressure, which shows certain softening characteristics. With the increase of confining pressure, the structure of these soil samples becomes more compact during consolidation, and the connection strength increases with the increase of interlocking force between particles, which decreases the extent of post-peak softening. When the matric suction is constant, bond strength and adsorption strength bond the particles. Under the same confining pressure and shear stress conditions, the loss of bond strength can be compensated for by the adsorption strength component, and the specimen exhibits certain strain hardening characteristics. The lower the suction, the smaller the confining pressure, the smaller the bond and adsorption strengths, and vice versa, therefore, the degree of soil hardening differs.

Effect of matric suction on deformation and strength characteristics of soil samples. Under the same confining pressure and different matric suction, the peak strengths of the soil samples are as summarised in Table 5, and the stress-strain curves are as shown in Figure 6: with increased matric suction, the peak strength of each soil sample increases and then decreases, and with increased confining pressure, the decrease of increase extent and the increase of decrease extent.

![Stress-strain curves at the same matric suction under different net confining pressures](image-url)
TABLE 5
Experimental results: peak strength of soil samples under different suction and confining pressures

<table>
<thead>
<tr>
<th>Matric suction (kPa)</th>
<th>Confining pressure (kPa)</th>
<th>100</th>
<th>200</th>
<th>300</th>
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<th>200</th>
<th>300</th>
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</thead>
<tbody>
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<td>214.168</td>
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<td>31.566</td>
<td>90.654</td>
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</table>

FIGURE 6
Stress-strain curves at the same net confining pressure under different matric suctions

FIGURE 7
Stress analysis diagram of unsaturated soil air-water interface
The analysis shows that, when the matric suction is zero, there is only the structural bond strength acting between the particles. When the stress increases to the yield strength of the soil structure, i.e., its peak strength, the inter-particle connections are destroyed, and the strength then decreases. In the presence of matric suction, adsorption strength between soil particles will be generated, and the loss of strength that beyond the bond strength of soil structure will be offset by the adsorption strength, thus the peak strength will be increased. When the matric suction reaches a certain value (at 200 kPa), under the effect of the matric suction, the influence of the water film on particles smaller, resulting in a smaller of decrease extent in cohesion. As the matric suction increases, the greater the surface tension, the more significant the changes in the geometry of closed bubbles and the curved pore water surface, which will make the area acted upon by the water film on particles smaller, resulting in a smaller of increase extent in cohesion. As the matric suction increases from 0 kPa to 50 kPa, the total cohesion becomes 4.35 times, and the increase increases first and then decreases. The effective internal friction angle becomes 0.96 times the original value, it increases and then decreases with the increase of matric suction and tends to be stable thereafter, therefore, the influence of matric suction on the shear strength of unsaturated silty clay mainly depends on the total cohesion.

According to the analysis, with the increase of matric suction, the adsorption strength increases, and thus the total cohesion increases; on the other hand, when the adsorption strength exists, the soil particles will have a tendency of relative movement and gradual compacting (Figure. 7): the larger the matric suction, the greater the surface tension, the more significant the changes in the geometry of closed bubbles and the curved pore water surface, which will make the area acted upon by the water film on particles smaller, resulting in a smaller of increase extent in cohesion. As the matric suction increases from 0 kPa to 50 kPa, and the pore water reduces lubrication, which leads to an increased internal friction angle. With increased matric suction, the pore air increases continuously: this decreases the interlocking force between particles and decreases the effective internal friction angle to some extent.

Shear strength parameters of soil samples. Based on the two-stress variable theory of net normal stress and matric suction [22], and according to the consolidation and drainage test data obtained by the GDS unsaturated soil triaxial test system, the expanded Mohr-Coulomb failure envelope of unsaturated soils under different matric suction is drawn (Figure. 8). The total cohesion and effective internal friction angle can be obtained (Table 6): the total cohesion of the silty clay changes significantly with matric suction. When the matric suction increases from 0 kPa to 200 kPa, the total cohesion becomes 4.35 times, and the increase increases first and then decreases. The effective internal friction angle becomes 0.96 times the original value, it increases and then decreases with the increase of matric suction and tends to be stable thereafter, therefore, the influence of matric suction on the shear strength of unsaturated silty clay mainly depends on the total cohesion.

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### TABLE 6

<table>
<thead>
<tr>
<th>Soil sample number</th>
<th>Matric suction $u_u - u_w$ (kPa)</th>
<th>Net confining pressure $\sigma_3 - \sigma_1$ (kPa)</th>
<th>Deviatoric stress $\sigma_1 - \sigma_3$ (kPa)</th>
<th>Total cohesion $c'$ (kPa)</th>
<th>Effective internal friction angle $\phi'$ (°)</th>
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<tbody>
<tr>
<td>1-1</td>
<td>0</td>
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<td>214.168</td>
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<td>26.9</td>
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<tr>
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<td>100</td>
<td>858.299</td>
<td>167.7</td>
<td>23.3</td>
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<td>858.299</td>
<td>167.7</td>
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<td>4-3</td>
<td>300</td>
<td>100</td>
<td>746.495</td>
<td>167.7</td>
<td>23.3</td>
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<td>100</td>
<td>746.495</td>
<td>167.7</td>
<td>23.3</td>
</tr>
<tr>
<td>5-2</td>
<td>200</td>
<td>300</td>
<td>858.299</td>
<td>167.7</td>
<td>23.3</td>
</tr>
<tr>
<td>5-3</td>
<td>300</td>
<td>200</td>
<td>723.649</td>
<td>167.7</td>
<td>23.3</td>
</tr>
</tbody>
</table>
GDS unsaturated soil triaxial equipment can dynamically monitor the change of void ratio during the test. According to the monitored data, the curves of void ratio with matric suction at the equilibrium stage under equal suction consolidation at different confining pressures are drawn (Figure 9): the void ratio decreases gradually with increasing matric suction, and the change in void ratio is most significant at matric suctions in the range of 50 kPa to 100 kPa. It can be seen from the analysis that the soil itself has a certain bond strength arising from inter-particle structural connections, and adsorption strength will only be generated between particles when matric suction is developed: when the matric suction increases from 0 kPa to 50 kPa, the structural bond strength plays a major role. The adsorption strength is insufficient to make the soil particles move and thus densify the soil mass, so the void ratio changes slightly. At this time, the effective cohesion remains unchanged, the apparent cohesion and the effective internal friction angle increase, and the soil strength increases; when the matric suction increases from 50 kPa to 100 kPa, the adsorption strength plays a major role, overcoming some unstable structural connections, making the soil particles move and become more densely packed and the void ratio decreases rapidly. At this time, the effective cohesion decreases slightly because of the increase in pore air pressure, but the apparent cohesion increases to a significant extent, and the strength of the soil increases accordingly; when the matric suction increases from 100 kPa to 200 kPa, the stable structural connection strength and adsorption strength play a joint role. There is no movement between particles and the change in void ratio is small. At this time, the effective internal friction angle is unchanged due to the increase of pore gas, and the decrease of pore water pressures, but the apparent cohesion increases and the strength of soil increases, therefore, when the matric suction is between 50 kPa to 100 kPa, the void ratio changes to a significant extent, and the effect of adsorption strength on soil strength is the most significant.

The curves of total cohesion and effective internal friction angle change with matric suction are shown in Figure 10: the total cohesion and the effective internal friction angle gradually stabilise when the matric suction is greater than 150 kPa, and there is a certain functional relationship between the matric suction and the total cohesion and the effective internal friction angle. The relationship between the matric suction and the total cohesion and the effective internal friction angle was fitted through analysis, as shown in Table 4, which provides the basis for the establishment of relevant models and the selection of parameters.
Variation curve of void ratio with matric suction at different confining pressures

Curves of total cohesion and effective internal friction angle with matric suction

Failure envelope of the matric suction shear stress plane

<table>
<thead>
<tr>
<th>Strength parameter</th>
<th>Functional relation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cohesion (kPa)</td>
<td>$c' = -5 \times 10^{-5} (u_d - u_w)^3 + 0.0152 (u_d - u_w)^2 - 0.6315 (u_d - u_w) + 28.691$</td>
<td>0.9825</td>
</tr>
<tr>
<td>Effective internal friction angle (°)</td>
<td>$\varphi' = 7 \times 10^{-6} (u_d - u_w)^3 - 0.0026 (u_d - u_w)^2 + 0.2227 (u_d - u_w) + 23.336$</td>
<td>0.9973</td>
</tr>
</tbody>
</table>
Figure. 11 shows the failure envelope of the matric suction-shear stress plane. It can be seen from the graph that the angle $\phi^b$ used to describe the increase of shear strength of soil with the increase of matric suction is a variable which has a non-linear relationship with matric suction, and they have a certain functional relationship. Regardless of the non-linearity of failure envelope, $\phi^b = 28.2^\circ$ can be chosen. According to Fredlund double-stress variable theory formula of unsaturated soil, referring to its form, the shear strength parameter formula of unsaturated silty clay under consolidation and drainage conditions can be obtained, as shown in formula (3).

$$
\tau_f = 27.3 + (\sigma - u) \tan 23.3^\circ + (u - u_w) \tan 28.2^\circ
$$

(3)

**CONCLUSION**

Based on triaxial shear tests of typical unsaturated silty clay in Changchun, China, under different matric suction conditions, the following conclusions were obtained:

1. Matric suction causes the soil to have certain hardening characteristics, and the higher the suction, the greater the confining pressure, the more pronounced the hardening characteristics. With the increase of matric suction, the peak strength increases first and then decreases, and with the increase of confining pressure, the decrease gradually of increase extent and the increases gradually of decrease extent.

2. When the matric suction increases from 0 kPa to 200 kPa, the total cohesion increases to 4.35 times its original value, and the effective internal friction angle becomes 0.96 times the original value. With the increase of the matric suction, the total cohesion increases first and then decreases, and the effective internal friction angle increases first and then decreases, thereafter tending to be stable. The effect of matric suction on the shear strength of the unsaturated silty clay depends mainly on the total cohesion.

3. Matric suction has the most significant effect on soil strength between 50 kPa and 100 kPa. The area of action of water film on particles determines the change in total cohesion, and the size of pore air and pore water determine the change in effective internal friction angle.

**ACKNOWLEDGEMENTS**

The authors are grateful for the financial support for the study presented in this paper from National Natural Science Foundation of China (Nos. 41472242, 51890914, 41602285), Science and Technology Development Program of Jilin Province, China (No.20180520064JH). We sincerely thank the editors and all anonymous reviewers for their constructive and excellent reviews that helped to improve the manuscript.

**REFERENCES**


MODULATION OF BIOELEMENT CONCENTRATION AND MACROMOLECULE CONFORMATION IN LEAVES OF SALVIA COCCINEA BY SALICYLIC ACID AND SALT STRESS

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2Center of Bioimmobilisation and Innovative Packaging Materials, West Pomeranian University of Technology, 35 Janickiego Str., 71-270 Szczecin, Poland
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ABSTRACT

We investigated the effects of salicylic acid (0.5 and 1.0 mM SA) and salinity (100 mM NaCl) on chemical composition of Salvia coccinea leaves. It is a little known species of sage grown as a medicinal and ornamental plant. We used standard chemical analyses to determine the content of micro- and macronutrients and advanced instrumental methods (FTIR and Raman spectroscopy) to assess the quantity and quality of biogenic compounds. Salt and SA treatment changed the levels of the investigated minerals, except for Zn and Cu. SA in salt-exposed plants enhanced the leaf content of P, K and K/Na ratio, as compared with salt-exposed plants not treated with the acid. Molecular spectroscopic analyses confirmed that SA affected protein, lipid, lignin and pectin content in S. coccinea leaves. We confirmed usefulness of FTIR and Raman spectroscopy for examining biochemical changes caused by SA and salinity stress within the plant tissues.

KEYWORDS:
Functional groups, sage, salicylic acid, spectroscopy, mineral uptake

INTRODUCTION

Salvia coccinea Buch'hoz ex Etl. (Lamiaceae) also called blood sage, banderilla, Texas sage, scarlet sage or tropical sage is one of less known but valuable sage species. It is a medical and ornamental plant common in south-east United States, Mexico, the Caribbean (Cuba), north America, south Africa and the eastern Himalayas [1, 2]. Depending on the site S. coccinea is an annual or perennial plant. Folk medicine uses S. coccinea as an analgesic, anti-inflammatory, antispasmodic and sedative agent. S. coccinea extracts help to cure nausea, bronchitis, flatulence, anorexia or ulcerative colitis. It has also been found to have antioxidant, antifungal, carminative, antiemetic, diaphoretic, stimulant, emmenagogue, and anticatarrhal activities [3]. Phytochemical studies [4] confirmed the presence of many secondary metabolites but comprehensive data on individual mineral content in S. coccinea are still missing.

Salicylic acid (SA), along with other phenolic acids, is an endogenous growth and metabolism regulator in vascular plants. It specifically regulates plant growth and development, participates in biochemical transformations and in various physiological processes [5, 6]. SA also protects plants against multiple stresses related to salinity, temperature, ultraviolet radiation, heavy metals and drought [7]. SA may affect nutrient uptake and transfer of assimilates [8] but previous studies in this field yielded ambiguous results [9]. We also have only limited information on quantitative and qualitative changes in individual functional groups in plants treated with SA.

Salt stress interferes with biochemical and physiological processes, causes growth and development disruption, and disturbs nutrient homeostasis. Plants show different tolerance to salt stress; they may either remove toxic ions or tolerate them by synthesizing compatible substances that protect membrane structures against salt excess [10, 11]. Treating plants with various growth regulators and biostimulators is one of the ways of limiting adverse effects of salt in agricultural practice [12-14]. Detailed studies on the role of elicitors in salt-exposed plants provide huge cognitive insight and may help in developing effective plant protection against stress.

S. coccinea responses negatively to excessive salt presence in the substrate, so looking for ways improving the species cultivation under this stress seems justified [15]. Our preliminary studies [16] showed that SA alleviates moderate salt stress in S. coccinea, enhances plant biomass, content of total chlorophyll, carotenoids, and polyphenols. However, molecular spectroscopic investigation in the SA
and NaCl-treated plants has not been reported. In this study we assumed that SA may modify mineral composition and macromolecule conformation of S. coccinea plants. To determine changes in lipids, proteins, carbohydrates and other organic polymers (e.g. lignin, pectin) we used Fourier transform infra-red (FTIR) and Raman spectroscopy.

**MATERIALS AND METHODS**

**Plant growth conditions and experimental design.** In the last decade of May 2014 S. coccinea plants grown from seeds (Legutko, Jutrosin, Poland) were planted into 2 dm³ pots containing deacidified peat of pH 6.5 containing (mg dm⁻³): 17 NO₃ – N, 7 NH₄ – N, 11 P₂O₅, 18 K₂O, 2.7 MgO, 8 S, 0.015 B, 0.2 Fe, 0.02 Mn, and 0.02 Zn. The plants were cultivated until 20 September 2014 under natural photoperiod in a two-layer plastic tunnel at an average temperature of 20.6 °C. Since the beginning of June they were sprayed three times (every seven days) with salicylic acid (Sigma Aldrich, St. Louis, MO, USA) at 0.5 and 1.0 mM, at about 20 cm³ of the solution per plant. Control plants were sprayed with distilled water. Since the beginning of July the plants were subjected to salt stress by watering with a solution of 100 mM NaCl pure p.a. 99.9%. We performed four salt treatments, every five days, with 250 cm³ of NaCl solution per plant. Control plants were watered with tap water of electrolytic conductivity (EC) 0.25 mS cm⁻¹. Each experimental variant included 20 plants, five plants per repetition. At the beginning of flowering the above-ground part was cut down, leaves were collected and dried in darkness for one month at 25-30 °C.

**Determination of nutrients.** The content of macronutrients (N, P, K, Ca, Mg, Na) and micronutrients (Mn, Zn, Cu, Mo, B) in the dried and ground leaves of S. coccinea was determined using atomic absorption spectroscopy (ASA), which consists in measuring the absorption of a cathode ray radiation by free elemental atoms in a sample mineralized in 1:1 mixture of nitric and perchloric acid. The analyses were performed in accordance with the methods [17] and each determination of the mineral component was carried out in four replications.

**Fourier transform infra-red (FTIR) measurements.** The spectra of S. coccinea samples were measured using a FTIR technique (Perkin Elmer Spectrophotometer, Spectrum 100, Waltham, MA, USA), operated at a resolution of 4 cm⁻¹, over 64 scans in order to minimize noise and improve band separation. Powdered samples were spread on a crystal as a thin layer. The spectra were recorded at a wavelength of 650–4000 cm⁻¹. All spectra were baseline corrected and vector normalized using SPECTRUM software.

**Raman spectroscopy measurements.** S. coccinea dried samples were analyzed using a Raman station (RamanStation 400F, Perkin Elmer) with point-and-shot capability (SuperMacro Point option) and an excitation laser source at 785 nm, 10% laser power (4 shots and 1 second exposition time per point). The resulting spectra were normalized, baseline corrected and analyzed using SPECTRUM software.

**Statistical analysis.** The results of chemical determinations were analyzed using univariate analysis of variance (ANOVA) and TIBCO StatisticaTM Professional 13.3.0 software (TIBCO Statistica, Palo Alto, USA). Significance of mean values variation was assessed by Tukey’s multiple comparison test at p ≤ 0.05.

**RESULTS AND DISCUSSION**

**Bioelement concentration.** Proper plant nutrition is a prerequisite for normal growth and survival under various environmental stresses. Macronutrients mainly serve as building materials, while micronutrients usually play catalytic and regulatory role [18]. Irrespective of treatment, S. coccinea leaves contained the following levels of macronutrients (in % dry weight): N 3.98-5.99 > K 1.71-2.98 > Ca 1.23-1.75 > P 0.46-0.64 > Mg 0.37-0.66 > Na 0.04-0.09 (Table 1). Micronutrient levels reached the following values (mg kg⁻¹ dry weight): Mn 97.4-149 > Zn 52.3-64.3 > B 7.7-31.4 > Cu 2.66-2.99 > Mo 0.24-0.36 (Table 2). The outcomes of our study demonstrated that the leaves of S. coccinea may be a valuable source of necessary dietary minerals, particularly K and Ca. High content and K and Ca in aerial parts is also characteristic of other species of sage, e.g. S. aucheri var. canescens [19], S. fruticosus, S. dichroantha, S. heldreichiana, S. tomentosa or S. halophila [20].

Research shows [6, 21, 22] that salicylic acid plays an important role in controlling plant homeostasis and regulating plant uptake of necessary functional elements. Our analysis of micro- and macronutrient content seem to corroborate this statement (Table 1 and Table 2). Foliar application of SA at 0.5 and 1.0 mM enhanced the leaf content of P (by 16.3% and 48.8%, respectively) and K (26.0% and 22.1%), and decreased the content of Ca (by 23.1% and 15.0%, respectively) and Mg (31.5% and 20.4%). Also, SA at 0.5 mM boosted the leaf N content by 9.9% and reduced Mn content by 19.5% as compared with control (Tables 1 and 2). Different effects of SA on mineral content were
also reported for maize, where it increased the concentration of N, Mg, Fe and Cu and reduced the levels of K, P and Zn [8]. In contrast, foliar spraying of tomato with SA did not change the nutritional status of leaves with respect to nutrients [9]. Biological effects of SA are dose-dependent; for some optimal concentrations they may be directly proportional to the concentration but usually this relationship is more complex [5]. Our results confirm this complexity, as lower SA concentration (0.5 mM) stimulated N accumulation in sage leaves, while higher concentration (1.0 mM) caused no such effect (Table 1).

Salt stress disturbs ion balance and unfavorably changes uptake intensity of important mineral elements [23]. Exposure to NaCl significantly reduced in the leaves of S. coccinea the concentration of N (by 27.0%) and K (26.0%), and increased the levels of P (16.3%), Mg (22.2%), Na (123%), Mn (35.5%) and Mo (50.0%) in comparison with control (Table 1 and Table 2). Studies in S. coccinea, S. farinacea and S. splendidus [15] also confirmed NaCl-triggered reduction of K levels and increase in Na accumulation.

S. coccinea plants exposed to salt and treated with SA accumulated more P and K (on average by 6.0% and 43.3%, respectively) and had significantly higher K/Na ratio (on average by 53.6%) than plants exposed to salt without SA treatment. Additionally, plants grown under stress and sprayed with 0.5 mM SA accumulated slightly less Na than the NaCl-treated plants (Table 1). Increased content of P and K in S. coccinea leaves may be due to their role in salt stress adaptation. Phosphorus takes part in transferring genetic information, formation of high-energy bonds, and as a component of phospholipids it considerably affects the properties of cytoplasmic membranes [24]. Maintaining appropriate concentration of K under salt stress is crucial, as this element is involved in controlling osmoregulation and ion balance. It also determines performance of stomata and consequently the course of CO2 assimilation [23]. Our earlier studies demonstrated that SA in S. coccinea plants treated with salt limited reduction of fresh herbal weight and a drop in the content of photosynthetic pigments, polyphenols, as well as antioxidant potential evaluated by ferric-reducing ability of plasma (FRAP) and 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diaminonium salt (ABTS) [16]. We believe the alleviating effects of SA in S. coccinea exposed to salt stress may be due to stimulation of nutrient uptake and their accumulation in plant tissues. Positive influence of SA on nutrient accumulation under different abiotic stresses was also observed by other researchers in Brassica napus [25], Iris hexagona [26] and Ocinum basilicum [27].

**TABLE 1**

Concentration (expressed in % dry weight) of macronutrients (N, P, K, Ca, Mg, Na) and K/Na ratio in the leaves of *Salvia coccinea* treated sodium chloride (NaCl) and salicylic acid (SA) determined at the beginning of flowering.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Na (%)</th>
<th>K/Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.45 ± 0.09b</td>
<td>0.43 ± 0.00d</td>
<td>2.31 ± 0.13b</td>
<td>1.60 ± 0.04a</td>
<td>0.54 ± 0.04bc</td>
<td>0.04 ± 0.00d</td>
<td>57.6 ± 6.94b</td>
</tr>
<tr>
<td>100 mM NaCl</td>
<td>3.98 ± 0.09c</td>
<td>0.50 ± 0.01c</td>
<td>1.71 ± 0.05c</td>
<td>1.75 ± 0.02a</td>
<td>0.66 ± 0.00ab</td>
<td>0.09 ± 0.00a</td>
<td>19.3 ± 1.29c</td>
</tr>
<tr>
<td>0.5 mM SA</td>
<td>5.99 ± 0.01a</td>
<td>0.64 ± 0.01a</td>
<td>2.91 ± 0.07a</td>
<td>1.23 ± 0.01b</td>
<td>0.37 ± 0.01a</td>
<td>0.07 ± 0.00c</td>
<td>51.8 ± 0.24b</td>
</tr>
<tr>
<td>1.0 mM SA</td>
<td>5.55 ± 0.02b</td>
<td>0.66 ± 0.03a</td>
<td>2.82 ± 0.05a</td>
<td>1.36 ± 0.07b</td>
<td>0.43 ± 0.01de</td>
<td>0.04 ± 0.00d</td>
<td>78.0 ± 3.32a</td>
</tr>
<tr>
<td>0.5 mM SA + 100 mM NaCl</td>
<td>4.06 ± 0.02c</td>
<td>0.54 ± 0.01b</td>
<td>2.41 ± 0.08b</td>
<td>1.62 ± 0.04a</td>
<td>0.50 ± 0.01c</td>
<td>0.08 ± 0.00b</td>
<td>30.3 ± 2.20c</td>
</tr>
<tr>
<td>1.0 mM SA + 100 mM NaCl</td>
<td>4.00 ± 0.05c</td>
<td>0.53 ± 0.02b</td>
<td>2.50 ± 0.12b</td>
<td>1.65 ± 0.05a</td>
<td>0.61 ± 0.01ab</td>
<td>0.09 ± 0.00a</td>
<td>29.0 ± 0.63c</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Values followed by differing letters in each column are significantly different at p ≤ 0.05 (ANOVA and LSD test)

**TABLE 2**

Concentration (expressed in mg kg⁻¹ dry weight) of micronutrients (Mn, Zn, Cu, Mo, B) in the leaves of *Salvia coccinea* treated sodium chloride (NaCl) and salicylic acid (SA) determined at the beginning of flowering.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
<th>Mo</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110 ± 3.35b</td>
<td>59.5 ± 3.56a</td>
<td>2.99 ± 0.29a</td>
<td>0.24 ± 0.03b</td>
<td>27.7 ± 0.01b</td>
</tr>
<tr>
<td>100 mM NaCl</td>
<td>149 ± 2.61a</td>
<td>58.5 ± 1.77a</td>
<td>2.85 ± 0.05a</td>
<td>0.36 ± 0.02a</td>
<td>29.9 ± 0.33ab</td>
</tr>
<tr>
<td>0.5 mM SA</td>
<td>97.4 ± 1.75c</td>
<td>52.3 ± 1.41a</td>
<td>2.94 ± 0.01a</td>
<td>0.29 ± 0.02ab</td>
<td>29.8 ± 0.65ab</td>
</tr>
<tr>
<td>1.0 mM SA</td>
<td>109 ± 3.10bc</td>
<td>60.6 ± 3.43a</td>
<td>2.66 ± 0.18a</td>
<td>0.26 ± 0.05ab</td>
<td>31.4 ± 1.36a</td>
</tr>
<tr>
<td>0.5 mM SA + 100 mM NaCl</td>
<td>116 ± 3.61b</td>
<td>64.3 ± 1.27a</td>
<td>2.66 ± 0.10a</td>
<td>0.33 ± 0.02ab</td>
<td>29.9 ± 0.66ab</td>
</tr>
<tr>
<td>1.0 mM SA + 100 mM NaCl</td>
<td>142 ± 3.12a</td>
<td>63.8 ± 1.10a</td>
<td>2.68 ± 0.06a</td>
<td>0.31 ± 0.03ab</td>
<td>31.1 ± 0.75a</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Values followed by differing letters in each column are significantly different at p ≤ 0.05 (ANOVA and LSD test)
Fourier transform infra-red spectroscopy (FTIR) and Raman spectroscopy analysis. Fourier transform infrared spectroscopy identified conformational changes in macromolecules of *S. coccinea* exposed to salinity and salicylic acid. For comparison purposes, we chose the specific spectral regions where lipids (3000-2800 cm\(^{-1}\)), proteins and lignin (1700-1500 cm\(^{-1}\)), lipids and pectin (1790-1720 cm\(^{-1}\)), cellulose and hemicellulose (1300-1180 cm\(^{-1}\)) and other carbohydrates (fingerprint region 1200-900 cm\(^{-1}\)) are presumably located [28]. We found no visible band shifts in the FTIR spectra of *S. coccinea* samples treated with sodium chloride and/or salicylic acid at different concentrations, but we observed changes in band intensities in all studied regions (Figure 1). The samples generated large numbers of sharp peaks in the mid-IR region (2000-1000 cm\(^{-1}\)), indicating a rich chemical composition of *S. coccinea*.

Figure 2 presents several identified absorption regions and the band assignments. In the higher region, the spectra predominantly show a large absorption band at approximately 3287.80 cm\(^{-1}\) due to hydroxyl and amine groups stretching vibrations denoting mainly proteins and carbohydrates. The bands at 2918.41 cm\(^{-1}\) and 2850.08 cm\(^{-1}\) are mainly due to CH and CH\(_2\) asymmetric and symmetric vibrations in lipids and carbohydrates. Absorption band around 1730.94 cm\(^{-1}\) corresponds to an isolated carbonyl group (COOR), indicating ester-containing compounds commonly found in membrane lipid and cell wall pectin. Other dominant bands include amide I that appears mainly due to C=O stretching vibrations at 1606.10 cm\(^{-1}\), amide II band (N-H) visible at 1546 cm\(^{-1}\), and amide III band (C-N) at 1255.24 cm\(^{-1}\). A band at approximately 1374.30 cm\(^{-1}\) represents the stretching vibrations of C-N from cytosine-guanine pairs in DNA. The peak at 1023.63 cm\(^{-1}\) resulted from an overlap of several bands, including absorption due to the vibration modes of CH\(_2\)OH and a C–O stretching vibration coupled with C–O bending mode of cell carbohydrates and nucleic acids.

Figure 3 shows Raman spectra obtained from all samples. The spectra display several signals derived from lignin, cellulose and other carbohydrates, lipids, amino acids, and carotenoids. Typical signals of lignin appear in a strong band between 1700 cm\(^{-1}\) and 1400 cm\(^{-1}\) overlapped by several bands generated by different stretching vibrations of an aryl ring of coniferyl aldehyde and coniferyl alcohol as well as other chromophores [29]. The band in the region 1440-1460 cm\(^{-1}\) can be attributed to vibrational modes of (C=O)O\(^{-}\) of aspartic and glutamic acids as well as CH\(_2\) groups from sugars [30]. The band between 1000 cm\(^{-1}\) and 1200 cm\(^{-1}\) can be assigned to a symmetric stretching mode of C-O-C in the glycosidic bond in cellulose as well as C-C ring modes in carbohydrates and/or carotenoids [31, 32]. The band in 730-740 cm\(^{-1}\) region is due to C-S groups from amino acid methionine as well as aromatic rings in tryptophan in proteins [30]. The band centered at about 380 cm\(^{-1}\) related to the ring deformational modes is characteristic for cellulose [33] but can be also a result of autofluorescence from laser-plant tissue interaction [34]. The band between 3282 and 3000 cm\(^{-1}\) is attributed to water molecules in the plant tissues.
FIGURE 2
Fourier transform infra-red spectroscopy (FTIR) spectra of the leaves of *Salvia coccinea* plants treated with sodium chloride (NaCl) and salicylic acid (SA) in the spectral region 3507 - 2650 cm⁻¹ (A); 1847-1466 cm⁻¹ (B), and 1478-652 cm⁻¹ (C): control (T-1), 100 mM NaCl (T-2), 0.5 mM SA (T-3), 1.0 mM SA (T-4), 0.5 mM SA + 100 mM NaCl (T-5), 1.0 mM SA + 100 mM NaCl (T-6)
changes in the investigated macromolecules. This suggests only conformational and not chemical sprouts, we noticed no significant band shifts. This evokes morphological changes that could impair may affect the levels of plant macromolecules and lighting different structures within the sample [29]. FTIR technique is appropriate for determining macromolecular changes in plant structural components as each functional group in a molecule exhibits characteristics absorption frequencies in the IR spectrum [28, 35]. Literature contains many reports on modifications of chemical groups in lipids, carbohydrates or proteins caused by plant exposure to salt or other exogenous compounds [36]. While hemicellulose, cellulose and pectins are structural components of primary cell wall, lignin provides rigidity to terrestrial plants [28, 37]. Due to the different underlying mechanisms the spectroscopic methods differ in their sensitivity towards different types of chemical bonds (selection rules). Amide I and amide II bands are particularly useful for determining the protein IR absorption changes. Amide I region (1700-1600 cm\(^{-1}\)) mainly represents C=O stretching vibrations of polypeptide chains, and may represent changes in overall protein conformation and content [38]. The most prominent change involved amide I band. At 1606.10 cm\(^{-1}\) there was a noticeable absorbance spike in \(S. \text{coccinea}\) samples treated with SA, which indicated its possibly stimulating effect on protein concentration in plant tissues. We saw a remote salt-induced decrease of absorbance in \(S. \text{coccinea}\) samples exposed to 100 mM NaCl. These results correspond with the outcomes of Afifi et al. [38], who also noted that salinity stress may modify plant protein levels. The addition of 1.0 mM SA significantly reduced this effect but also markedly decreased the absorbance in plants exposed to 1.0 mM SA and 100 mM NaCl.

The IR spectrum between 3000 and 2800 cm\(^{-1}\) represented mainly lipids. We observed intensity changes in the bands at 2918.41 cm\(^{-1}\) and 2850.08 cm\(^{-1}\) attributed to C-H asymmetric and symmetric stretching vibrations that belong to lipid methylene groups. Salt stress at 0.5 mM and 1.0 mM significantly enhanced absorption in both bands, suggesting increased lipid accumulation in plant tissues. Also, the application of NaCl and SA at both concentrations resulted in an increase of methylene group content.

The IR spectra between 1200 and 1000 cm\(^{-1}\) represented mainly carbohydrates. They displayed three bands located approximately at 1154.72 cm\(^{-1}\), 1100.31 cm\(^{-1}\) and 1022.63 cm\(^{-1}\). We observed a noticeable increase in absorbance in the samples exposed to 0.5 mM and 1.0 mM of SA at 1154.72

Both IR and Raman spectroscopy probe molecular vibrations but they differ in underlying physical mechanisms: absorption of light quanta and inelastic scattering of photons, respectively [29]. FTIR technique is appropriate for determination of macromolecular changes in plant structural components as each functional group in a molecule exhibits characteristics absorption frequencies in the IR spectrum [28, 35]. Literature contains many reports on modifications of chemical groups in lipids, carbohydrates or proteins caused by plant exposure to salt or other exogenous compounds [36]. While hemicellulose, cellulose and pectins are structural components of primary cell wall, lignin provides rigidity to terrestrial plants [28, 37]. Due to the different underlying mechanisms the spectroscopic methods differ in their sensitivity towards different types of chemical bonds (selection rules). IR is sensitive to bond polarization (change in dipole moment), while Raman is sensitive to its polarizability (distortion of electron cloud). Therefore, IR gives strong signals for polar functional groups, and Raman primarily shows vibrations of, e.g., the carbon backbone and vibrations of (nearly) symmetrical structures such as double bonds. Due to this fundamental difference, IR and Raman are often used as complimentary techniques, each highlighting different structures within the sample [29]. Environmental stresses and exogenous compounds may affect the levels of plant macromolecules and evoke morphological changes that could impair normal plant development. Similarly to observations of Zuverza-Mena et al. [28], who investigated the influence of silver nanoparticles on radish sprouts, we noticed no significant band shifts. This suggests only conformational and not chemical changes in the investigated macromolecules.

Figure 1 presents a few significant observations. Amide I and amide II bands are particularly useful for determining the protein IR absorption changes. Amide I region (1700-1600 cm\(^{-1}\)) mainly represents C=O stretching vibrations of polypeptide chains, and may represent changes in overall protein conformation and content [38]. The most prominent change involved amide I band. At 1606.10 cm\(^{-1}\) there was a noticeable absorbance spike in \(S. \text{coccinea}\) samples treated with SA, which indicated its possibly stimulating effect on protein concentration in plant tissues. We saw a remote salt-induced decrease of absorbance in \(S. \text{coccinea}\) samples exposed to 100 mM NaCl. These results correspond with the outcomes of Afifi et al. [38], who also noted that salinity stress may modify plant protein levels. The addition of 1.0 mM SA significantly reduced this effect but also markedly decreased the absorbance in plants exposed to 1.0 mM SA and 100 mM NaCl.

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FIGURE 3
Raman spectra of the leaves of \(S. \text{coccinea}\) plants treated with sodium chloride (NaCl) and salicylic acid (SA): control (T-1), 100 mM NaCl (T-2), 0.5 mM SA (T-3), 1.0 mM SA (T-4), 0.5 mM SA + 100 mM NaCl (T-5), 1.0 mM SA + 100 mM NaCl (T-6)

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cm$^{-1}$ attributed to C-O-C (ether groups) from lignin [28, 39]. The sample exposed to 100 mM NaCl showed a slight shift and a decrease in absorbance.

The spectra of $S$. coccinea samples exposed to salinity and SA at 0.5 mM and 1.0 mM were comparable to the control samples suggesting that SA reduces the negative effect of sodium chloride on lignin accumulation in plant tissues. At 1100.31 cm$^{-1}$ the control sample and the sample treated with salt and SA at 0.5 mM were similar, while absorbance increased in all other variants indicating accumulation of carbohydrates [35]. The spectra showed no differences in peak intensity at 1022.63 cm$^{-1}$. These data are consistent with the results of Afifi et al. [38] and Hoang et al. [36], who noted that salinity stress may affect plant carbohydrate metabolism and concluded that carbohydrate synthesis pathways or some carbohydrates may play an important role in plant response to salt stress. Different capacity of plant metabolism adjustment to salt stress results in various changes to carbohydrate profile [38]. Remarkably strong spectral contributions from lignin visualized by Raman spectroscopy were reported also by Zeise et al. [32].

The band around 1730.94 cm$^{-1}$ represents – COOR stretching vibration attributed to cell wall pectin [38]. The rate of cell wall synthesis and pectin accumulation observed in ester analysis is a meaningful indicator of stress [36]. Studies by Yang and Yen (2002) demonstrated that Arabidopsis plants sensitive to salt treatment did not accumulate cell wall precursors. Contrary to that, tolerant ice plants (Mesembryanthemum crystallinum) continue to produce pectins and other cell wall precursors. Figure 2 presents a visible growth in absorbance caused by all experimental variants versus the control sample. The band absorption was the highest in the sample exposed to NaCl and 0.5 mM SA, followed by the sample exposed to NaCl and 1.0 mM SA and that treated with 1.0 mM SA alone. It is therefore tempting to suggest that SA may stimulate pectin accumulation in plant tissues exposed to salinity stress.

Altangerel et al. [40] suggest that Raman spectroscopy may be used to detect an early abiotic stress response through a measurement of anthocyanins and carotenoids in plant tissues. Carotenoids exhibit two strong Raman peaks in two separate spectral regions, 1100–1200 and 1400–1600 cm$^{-1}$, due to the stretching vibrations of the carbon–carbon bonds in the polyene chain. These spectral features can be used to identify and quantify carotenoids in various plant tissues. Qin et al. [34] used Raman spectroscopy to determine carotenoids in tomato. The intensity changes in the bands related to carotenoids are in line with the results of a previous study showing the influence of salinity stress and salicylic acid on $S$. coccinea total carotenoid content [16].

Given the complex biological matrix, the changes in specific functional groups cannot be assigned to particular molecules in the plant cells. However, the changes in chemical composition reflect the general shift in the metabolic processes and indicate plant response to exogenous stimulants [35].

**CONCLUSION**

The study lets us conclude that the leaves of $Salvia coccinea$ may be a valuable source of necessary dietary minerals, particularly potassium and calcium. Foliar spray with SA is a promising bioregulatory approach limiting negative effects of salinity in $S$. coccinea crop. In NaCl treated plants SA stimulated leaf accumulation of basic macronutrients, limited the decrease of K/Na ratio and affected the content of lipids, lignin, proteins and pectins. We confirmed usefulness of FTIR and Raman spectroscopy as tools for examining biochemical changes caused by salicylic acid and salinity stress within the plant tissues.

**REFERENCES**


SELECTION OF THE MOST SUITABLE SENTINEL-2 BANDS AND VEGETATION INDEX FOR CROP CLASSIFICATION BY USING ARTIFICIAL NEURAL NETWORK (ANN) AND GOOGLE EARTH ENGINE (GEE)

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1Namik Kemal University, Agricultural Faculty Department of Biosystem Engineering Tekirdag, Turkey
2Republic of Turkey Ministry of Agriculture and Forestry Ataturk Horticultural Central Research Institute Yalova, Turkey

ABSTRACT

The aim of this study is to determine the most suitable date, band and vegetation indices for the crop classification by using the Artificial Neural Networks method in irrigated agricultural areas and the usage possibilities of the web-based remote sensing platform GEE. For this purpose, the crop pattern of Cifteleg irrigation scheme is located in Eskişehir, Turkey has been investigated. During the study, GEE has shown ability as an extremely powerful tool for obtaining high-resolution Sentinel 2 satellite images on a very wide area, for processing these images and for the use and export of results in classification study. It was seen that, because of GEE is cloud-based, it performs tasks quickly and conveniently with a large number of servers without depending on the performance of local computers. 12 band and 21 vegetation index values for each of the irrigation parcels were transferred to the MS Excel for use in the JMP statistical program. During the study, classification was performed with Artificial Neural Networks. During the classification process, Generalized $R^2$ and overall accuracy calculated respectively 96% and 94%.

KEYWORDS:
Google Earth Engine, Artificial Neural Network, Crop Classification, Sentinel-2

INTRODUCTION

Irrigation networks have an important place in meeting the increasing food need. The identification of the crops in the irrigation area serves to determine the irrigation program and to make agricultural and environmental planning on a large scale. However, it is extremely difficult to accurately and quickly identify the crop pattern of irrigation networks using conventional methods. At this point, remote sensing technology can provide vital information to researchers and managers.

Remote sensing is an important data source to estimate the vegetation matter in wide areas and satellite-based indices have been used in many types of research to estimate vegetation cover. Most of the research efforts have been made for mapping urban landscapes at various scales and on the spatial resolution requirements of such mapping [1]. However, while working in a wide range of areas high-resolution satellite images such as Sentinel-2 creates "big data" problem. Big data refers to a collection of data sets so large and complex that it is difficult to employ traditional data processing algorithms and models [2]. Mandal et al. (2017) stated that this problem can only be eliminated with a platform such as Google Earth Engine (GEE) [3]. GEE is used by different disciplines as petabyte-sized multi-talented tools that allow the cloud-based study of many satellite data such as Landsat, Modis, NOAA, and Sentinel [4].

GEE, a web-based remote sensing platform, can perform simultaneous complex spatial and temporal analysis on its nearly 40 years of satellite image collection [5]. GEE has easy access to remote sensing data on a global scale. GEE is a cloud-based platform for planetary-scale environmental data analysis. So, processing large data and solving complex problems can be performed without depending on the performance of your personal computer [5]. Through code editor, researchers have the opportunity to write, store and share their own code with other users. [6, 7]. The GEE Code Editor is a web-based integrated development environment (IDE) for writing and running scripts. Code examples can be found from https://code.earthengine.google.com [8]. Hansen et al. (2013), in their study on the global change of forest cover, investigated 707 terabytes Landsat data by working on GEE [9].

Image classification is an important task for many aspects of agricultural applications. Until today, several classification algorithms like Rule-Based Classifiers, Bayesian Networks (BN), Decision Tree (DT), Nearest Neighbour (NN), Genetic
Algorithms, Support Vector Machine (SVM), Rough Sets, Fuzzy Logic and Artificial Neural Network (ANN) are developed and applied for various studies [10-18]. Artificial neural networks (ANNs) are computer-based algorithms which are modeled on the structure and behavior of neurons in the human brain and can be trained to recognize and categorize complex patterns [19]. In most cases an ANN is an adaptive system that changes its structure based on external or internal information that flows through the network during the learning phase [20]. Antonio et al. (2003) reported that they obtained 87% overall accuracy in their crop classification with ANN [21]. Kumar et al. (2015) stated that they obtained an accuracy of 71 to 88% in classification study with Landsat 8-OLI images by using ANN [22].

In the classification process, the spatial and spectral resolutions of the satellite images and the image acquisition date directly affect the result. On the other hand, band and vegetation indices also play an important role in the classification process. Vegetation indices use the reflectance of bands such as RED, NIR, RED EDGE, and TIR, which are in direct contact with leaf and canopy while revealing crop growth and stress status [23]. Remote sensing images have been focused on these specific band spacing, especially in order to reveal plant physiological changes over the years [24].

A number of techniques have evolved in the past to derive the biophysical variables of vegetation using remote sensing data and one of them is Vegetation Indices (VIs) [25]. VIs have widely used method measurements of the biophysical parameters of vegetation by contrasting specific spectral reflectance characteristics of vegetation due to the benefit of being computationally simple [26]. To date, many studies have been done to detect different plant biophysical activities with a lot of vegetation index as simple Ratio (SR), Modified Simple Ratio (MSR), Red edge Normalized Difference Vegetation Index (NDVI), Soil Adjusted Vegetation Index (SAVI), Normalize Difference index (NDI), Renormalized Difference Vegetation Index (RDVI), Green red Vegetation Index (GRVI), MERIS Terrestrial Chlorophyll Index (MTCI) etc. [27-32]. On the other hand, Normalized Difference Water Index (NDWI) and Modified Normalized Difference Water Index (MNDWI) were used for the determination of irrigated farmland and wetlands [32-34]. The effect of various indices SR, NDVI, TNDVI, SAVI, and TVI to identify cotton crop using temporal multi-spectral images was investigated by [35]. Zhang et al. (2017) tried to determine the land use and vegetation with the different bands of Sentinel-2 and NDVI and NDWI vegetation indices [24]. Ferrant et al. (2017) reported that they determined to succeed of dominant crops in the irrigation area by using Sentinel-1, Sentinel-2 satellite imagery bands, NDVI, and NDWI vegetation indices [36].

In this study, it was tried to determine which data was obtained with the highest success in crop classification using Sentinel-2 bands and numerous vegetation indices obtained at different dates. For this purpose, the Sentinel-2 satellite image catalog for Cifteler irrigation in Eskişehir in 2018 was created with Google Earth Engine. A large number of vegetation indices were created from the generated catalog and the band and vegetation index values for each parcel in the irrigation area were exported with GEE for use in the JMP statistical program. This data was used in the 30-day trial of the JMP program. Firstly, only the bands of Sentinel-2 satellite image, then only the vegetation indices and finally the band and vegetation indices together were classified using the artificial neural networks method. The success of the study was tested with accuracy analysis.

MATERIALS AND METHODS

Study area. Cifteler irrigation scheme was put into service by State Hydraulic Works (SHW), In 1966. Cifteler irrigation is located in Eskişehir and operated by Sakaryabası irrigation association. total irrigation area is 6200 ha, 5300 ha is irrigated with gravity and 900 ha is pressurized irrigation methods. Crop pattern is cereals, Sugar Beet, Watermelon, Corn, Sunflower and fodder plant [37].

Methodology. General Architecture overview. A three-level architecture is proposed for the classification of crop types from multi temporal satellite imagery. These levels are Datasets and Pre-Processing (multispectral image collection, Bands Stacking, Vegetation Indices field survey), Classification and Accuracy assessment. The methodology followed during the study is given in Figure 1.

Datasets and Pre-Processing. An image collection which includes the boundary file of the Cifteler irrigation and provided the condition of non-clouding was created with the help of Code Editor on GEE, including March and September 2018. At this stage, radiometric and geometric corrected L1C level Sentinel-2 are transferred to the UTM / WGS84 coordinate system. The root means square errors (RMSE) less than 1.0 pixel in the geometric correction indicating that the images are located with an accuracy of less than a pixel.

In the studies related to plant development, the spatial and spectral resolution of the satellite image to be used is extremely important in terms of performance of the study [38]. Sentinel 2 provides multi-spectral data with a large swath width (290 km) and short revisit time. Sentinel-2 is very suitable satellite for vegetation monitoring due to having a spatial resolution of 10 m or 20 m, others have 60
m resolution. Radiometric accuracy of <5% and operate at 12-bit radiometric resolution [39]. It is designed to give a high revisit frequency of 5 days at the Equator [40]. Band properties of Sentinel-2 is given in Table 1.

As shown in Table 1, the Sentinel-2 multispectral image has 13 bands in total, in which four bands (blue, green, red and NIR) have a spatial resolution of 10 m and six bands (including SWIR band) have a spatial resolution of 20 m [34].

Very careful examination of the biophysical changes of each stage of agricultural plant production provides vital information on the plant physiological state [41]. Vegetation activities can be measured comprehensively through semi-analytical methods of spectral band ratios that have been extensively used to detect not only seasonal variability of the vegetation cover but also local scale spatial variability [42].

Several different indices concerning the chlorophyll concentration [28], water content [32], leaf pigments and carbon in lignin [43] were evaluated. Vegetation indices can be used for many different purposes like detection of vegetation, land cover changes or soil type detection. In this case, vegetation indices were used for determination of crop types in the irrigation scheme. Twenty-one vegetation indices were used in order to determine and evaluate the crop growth in this research (Table 2).

GEE can ability creates all of these filters and indices, as well as visually showing results as maps. In addition, all image collection data and the time series data of the band and vegetation index can be automatically plotted.

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![Flow Chart of Methodology](image)

**FIGURE 1**
Flow Chart of Methodology

<table>
<thead>
<tr>
<th>Band Number</th>
<th>Spatial Resolution (m)</th>
<th>Central Wavelength (nm)</th>
<th>Bandwidth (nm)</th>
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<td>B1</td>
<td>60</td>
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</tr>
<tr>
<td>B2</td>
<td>10</td>
<td>490</td>
<td>65</td>
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<td>B3</td>
<td>10</td>
<td>560</td>
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<tr>
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<td>10</td>
<td>665</td>
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<td>842</td>
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<td>865</td>
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<td>60</td>
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</tr>
<tr>
<td>B10</td>
<td>60</td>
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</tr>
<tr>
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<td>20</td>
<td>1610</td>
<td>90</td>
</tr>
<tr>
<td>B12</td>
<td>20</td>
<td>2190</td>
<td>180</td>
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**TABLE 1**
Band spatial resolution, central wavelength, and bandwidth of the Sentinel-2 image.
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Codes were prepared for this study with the help of sample codes shared on the GEE platform and the indices defined in the Sentinel Hub pages (Sup-1). Sentinel-2 images and other created layers displayed on the main screen. On the other hands, two panels are defined as left and right on GEE. These panels display some important graphs as Sentinel-2 band’s reflectance, NDVI, and NDRE, etc. and there are also thumbnail images for testing cloudless condition. Using this thumbnail, firstly images were visually examined one by one and dates that provided cloudiness conditions for the study area were determined. Based on the pixel average within each irrigation parcel, the band reflection and index values of the parcel were exported in MS Excel format to JMP program for statistical analysis.

**Field Survey and Creating Reference data.** Crops in each parcel of the entire irrigation area have been recorded with the field studies. This data was converted to MS Excel format and imported to the parcel information system in ArcMap program. Thus, the spatial distribution of each crop in the irrigation area was revealed. The created parcel information system was transferred to GEE environment as shp. file format. Thus, the band and VIs values of each parcel were exported in CSV format to be used in the JMP program for crop classification.

**Image Classification.** In this section, all the dates that were displayed during the plant development period of the study area and the band and index values measured from each parcel at these dates were classified. The classification process was performed using the 30-day trial version of the JMP statistic program. The classification process was done separately for 3 different groups. These groups are a) only bands b) only vegetation indices, and c) all band and vegetation indices.

### TABLE 2

<table>
<thead>
<tr>
<th>Vegetation index</th>
<th>Formulation</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Ratio Vegetation Index</td>
<td>GRVI = NIR / GREEN</td>
<td>[44]</td>
</tr>
<tr>
<td>(Normalized Difference Vegetation Index</td>
<td>NDVI=(B08-B04)/(B08+B04)</td>
<td>[27]</td>
</tr>
<tr>
<td>Soil Adjusted Vegetation Index</td>
<td>SAVI = (1 + L \times (\frac{NIR - RED}{NIR + RED + L}))</td>
<td>[45]</td>
</tr>
<tr>
<td>Leaf Area Index - Soil Adjusted Vegetation Index</td>
<td>LAISAVI = (-log\left(0.371 + 1.5 \times (NIR - R) / (NIR + R + 0.5)\right))</td>
<td>[46]</td>
</tr>
<tr>
<td>Modified Simple Ratio</td>
<td>MSR=(R/\left(NIR/RED+1\right))</td>
<td>[47]</td>
</tr>
<tr>
<td>Renormalized Difference Vegetation Index</td>
<td>RDVI=(NIR-R)/(NIR+R)*0.5</td>
<td>[48]</td>
</tr>
<tr>
<td>Simple Ratio</td>
<td>SR=(B08 / B04)</td>
<td>[49]</td>
</tr>
<tr>
<td>Enhanced Vegetation Index</td>
<td>EVI = 2.5*((B08 - B04)) / ((B08 + 6\times B04 - 7.5\times B02 + 1))</td>
<td>[50]</td>
</tr>
<tr>
<td>Normalized Difference Built-up Index</td>
<td>NDBI = ((B11 - B08) / (B11 + B08))</td>
<td>[51]</td>
</tr>
<tr>
<td>Modified Normalized Difference Water Index</td>
<td>MNDWI = ((B03 - B11) / (B03 + B11))</td>
<td>[33]</td>
</tr>
<tr>
<td>Normalized Difference Infrared Index</td>
<td>NDII = ((B08 - B11) / (B08 + B11))</td>
<td>[52]</td>
</tr>
<tr>
<td>Moisture Stress Index</td>
<td>MSI=((B11 / B08))</td>
<td>[53]</td>
</tr>
<tr>
<td>Structure Insensitive Pigment Index</td>
<td>SPI = ((B08 - B01) / (B08 - B04))</td>
<td>[43]</td>
</tr>
<tr>
<td>Normalized Difference Water Index</td>
<td>NDWI = ((B08-B11) / (B08+B11))</td>
<td>[32]</td>
</tr>
<tr>
<td>Chlorophyll Red-Edge</td>
<td>CHL-RE=([760:800]+[690:720]) / (-1)</td>
<td>[54]</td>
</tr>
<tr>
<td>Modified Chlorophyll Absorption in Reflectance Index</td>
<td>MCAI = ((B05 - B04) - 0.2 \times \left(B05 - B03\right)) / (B05 / B04)</td>
<td>[28]</td>
</tr>
<tr>
<td>Red Edge Normalized Difference Vegetation Index</td>
<td>RENDWI = ((B3-B5)/(B3+B5))</td>
<td>[55]</td>
</tr>
<tr>
<td>Red-Edge Inflection Point Index</td>
<td>REIP=(700+40\times\left(B04+B07\right)/2\times B05) / (B06-B05)</td>
<td>[56]</td>
</tr>
<tr>
<td>MERIS Terrestrial Chlorophyll Index</td>
<td>MTCI=((B06-B05) / (B05-B04))</td>
<td>[30]</td>
</tr>
<tr>
<td>Modified Simple Ratio 705</td>
<td>MSR705=((B06-B01) / (B05-B01))</td>
<td>[57]</td>
</tr>
<tr>
<td>Sentinel-2 red-edge position</td>
<td>S2REP= (705 + 35 \times \left(\left(B7 + B4\right)/2 - B5\right) / (B6 - B5))</td>
<td>[25]</td>
</tr>
</tbody>
</table>
An ANN classification algorithm was used in this investigation. ANN are computer programs that are designed to simulate human-learning processes through the establishment and reinforcement of linkages between input data and output data. ANN is used as a powerful tool for pattern classification and has been found to be accurate in the classification of remotely sensed data [58]. Typically, a minimum of three layers which are the input layer, the hidden layer, and the output layer is required to develop an ANN system [59] (Figure 2).

The layers between input and output layers are referred to hidden layer that may be one or more, helps to capture nonlinearity and is not directly observed. In theory, ANNs can be contained an arbitrary number of input and output variables [60].

Each input layer node is connected to each hidden layer node and each connection has a weight, and each hidden layer node has a hidden term. Each hidden nodes have their own activation function. NTanH activation function is used in this study. NTanH activation corresponds to how neural models were implemented in the legacy platform. The way that each input variable contributes to the design row depends on whether the independent variable is continuous or categorical, and various modeling options in the platform [61].

**Accuracy Assessment.** In order to achieve high success in the classification process with artificial neural networks, good selection of training data is directly related to the training process [62].

Accuracy analysis of classification results was performed by K Fold Cross Validation method. In K Fold validation method, first, created k group are included the same amount of observation and then, k-1 group is used as a training data set, kth the group is used for validation. K times validation is calculated and different groups are considered in each calculation, so, average the k measures of accuracy to be obtained an overall estimate of the accuracy.

For each of the training and validation sets a variety of diagnostic measures are provided. These diagnostic measures differ whether the response is categorical or continuous. the cross-validation statistics include Generalized $R^2$, Entropy $R^2$, the Root Mean Squared Error (RMSE) and Mean Absolute Deviation (MAD. The formulas of the parameters of accuracy analysis are given below [61].

\[
R^2_{\text{generalized}} = 1 - \frac{\sum_{i=1}^{k} L_{\text{null}}}{L_{\text{null}}} 
\]

\[
R^2_{\text{Entropy}} = 1 - \frac{L_{\text{null}}}{L_{\text{null}}} 
\]

\[
\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (y_i - \hat{y}_i)^2} 
\]

\[
\text{MAD} = \frac{1}{N} \sum_{i=1}^{N} |y_i - \hat{y}_i| 
\]

Where,

Generalized $R^2$ measure simplifies to the traditional $R^2$ for continuous normal responses in the standard least squares setting.

Entropy $R^2$ Compares the log-likelihoods from
the fitted model and the constant probability model. Values closer to 1 indicate a better fit. A measure that can be applied to general regression models. It is based on the likelihood function \( L \) and is scaled to have a maximum value of 1 [63-64].

\[ L = \beta \] is the negative loglikelihood

\[ \hat{L}_{null} \] is the simple mean and variance normal distribution model loglikelihood.

\( X_i \) is input

\( Y_i \) is response

RMSE (Root Mean Square Error), where the differences are between the response and \( P \) (the fitted probability for the event that actually occurred). Smaller values indicate a better fit.

MAD (Mean Absolute Deviation), The average of the absolute values of the differences between the response and \( P \) (the fitted probability for the event that actually occurred). Smaller values indicate a better fit.

When the response is categorical, the misclassification rate is given in addition to the model fit measures described above for continuous responses. The misclassification rate is the proportion of times the actual level of the response is not the one assigned the highest probability by the neural model. Smaller values indicate a better fit [65].

The performance of the classification system is evaluated by the construction of a confusion matrix [66]. Confusion matrices record the number of times each level of the response is actually one level and predicted under the model to each of the levels.

**RESULTS AND DISCUSSION**

Sentinel-2 image catalogue which provides of the cloudless condition of the study area in 2018 was created using the GEE platform (Figure 3). Parcel information of the study area was transferred to GEE in shp file format. Thus, the centroid or the average reflection values of the entire parcel determined for each parcel. The band and index values from each parcel are displayed on both sides of the screen. These values for each parcel on GEE were exported in CSV format for use in the classification process in JMP program.

Crop development periods and image dates that provide the non-clouding condition of the study area are given as follows (Figure 4). Sidhu et al. (2018) stated that they were used GEE for investigation of land cover in Singapore [67]. Shelestov et al. (2017) highlight that GEE is one of the most useful platforms to effectively execute complex workflows of satellite data processing required with large scale applications such as crop mapping [4].

**Bands based classification.** All bands belonging to the Sentinel-2 satellite (except the 10th band) were used to classify with the artificial neural networks in the JMP program. As a result of this classification process, Generalized R² has been calculated between 0.59-0.93 in the whole period. MAD value was calculated as 0.21 in the classification with the highest accuracy. The most effective band values for the determination of plant classes in artificial neural networks were determined as B11, B06, B03, B08, B08A, B07, B12, B04, B02, B09, B05, B01, respectively.

**FIGURE 3**

Band and vegetation indices of image catalogue created on GEE
FIGURE 4
Image Dates and Crop Calendar in Research Area

TABLE 3
Classification results in training and validation

<table>
<thead>
<tr>
<th>Training</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalized R²</td>
<td>0.9244687</td>
</tr>
<tr>
<td>Entropy R²</td>
<td>0.7140603</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.3701746</td>
</tr>
<tr>
<td>MAD</td>
<td>0.2474034</td>
</tr>
<tr>
<td>Misclassification Rate</td>
<td>0.1478873</td>
</tr>
<tr>
<td>Sum Freq.</td>
<td>142</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Validation</th>
<th>Generalized R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entropy R²</td>
<td>0.8248195</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.2738763</td>
</tr>
<tr>
<td>MAD</td>
<td>0.1919283</td>
</tr>
<tr>
<td>Misclassification Rate</td>
<td>0.0571429</td>
</tr>
<tr>
<td>Sum Freq.</td>
<td>35</td>
</tr>
</tbody>
</table>

TABLE 4
Confusion matrix for training

<table>
<thead>
<tr>
<th>Actual</th>
<th>Predicted Count</th>
<th>Sunflower</th>
<th>Watermelon</th>
<th>Cereal</th>
<th>Maize</th>
<th>Sugarcane</th>
<th>Alfalfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Watermelon</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Cereal</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Maize</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Vegetation Indices based Classification. In the classification using a large number of plant indices generated by different proportions of the bands of the Sentinel-2 satellite, the Generalized R² value was calculated between 0.72 and 0.92 for different dates throughout the plant growth period. MAD value was calculated as 0.25 for the highest accuracy classification where Generalized R² was calculated as 0.92. During the study, the most effective indices on the classification were listed as NDVI7052, NDVII, PSRIN, MSI, RDVI, SAVI, REIP, IRECI, GRV11, MNDWI, PSRI, LAISAVI, CHLRE, respectively.

Bands and Vegetation Indices based classification. The highest accuracy was obtained at 30.4.2018 date, in classification in which band and indices are used together. Table 3 shows the results on classification in training and validation.

In the KFold validation method, 142 samples for training and 35 samples for validation are selected randomly. It is seen that the highest accuracy value is obtained by using the band and plant indices together among three classifications. In this classification, the highest Generalized R² value was determined as 0.96. The lowest MAD value achieved as 0.19 in all classifications. Also, the Misclassification rate was set to an extremely low value of 0.05. This indicates the effectiveness of the classification. ANN’s confusion matrix for classification is shown in Table 4 and Table 5.
In the classification made with all bands and indices, the overall accuracy of 94.2% in the validation test was reached. Accordingly, it was observed that more information increased the success.

As can be seen in Table 5, foolproof results for Cereal, Maize, Sugarcane and Alfalfa have been reached. It has been determined that both the spectral bands and the plant indices formed from these bands affect the classification result positively in the classification performance with artificial neural networks. Congruently, Ferrant et al. (2017) indicated that they have achieved success in the detection of irrigated plants in their study with Sentinel 2 satellite band and some indices [36]. Similar, Zhang et al. (2017) indicated that they have achieved a higher success by increasing the amount of data in the classification procedure, in their study with Sentinel 2 satellite images [24].

On the other hand, selecting variables for inclusion in a neural network is always difficult because there is no test for whether a variable makes a contribution to a model. To obtain the most affected variables a tree to the data can be applied then check the “variable importance” measures from the tree, and use the most important variables from the tree as the variables to be included in the neural network [65]. According to this study, the most effective parameters for each crop are given in the table below (Table 6).

When Table 6 is examined, it is seen that especially indices as MSR705, MCARI, MTCI, and RENDW1 come into prominence in the classification study with the artificial neural networks. On the other hand, B09 and B01 bands were determined as the most effective variables in the classification process.

**CONCLUSION**

This study focuses on two main topics in plant classification studies. The first one is to determine the capabilities of the GEE platform in the processing of a large number of satellite data, and the second one is the determination of which type of data is most suitable for the most accurate classification with artificial neural networks. For this purpose, twenty-one Sentinel-2 satellite images that provide non-clouding conditions were processed with open source codes directly on the platform without any downloading. These codes were then put into use in such a way that even researchers with the lowest level of software knowledge doing similar studies can easily conclude so, researchers will be able to use all or part of this code according to their work.

In this study, the crop pattern consisting of six classes, the best estimate was reached in the group where all band and vegetation indices were processed. It was found that both the raw data and the different vegetation indices showing the crop physiological status increased the success of classification. Besides, Sentinel 2 images, which is used as a satellite image, has been found to be highly suitable both for spectral and spatial studies for vegetation monitoring.

On the other hand, it is seen as a great convenience that the JMP program, in which all statistical operations are performed, can directly import data transferred to Excel format. At the same time, it is among the positive features of the program that it can produce the results by processing all the data obtained for 25 different dates very quickly at the same time. It will also increase its use in GIS stud-
ies such as crop classification or land use change due to the fact that it can process the map.

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STUDY ON EMERGENCY MECHANISM OF SUDDEN ENVIRONMENTAL POLLUTION EVENTS IN DANJIANGKOU SECTION

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ABSTRACT

In order to cope with the sudden environmental pollution accidents that may exist in the trunk line of the South-to-North Water Diversion Project, strengthen the emergency prevention and management of sudden environmental pollution accidents, investigate the risk sources of the Danjiangkou section, and analyze the possible sudden environmental pollution incidents in the Danjiangkou section. Based on the probability and frequency of its occurrence, it summarizes the main problems in the construction of the emergency mechanism for environmental pollution emergencies in Danjiangkou, and establishes a burst of five major links: “Numerical Simulation-Evaluation and Diagnosis – Traceability Prediction – Emergency Control – Pollution Disposal”. Environmental pollution emergency control and disposal technology system is built. Through the research on the emergency mechanism of sudden environmental pollution incidents in Danjiangkou, it is possible to ensure the safety of water supply in the trunk line of the South-to-North Water Transfer Project and achieve the goal of “sending water to the north”.

KEYWORDS:
Danjiangkou, South-to-North Water Diversion, environmental pollution, mechanism

INTRODUCTION

China is a country with very poor water resources. The per capita water resources of the country is 2163 m³, accounting for 1/4 of the world average. The uneven distribution of water resources in time and space further aggravates the seriousness of the problem. The water resources in the northern region are particularly poor, and the per capita water resources of the Huang-Huai-Hai River Basin is one-fifth of the national total. The population, grain output and GDP of the Huang-Huai-Hai River Basin account for 1/3 of the national total [1].

The amount of water resources is only 7.2% of the national total. The serious shortage of resources has become a bottleneck for economic and social development in the northern region.

In order to resolve the contradiction of water shortage in the northern region, the state decided to transfer water from the Yangtze River basin with relatively abundant water volume to the Huanghuaihai area to implement the South-to-North Water Diversion Project. The Middle Route of the South-to-North Water Transfer Project is an integral part of the entire South-to-North Water Diversion Project. The plan is to divert water from the Danjiangkou Reservoir in the Han River in the near future. The annual water transfer is 95 × 10⁶m³. The project was completed before 2014. The scale of the Han Dynasty will be further expanded in the later period. The average annual water transfer will reach 130 × 10⁶m³. The project is expected to be completed in 2030. The prospect is from the Yangtze River. Three Gorges water transfer. The mid-line project can alleviate the water crisis in Henan, Hebei, Tianjin and Beijing, improve the ecological environment and promote sustainable economic and social development. It has important strategic significance.

The main channel of the South-to-North Water Transfer Project is from the west of the Danjiangkou Reservoir Tao Road and north to the Beijing Tuancheng Lake. The total length of the water main line is 1,267km. There are 64 and 88 water diversion gates along the trunk line of the South-to-North Water Diversion Project. It is a long-distance complex water delivery system consisting of open channels, channel aqueducts, channel inverted siphon culverts, tunnels, pressurized pipe culverts and several control buildings.

In the process industry, environmental pollution accidents occur frequently, especially in the industries such as chemical industry, agricultural chemicals production, natural gas mining, etc. [2]. There has been a large increase in public awareness of the potential dangers posed by the environmental pollution accidents and their effects to both human beings and the environment [3]. Taking P.R. China as an example, the number of pollution accidents reported to and acted upon by the Ministry of Environmental Protection rose from 64 in 2003 to 171 in 2009. A total of 784 environmental pollution acci-
sidents were responded to from 2003 to 2009 [4]. In various pollution incidents, accidental water pollution events pose a very specific threat [5]. Over the past decade, the Ministry of Environmental Protection, P.R. China responded directly to 558 water pollution accidents, corresponding to 52% of the total number of environmental emergencies. In the U.S.A., water pollution incidents comprised 79% of the total number of environmental emergencies reported in 2014 [6]. When a water pollution accident occurs, the physical, chemical, and biological properties of water body will be changed, which may cause more serious effects, such as threatening residents’ health, inducing economic loss, incurring social instability, and destroying the aqueous ecological balance [7-9]. Common and dangerous pollutants in water pollution accidents include organic matter and heavy metal, and organic matter includes benzene, phenol, alcohol, naphthalene, anthracene, etc. Benzene, as a typical hydrocarbon, is highly toxic and carcinogenic. It can enter the human body through the skin and respiratory tract. Moreover, because benzene is so volatile, it’s very easy to spread in the air. A substantial proportion of leukemia patients have a history of exposure to benzene [10-12].

At present, many studies have been carried out by developing simulation programs to simulate spatial and temporal distributions of pollutants during various pollution incidents. For example, Wang et al. [13] used the one-dimensional (1D) MIKE-11 model to simulate the pollutant arrival time and concentration at various locations after water pollution accidents. He et al. [14] used several models to reveal transport processes of various pollutants in different types of water bodies within the Three Gorges on the Yangtze River. Grifoll et al. [15] studied the extent and risk of pollution for various water bodies in Barcelona after a water pollution accident. Ani et al. [16] presented a mathematical model for simulating pollutants transport in rivers that was calibrated by MATLAB. Tao et al. [17] proposed a method for determining the degradation coefficients of pollutants in a conventional water quality model and simulated a pollution accident quantitatively. Ding et al. [18] developed a simulation program to simulate the change trend of pollutant concentration after water pollution accidents and apply it to the Heshangshan drinking water source area.

MATERIALS AND METHODS

Study area. The Danjiangkou Reservoir, situated in the center of China and spanning Xichuan (Henan Province) and Danjiangkou Counties (Hubei Province), is the source reservoir for China’s South-to-North Water Diversion Middle Route Project (S-N-M Project). The reservoir was constructed in 1958 and was one of the largest reservoirs in Asia at that time. It has two main tributaries, which join at the Danjiangkou Dam and form two main parts of the reservoir, namely the Han Reservoir and the Dan Reservoir. The S-N-M Project’s canal head (Taocha) is located at the northeast of the Dan Reservoir. Because 90% of the inflow comes from the Han River, the reservoir owns both rivers and lakes characteristics.

Model description. A 3-D eutrophication model was built based on the well accepted platform of the EFDC (Environmental Fluid Dynamic Code). The EFDC is an open-source, three-dimensional software package for the analysis of hydrodynamic and water quality models.

RESULTS AND DISCUSSION

The possibility exists that the reservoir inflow would be substantially decreased in the future because of climate change and the dam operation upstream of the Danjiangkou Reservoir. However, there is no end to the human demand for water resources. The contradiction between the water requirement for water diversion from the Danjiangkou Dam and the water requirement for the downstream ecology of Han River from the Danjiangkou Dam will continue. This study analyzed the effects of the inflow decrease on the eutrophication in the Taocha area. The results, shown in Fig. 1, indicate that inflows that decreased by 50% obviously influenced the Chl a in the Taocha area, compared with the reference scenario. However, subsequently, the Chl a concentration decreased sharply and approached zero. The reasons are analyzed as follows: (1) the nutrients’ concentration in the reservoir will increase as the inflow decreases and the nutrients’ concentration in the inflows does not changed, which benefits the algae and makes the Chl a concentration increase in the Taocha area; (2) when the inflows continue to decrease, but with the outflows remain unchanged, the water level will continue to decline. This effect will result in the drying up of water and the decline of algae in the Taocha area in view of the higher elevation of the reservoir bottom in that area than in the Danjiangkou Dam area. Therefore, the water quantity for water diversion and discharge from the Danjiangkou Dam should be reduced accordingly in dry years to avoid an algae bloom and a threat to the safety of the water resource. The effects of the TN concentration increase associated with the inflow decrease on the Chl a in the Taocha area were analyzed. The results were presented in Fig. 1, which shows the TN concentration increase of 50% associated with each inflow has no additional effects on the Chl a concentration in the Taocha area. Therefore, it is unlikely that the TN pollution in the Danjiangkou
Reservoir basin directly disturbs the algae growth in the Taocha area.

Main problems in the construction of emergency mechanism for environmental pollution emergencies in Danjiangkou. (1) The crisis awareness is weak, and there are hidden dangers in environmental safety. The environmental emergency management system is based on a normal assumption. From the government to the ordinary citizens, they are used to working in the normal state. When emergencies occur, especially when emergencies are rapidly transformed into crises, they can only passively adopt the "impact" response mode of "headaches and pains." A peaceful and stable development environment often leads people to gradually relax their awareness of the crisis. Although environmental emergencies can be prevented to a certain extent, the existing systems and mechanisms are still not enough to make the crisis awareness deep into the government, communities and individuals. Level. The faint awareness of the government crisis and the weak awareness of the public crisis have directly affected the efficiency of China's response to emergencies. And China is currently in a critical period of "economic transition and market transformation". The society is prone to disorder and the people's psychology is easily out of balance. In addition, the environmental industry is the speciality and importance of the lifeline system. It is easy to cause major hidden dangers to the country's environmental safety and sustainable development.

(2) Ineffective emergency command and weak professional ability. Conduct command according to the emergency plan. The contingency plan is the procedural norm and overall plan of the government to coordinate and organize, organize and manage emergency actions before emergencies in response to emergencies. Emergency plans must be scientific, systematic and operational. However, at present, China's emergency plan still lacks in these three aspects, which directly affects the efficiency of emergency command.

The lack of unity and coordination of the command ultimately led to many problems. The prerequisites for successful response to emergencies are strong command measures, unified and efficient actions, coordination and coordination among departments, and active and steady command of all parties in the society to carry out effective and effective work, all of which require a unified emergency command. The agency is responsible for coordinating and integrating emergency resources of multiple departments. The response of emergencies requires the participation of multiple departments. However, China’s emergency management model is a coping mode for sub-sectors and sub-disasters that have been formed for a long time. According to the types of emergencies, which are undertaken and set up by the government and other social organizations, each functional department is independent and independent, and lacks a unified command and coordination mechanism.

It can be seen that the emergency management system of various policies and divisions that have been formed for a long time in the response to sudden environmental incidents is not conducive to the government's timely grasp of information, and it is difficult to make unified and efficient decision-making, thus resulting in the overall social resources. Idle and waste. The effective response to sudden environmental incidents requires the government to establish a unified, efficient, and authoritative permanent command coordination agency that has the ability to coordinate the communication of government parties and make quick decisions. And after the incident, the information can be accurately and objectively released in the first time, ensuring the smooth flow of information channels and the rapid transmission of information, and timely conveying corresponding prevention and self-rescue and mutual rescue measures to the pub-
lic, and at the same time, in a short time. Gather the power of all parties, unified command, and rational allocation of resources. If the current emergency response system for environmental emergencies is maintained and the various departments do not work together, it will be more difficult to deal with sudden environmental pollution.

(3) Poor information communication and insufficient information disclosure. First, under the existing emergency system, the information reporting and notification mechanisms between local governments, enterprises and institutions are not perfect, and a best-selling information communication mechanism has not yet been formed, and the definition of responsibility of all parties is not clear. The specific provisions of the content and responsible persons are lacking. At present, when various emergencies occur in the environmental protection industry, they are still reported in the form of administrative units reporting step by step or internal reporting, and there is a lack of unified and fast communication channels, resulting in poor information communication or scattered distribution channels. The government department cannot understand the development process of the emergency in a timely manner and make decisions quickly according to the situation. The relevant disposal units are unable to get the government and the command and coordination of the time and the support of relevant departments make it difficult to effectively deal with emergencies, which seriously affects the efficiency of event handling.

Second, the media's information reporting on emergencies and the government's timely release of emergency information are not sound. At present, the environmental protection industry lacks an information release system and a spokesperson system, and lacks centralized management and organization coordination for news reporting. The responsibility and rights of the media in the report of the emergency have not yet been fully clarified, and the rapid response to heavy news reports on major and catastrophic events has not yet been established. After the emergencies, the information released by the government and the information that the public understands are biased, resulting in asymmetric and incomplete information, which causes the public to panic. This not only led to the continuous escalation of the crisis caused by the incident, but also the lack of authoritative voice, the public's self-rescue action and the government's rescue measures could not be consistent, and the government's credibility was seriously challenged.

Study on Emergency Mechanism of Environmental Pollution Accidents in Danjiangkou.
(1) Innovative crisis management system. Compared with the achievements of China's reform and opening up in recent years, China's emergency environmental emergency response system reform has lagged slightly, and progress has not been great. To a certain extent, the backwardness of the emergency response mechanism has hindered or hindered the coordinated development of the social economy, and at the same time weakened the government's ability to govern and emergency management. The emergency management system reform of emergencies should focus on the transformation of government management functions. As a large country in the period of rapid development, there should be a higher target and updated crisis in the management system reform of emergency response. The management concept is to break the traditional single management model that has been formed for a long time, and establish a comprehensive emergency management department with unified command and coordination; local governments at all levels should also actively construct a “big government” with service-oriented “small government” and full development of social organizations. In order to construct and realize an emergency management system in which the government and the social pluralists participate together.

(2) Scientific preparation of emergency plans. In the study of the plan, the organic combination of plan preparation, drill, correction and emergency resources should be fully considered; in the preparation process of the plan, the various resources required for the emergency should be listed in detail, and the relevant information should be managed in a unified manner; During the process of the pre-planning exercise, the resources listed previously were tested in actual combat; in the process of repairing the plan, the personnel and material preparations formulated in the original plan were timely adjusted and supplemented according to the situation exposed by the exercise. Through such a process, both the plan is improved and the operability and systemicity of the emergency plan are ensured.

"Deep and simple" combination. Formally speaking, the plan is only a static written text, but the crisis often has no signs, and it exists in the dynamic changes of the moment. Therefore, even if the plan is elaborated, it is difficult to be comprehensive and foreseeable. Everything must have a certain lag in time. Moreover, there are various kinds of environmental incidents, and the plan system is numerous and the capacity is large. Therefore, it is necessary to continue to "deepen", "strengthen", research and gradually improve, and at the same time, "differentiate" and "simplify" the plan. Simplify the plan into a program text, and make it easy to implement through software development, setting up public inquiry devices (touch-type electronic display) in public places, and so on.
(3) Sound monitoring and forecasting mechanism. Establish special detection and prediction systems for disaster accidents and natural disasters based on the classification of emergencies. Extensively collect information on the signs of emergencies in natural and social environments, timely discover various safety hazards and detect the development trend of tracking events, and predict upcoming emergencies, and report to relevant functional departments at the same time, and the occurrence of an emergency is evaluated. First of all, local governments at all levels should comprehensively carry out the census and monitoring of hazards, pollution sources and hidden dangers of various environmental emergencies. As a normal management tool, gridding can make a difference in the early warning and early disposal of emergencies, and it can help. Secondly, on the basis of comprehensively grasping the sources of danger and sources of pollution, we will assess the hazards that may be caused by these sources and sources of pollution, establish a classification and classification management system, update records regularly, and uniformly mark the hazard levels of hazards and sources. For potential risks that may trigger unexpected environmental incidents, it is necessary to organize regular inspections and find problems to be treated within a limited time. Again, strengthen assessment and statistical analysis. While investigating and dealing with all kinds of sudden environmental incidents, it is necessary to make an assessment of the relevant links and prevention work in the event handling. Strengthen the statistical analysis of emergency management, clarify the responsible departments and personnel, improve the classification and grading standards, and timely, comprehensively and accurately count the number of sudden environmental incidents, the number of casualties, the direct economic losses caused, and the possible derivative pollution. And other relevant circumstances, set the weight coefficient, and be included in the annual economic and social development statistical indicators assessment system.

(4) Establish an efficient disposal mechanism. Since sudden environmental events differ in the degree of hazard, there are bound to be significant differences between general emergency environmental events and emergency measures for sudden environmental events that may cause serious consequences. According to the severity classification of the event, the classification and grading of the emergency response mechanism is of great significance for improving the efficiency of the emergency handling and the execution of the government.

It is recommended that the environmental protection industry deal with emergencies at two levels on the basis of classification and classification of emergencies and emergency agencies. (a) Early emergency response. After the general and major emergencies occur, the emergency response center where the incident occurs and the environmental protection bureau of the area are responsible for coordination, and the emergency unit and responsible unit are required to conduct emergency response according to their respective duties, and mobilize emergency teams to carry out emergency response. Rescue and aftercare, organize and guide the masses to carry out self-help and mutual rescue. (b) Emergency response disposal. After the incident occurs, the early disposal can not effectively control the emergency situation, and the situation has further development and expansion trend, that is, when it is transformed into a major emergency, the environmental protection department should assist the local government to carry out emergency work and determine the severity of the incident. Identify the emergency response level and scope, initiate emergency plans, and participate in the establishment of emergency response coordination headquarters when necessary to implement coordinated command and emergency response.

(5) Strengthen external communication and cooperation. The earth is a whole, a total ecosystem, regardless of national boundaries. Therefore, the harm caused by sudden environmental incidents in any country or region has already spread across national borders and spread and spread around the world. The environmental pollution caused by emergencies has evolved into a global problem, mainly reflected in the following four aspects: (a) Cross-border transfer by means of wind, atmosphere, rivers and other media has accelerated the spread of pollution, while exacerbating the global The deterioration of the environment; (b) the advancement of the “global integration” process has exacerbated the environmental burden of developing countries and accelerated the rapid consumption of biological resources; (c) some countries are geographically adjacent to each other, giving cross-border pollution Diffusion provides convenient conditions; (d) Due to the limited economic strength of some developing countries, there is no advanced technology and abundant material resources to deal with sudden environmental events. Therefore, the emergency mechanism for sudden environmental incidents cannot be confined to the scope of individual countries. It should follow the relevant spirit of the International Law and strengthen international cooperation and exchanges. It can build an international early warning mechanism for environmental incidents. The establishment of an international information communication mechanism and the construction of an aid mechanism will be completed together.
CONCLUSIONS

Aiming at all aspects of the prevention and management of sudden water pollution accidents, the establishment of a five-step link of “Numerical Simulation—Evaluation and Diagnosis – Traceability Prediction – Emergency Control – Pollution Disposal” is integrated into the emergency water pollution emergency control and disposal technology system. Environmental pollution emergency control and disposal technology system is built. Through the research on the emergency mechanism of sudden environmental pollution incidents in Danjiangkou, it is possible to ensure the safety of water supply in the trunk line of the South-to-North Water Transfer Project and achieve the goal of “sending water to the north”.

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LOSS OF GIT1 GENE IN FISSION YEAST PROVIDES RESISTANCE TO ENDOPLASMIC RETICULUM STRESS

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ABSTRACT

Schizosaccharomyces pombe detects glucose G-protein coupled receptor and transmits signal transduction via a cAMP-dependent protein kinase A (PKA) pathway. Also, Git1 C2 domain protein is required for maintaining basal cAMP levels and for producing glucose-triggered cAMP response. Actually, Git1 is a member of the UNC-13/Munc13 protein family that are conserved in eukaryotes and functionally included in synaptic vesicle exocytosis. We aimed to investigate the impact of Git1 on the vesicular trafficking dependent on endoplasmic reticulum and on the oxidative stress response. In this study, we cloned the full-length (git1T) of S. pombe git1 gene into pSGP572 plasmid, as a S. pombe expression vector carrying thiamine-repressed promoter, nmt1 and C-terminal GFP. Then, we compared the viability of S. pombe strains (wild type, gitΔ, pSGP572/gitΔ, pG1T/gitΔ) against to H2O2-induced oxidative stress and tunicamycin-induced ER stress to that of their control groups. We found that deletion of git1 gene brought S. pombe resistance to ER stress, but not oxidative stress in S. pombe cells.

KEYWORDS:
Schizosaccharomyces pombe, git1, tunicamycin, ER stress, oxidative stress

INTRODUCTION

Yeasts including Schizosaccharomyces pombe regulate their growth and metabolism presence of glucose in their medium [1]. S. pombe detects glucose via a cAMP-dependent protein kinase A (PKA) pathway, which consist of Git3 G-protein-coupled receptor, Gpa2 alpha subunit, Git5 beta subunit, and Git11 gamma subunit of a heterotrimeric G protein, Cgs1 regulatory subunit, and Pka1 catalytic subunit of PKA [2]. In addition, Git1 C2 domain protein is required for maintaining basal cAMP levels and for producing glucose-triggered cAMP response [3].

Git1 is a member of the UNC-13/Munc13 protein family that are conserved in eukaryotes and functionally included in synaptic vesicle exocytosis. In fungi, these proteins contain a C2 domain and two Munc13-homology domains (MHD 1 and MHD 2) near their carboxy-terminus [4]. In mammalian, C1 and C2 domains bind to phospholipids in a calcium dependent manner, and facilitates MUN domain functions by tethering vesicles to the plasma membrane [5-9]. These proteins have vital roles in both neuronal and other secretory cells for synaptic vesicle priming, large dense-core vesicle exocytosis, neurotransmitter secretion [10-13].

In S. pombe, there are two proteins involved in UNC-13/Munc13 protein family. One is Git1, which is a protein of 1097-amino-acid in length, and contains a C2, MHD1 and MHD2 domains [14]. The second is Ync13, which is essential for late stages of cytokinesis in fission yeast, and localizes to the expanding plasma membrane including the division site [15]. Also, Ync13 does not share the functional homology with Git1 [3]. In addition to the role of Git1 in glucose signaling, the C2 domain is also important for the posttranscriptional regulation that controls the level of Git1 in the cell. Also, MHD2 domain is critical for Git1 function [3].

In S. pombe, it has been known that glucose depletion or non-fermentable carbon source triggered the stress response pathway [16, 17]. The mitogen activated protein kinase (MAPK) pathway occurs under various adverse environmental conditions. MAPK pathway includes a set of three conserved protein kinase, of which is the stress-activated kinase, Sty1 [18]. Also, glucoseogenesis is regulated via Sty1 and cAMP [19].

The endoplasmic reticulum (ER) is functional in cell physiology, including vesicle trafficking and lipid and membrane biogenesis, as well as protein targeting and secretion [20]. The accumulation and aggregation of unfolded proteins causes ER stress. To fold proteins properly, a highly conserved unfolded-protein response (UPR) signal-transduction pathway is activated. The UPR pathway is basically conserved from yeast to mammalian cells [21, 22]. Because, tunicamycin (Sigma-Aldrich, T7765) inhibits N-linked glycosylation of lipids and pro-
teins in ER [23, 24], it was used for an ER stress agent.

In this study, due to Git1 is a member of UNC/Munc13 protein family, a long side its role in glucose signaling, we investigated the impact of Git1 on vesicular trafficking dependent on endoplasmic reticulum. We also examined to link between Git1 and the oxidative stress response. For this purpose, the full-length (git1T) of S. pombe git1T gene was cloned using S. pombe expression vector, pSGP572 carrying thiamine-repressed promoter, nmt1 [25] and as reporter protein, C-terminal GFP [26, 27]. Then, the viability of the transformants and the wild type cells were compared under H2O2-induced oxidative stress and tunicamycin-induced ER stress. We found that loss of Git1 gave resistance to ER stress in S. pombe cells.

**MATERIALS AND METHODS**

*S. pombe* strains and growth conditions. Of the purchasing haploid deletion library from BI-ONEER (http://pombe.bioneer.co.kr/), S. pombe git1A strain and transformant strains (pSGP572/git1A, pG1T/git1A), which were derived from the git1A strain used in this study are listed in Table 1.

These strains (10^6 cells/mL) were incubated in liquid and solid minimal (EMM) or enriched (YES) media [28]. 50 mg/L each of adenine, uracil, leucine as supplements; 200 mg/L genetin (G418) sulfate as selective marker.

**Table 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pombe 972 wild type</td>
<td>r</td>
</tr>
<tr>
<td>S. pombe git1A</td>
<td>git1A ade6-M216 leu1-32 -ura4-18</td>
</tr>
<tr>
<td>S. pombe pSGP572/git1A</td>
<td>pSGP572/git1A</td>
</tr>
<tr>
<td>S. pombe pG1T/git1A</td>
<td>git1T-pSGP572/git1A</td>
</tr>
<tr>
<td>E. coli DH5α</td>
<td>F′ endA1 histR1 (E. coli)</td>
</tr>
<tr>
<td></td>
<td>supE44 thi-1 recA1 gyrA (matt)</td>
</tr>
<tr>
<td></td>
<td>rplA1A (lacZVX-argF) U169</td>
</tr>
<tr>
<td></td>
<td>deoR [p80 d LacA (LacZ) M15]</td>
</tr>
</tbody>
</table>

**General methods in gene cloning.** Escherichia coli DH5α strain used for recombinant plasmid preparation and amplification was grown on Luria-Bertani medium (LBA) [29, 30]. The selection of bacterial transformants was done by using LBA supplemented with ampicillin (50 mg/L). High-efficiency transformation of S. pombe and S. pombe DNA isolations were performed according to standard protocols as described by Sabatinos and Forsburg (2010) [31].

**Plasmid constructions.** S. pombe git1T (git1T) was amplified by PCR using the following oligonucleotide primers: 5’-TTTTGTCGACATGGGATTCACATGTTGA-3’, P1, forward; 5’-AAGGATCCCTAATCTACTCACTCTTTTGAC-3’, P2, reverse. P1 and P2 primers contain *SalI* and *BamHI* restriction sites at 5’ and 3’ ends, respectively. In these PCR reactions, S. pombe wild type genomic DNA was used as a template. Phusion High-Fidelity DNA Polymerase (Thermo Fisher) was used for PCR according to the manufacturers’ protocols. PCR conditions were performed using 35 repetitions of following temperature cycle: at 98 °C for 10 s, at 51-53 °C for 30 s, at 72 °C for 1 min and a final elongation step at 72 °C for 10 min. The reaction products were resolved by electrophoresis in a 0.8% agarose gel. The fragment of git1T was recovered as described by a commercial kit (High Pure PCR Product Purification Kit, Roche). PCR product (git1T) was cut by *SalI* (Fermentas), *BamHI* (TaKaRa) restriction endonucleases, while pSGP572 plasmid was cut with *XhoI* (Fermentas), *BglII* (Fermentas) restriction endonucleases in its multiple cloning sites. The PCR product was ligated into pSGP572 plasmid by T4 DNA ligase (invitrogen) to generate plasmid pG1T.

**Microscopy.** S. pombe cells with GFP fluorescence were visualized using a 63 x oil-immersion objective on a Leica SPE2 confocal microscope. Images were captured using LASAF software (Leica Microsystems). For this purpose, fresh cultures cultured in liquid EMM medium were harvested by centrifugation and fixed using 10% formaldehyde for 20 min as described [32]. After cells washed once in PBS, they were stained with 4’,6-diamidino-2-phenylindole (DAPI, Sigma) at a final concentration of 1 μg/mL stock in PBS and incubated for 1h at room temperature in dark.

**Induction of Oxidative Stress.** S. pombe cells (10^6 cells/mL) were grown in liquid EMM with supplemented, without thiamine on an orbital shaker at 30°C at 180 rpm until their mid-log phase. Each culture was split into two tubes as the experimental and the control groups. 2 mM H2O2 (Sigma-Aldrich H11009) was added to the experimental group and incubated for 1 h as described by Pekmez et al. (2008) [33]. The control culture was not exposed to oxidative stress. Then the cultures were harvested by centrifugation (400 xg for 5min), washed once in sterile water, and diluted (10^3) and 100μl spread onto EMM plates. Plates were incubated at 30°C for 3–5 days. The cell viability (%) was calculated from the numbers of colonies obtained from control and experimental groups.

**Induction of Endoplasmic Reticulum Stress (ER).** After fresh S. pombe cell culture was split into two tubes as the experimental and the control groups. Tunicamycin (Sigma-Aldrich, T7765) was added to the experimental group at final concentration of 1 μg/mL stock in 1 mg/mL DMSO (dimethyl sulfoxide, Sigma-Aldrich), and incubated 24 hours. The control culture was not exposed to ER
stress. Then cell cultures of 1, 4 and 24 hours were harvested by centrifugation (400 xg for 5 min), washed at least twice in sterile water, and five 10-fold serial dilutions were made, and 5 μl aliquots from each spotted onto EMM plates with/without thiamine. Plates were incubated at 30°C for 3–5 days.

RESULTS AND DISCUSSION

Construction of git1 gene. The full straight (git1T) was cloned using pSGP572 expression plasmid (Fig. 1). After transferring the new construct (pG1T) and pSGP572 into S. pombe git1Δ strain, selection of transformants are based on ura4+ auxotrophic marker [27]. Also, in this construct, the expression of inserted gene is controlled by the nmt1 promoter [25, 26]. When the transformants monitored by confocal microscope with DAPI staining, as shown in Fig. 2, the transformants with pG1T plasmid showed GFP fluorescence, while pSGP572 did not (data not shown).

The growth rates of S. pombe cells. S. pombe wild type (wt), host (git1Δ) and transformant (pSGP572/git1Δ, pG1T/git1Δ) strains grown in liquid EMM with proper supplements without thiamine were monitored for 48 hours. The growth rates of these cells were given in a graph, as shown in Fig. 3. Generation times of cells is 4 h for wt, 4,2 for git1Δ, 5,2 h for pSGP572/git1Δ, 5 h for pG1T/git1Δ (Fig. 3). In the transformants with pSGP572 and pG1T plasmids, it was calculated slower growth rates than that of host cells in the same media.

In the fact that it was calculated slower growth rates of transformant cells than that of healthy haploids in the same media (Fig. 3) may be arise from the genotype of S. pombe host strain, or the plasmid DNA, as a big extra chromosomal structure [34].
Viability of *S. pombe* cells under oxidative stress and ER stress. It has been known that the glucose sensing/signaling mechanisms leading to glucose repression take part in the regulation of the oxidative stress response [35-37]. For this reason, the viability of all *S. pombe* strains was compared to each other under a mild level of oxidative stress. In this condition, there were no significant differences between among of these cells (Fig. 4). Based on these findings, it is suggested that Git1 C2 domain protein may not be associated with the oxidative stress response pathway.
At this time, it has been known that UNC/Munc13 family proteins, of which Git1 is a member, participate in the vesicular exocytosis process, we investigated whether any effect of the ER stress on the viability of S. pombe cells. For this purpose, we observed the reproductive status of the spots in EMM plates of the cells exposed to 1 μg/mL tunicamycin for 1, 4, and 24 hours after growth until mid-logarithmic phase in liquid EMM medium containing required supplements depending on S. pombe strain, but no thiamine to induction of nmt1 promoter in new constructs. Under the ER stress for 1 hour, it was observed that the difference obtained between the experimental and their control groups in each strain was to be similar in all strains (Fig.5). When the exposure time of the tunicamycin is increased up to 4 and 24 hours, each strain displayed different growth pattern against tunicamycin. It was observed that especially 24-hour application increased the sensitivity level in each cell, except for S. pombe git1Δ strain. In other words, because the most dramatically difference between the viability of the experimental and control groups were observed in a 24-hour application, the tolerances of the strains against the ER stress were compared under this condition. As shown in fig.5, there was not any tolerance to tunicamycin in wild type. In contrast, S. pombe git1Δ strain was found to be highly tolerant to tunicamycin. Interestingly, while the survival profile of the transformant with pSGP572 plasmid was similar to that of the host cell (git1Δ), the viability profile of the transformant with pG1T plasmid was found to be similar to that of the wild type with a slightly different.

In conclusion, our results suggested that Git1 protein, which was required for maintaining basal cAMP levels and for producing glucose-triggered cAMP response [3], might also play an effective role in the development of tunicamycin-induced ER stress. Comprehensive experiments are needed to understand which region(s) of Git1p is a factor in the development of ER stress induced-tunicamycin. For example, because the C2 domain is also important for the posttranscriptional regulation that controls the level of Git1 in the cell [3], C2 domain may be a candidate.

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TRAFFIC-INDUCED AIR POLLUTION EFFECTS ON PHYSIO-BIOCHEMICAL ACTIVITIES OF THE PLANT EUCALYPTUS CAMULDENSIS

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ABSTRACT

Air pollution in the urban area is a major concern globally, which causes generally by natural or anthropogenic effects. Increased traffic activities cause an increase in the amount of exhaust harmful gases such as CO₂ (carbon dioxide), HC (hydrocarbons), and NO (nitrogen oxides) release to the environment; which are responsible for both increases and decreases in rates of photosynthesis, stomatal conductance, responsible for vegetation injury, which lead to decrease crop yield. Considering these important aspects, the present study was undertaken to determine the effects of air pollution caused by exhaust emissions of harmful gases on plants. For this purpose, stomata intensities, proline, total soluble sugar and amino acid contents and some heavy metal accumulations were recorded on Eucalyptus camuldensis which were located in different distances (i.e., 0, 10, 30, 50, 70, 90 and 110 meters) from the highway of two cities namely Adana and Tarsus of Turkey. Data on opened stomata intensities, proline content and heavy metal concentrations (such as Zn, Fe, Pb, and Cd) were shown a statistically meaningful decreasing trend when distances from the highway were increased. It was also seen as meaningful increasing on total soluble sugar and amino acid contents. It was also recorded the concentration of soluble sugar and proline in polluted leaves of Eucalyptus camuldensis were significantly increased as compared to plants grown under control condition, indicating that the activation of protective mechanism in plants under air pollution, and the plant may act physiological adjustments to compensate for that air pollution stress.

KEYWORDS:
Harmful gases, traffic activities, photosynthesis, stomatal conductance, vegetation injury

INTRODUCTION

Plants are the main important parts of the ecosystem, and the concern to their sensitivity to air pollution is more significant than standards of air pollution [1]. Air pollution is one of the severe problems globally today. In recent past, air pollutants, responsible for vegetation injury and crop yield losses, are causing increased concern [2]. Air pollution is defined in many air pollution control laws as the presence of one or more contaminants, in such quantities and of such duration as they are or tend to be injurious to human life or property. Air quality is a global problem related to urbanisation and population growth that depend on rising of traffic, industrialisation and energy use [3]. Environmental stress, such as air pollution is among the factors most limiting plant productivity and survivorship. Increased traffic activities will cause an increase in the amount of exhaust gas released to the environment. The main harmful gases from exhaust emissions are CO₂ (carbon dioxide), HC (hydrocarbons), and NOx (nitrogen oxides) [4]. Controlled studies about nitrogen dioxide and nitric oxide demonstrated that responses for this pollutants include both increases and decreases in rates of photosynthesis and stomatal conductance in plants [5].

Adverse effects of air pollution on biota and ecosystems have been demonstrated worldwide. Many experimental works have been conducted on the analysis of air pollutant effects on crops and vegetation at various levels ranging from biochemical to ecosystem levels [6]. The studies on the effect of air pollution due to industrial activities on morphologic and physiologic factors of plants have been carried out [2], but the responses of trees to air pollutants may vary widely and these variations can be caused by many factors, such as differences in pollutant concentrations and distribution in time, the genetic origin, physiological activity, phenological stage and nutritional status of plants as well as
effects of various environmental factors [1].

In this context, the determination of plants that are both negatively affected by these pollutants is very important for roadside planting. Because roadside plants are important both for absorbing exhaust emissions and for keeping noise. In this study, Eucalyptus trees on the E5 highway (Adana-Tarsus) were selected. The aim of the study was to determine the effects of vehicle exhaust gases on the density of stomata and opened-closed stomata numbers.

**MATERIALS AND METHODS**

**Locations and treatments.** The study was carried out in the Department of Biology of Cukurova University, Faculty of Arts and Sciences. Leaves of evergreen Eucalyptus camaldulensis have been widely used as plant material in the Mediterranean region. In the study, trees of the same age were sampled that found in the refuges on the highway (Adana-Tarsus, E5) and in the distance of 0, 10, 30, 50, 70, 90 and 110 meters toward the agricultural areas from the road. Leaf samples were taken from the upper and lower parts of the canopy with 4 replications. The molds were removed from the leaf samples by using Silicon Rubber (RTV 11). Stoma counts were made from cellulose acetate films obtained from these molds [7]. For general stoma distributions, 25 counts were made on each leaf and the standard error of the 4 repetitions mean was calculated. In addition, for the open and closed stoma counts, 25 counts were made in two different regions on the top and bottom of each leaf and the average of these counts was used as a repeat value.

**Measurement of Proline.** It was determined according to the following method [8]. In the case, 500 mg of fresh plant material was homogenized with 3% sulpho-salicylic acid and filtered at blue band filter paper. Then 2 ml sample was put in a glass tube, then 2 ml ninhydrin solution (which contains ninhydrin, orthophosphoric acid, and acetic acid) and 2ml acetic acid were added into the tube. After that it was incubated in boiling water for one hour, samples put into an ice bath. Then, 4 ml toluene put on the samples and read at 520 nm spectrophotometrically. Proline content of samples was calculated according to the standard curve which drawn with L-proline.

**Soluble sugar analysis.** It was determined by using the following method [9]. For this purpose 100 mg dry powdered leaf material, homogenised with % 80 ethyl alcohol. 2.5 ml anthrone solution (150 mg Antron, solved until 100 ml with H2SO4) added on to 500 μL sample in a glass test tube and incubated in boiling water 5 minutes for glucose analysis. For fructose, 500 μL sample put again in another tube, and the same step was repeated. With the difference of glucose, samples incubated at 40 °C water bath 30 minutes. Later whole samples were read at 625 nm spectrophotometrically. The sugar content of samples was calculated according to the standard curve which drawn with D-glucose.

**The total amino acid analysis.** It was determined by using a modified Spies’s protocol [10]. Dry powdered leaf material of 1 g was homogenised with distilled water. Reaction reagent was prepared by using different solutions. The first solution, pH 5 citrate buffer, contains citric acid monohydrate and sodium hydrosulphate in it. The second solution had SnCl2·2H2O and prepared with the first solution. For the third solution, ninhydrin solved in ethanol. Reaction solution gets ready by mixing second and third solutions equal volume. Then added 1 ml sample into a glass test tube and added2 ml reaction reagent on it. After 15 minutes in boiling water bath and cooling, 3 ml diluted (1/1) propanol added on samples and they read at 570 nm spectrophotometrically. The amino acid content of samples was calculated according to the standard curve which drawn with glycine.

**Heavy Metal Analyses.** For element analyzes [11], dry plant material of 0.2 gram was burned five hours in 550 °C ash oven, then added in 0.5 ml HCl and evaporated. Then Zn, Fe, Cu, Cd, Pb elements were read with atomic absorption spectrophotometer.

**Statistical Analyses.** ANOVA test was used for statistical analyses that performed with IBM SPSS 20 packet program. In this context, Tukey or Tamhane’s T2 tests were used according to whether the variances were equal distributed or not [12].

**RESULTS AND DISCUSSION**

**Stoma Intensity of Leaves.** Opened stoma intensities were increased as the distance increased in this study (Table 1). And its found significant according to statistical analyses, changing of total opened stoma intensities in 90 and 110 meters disturbances are important. Also, the changes between all distances were significant according to ANOVA test which performed for total stoma intensities. But changing in total closed stoma intensities were not found significant according to statistical analyses. Stomatal and epidermal cell size, lower recurrence, thickening of the cell wall, epicuticular wax deposition alterations and chlorosis are among the auxiliary alterations in leaves subjected to air pollution [13]. Pollutants can cause leaf injury, stomatal damage, premature senescence, decrease photosynthetic activity, disturb membrane permeability and
reduce growth and yield in sensitive plant species [10]. Reductions in leaf area and leaf number may be due to decreased leaf production rate and enhanced senescence. The reduced leaf area results in reduced absorbed radiations and subsequently in reduced photosynthetic rate [6].

Earlier researchers [14] stated that leaf size, stomatal pore width and stoma density of plane were lower at the urban site. Air pollution particulates may cause stomatal occlusion and lead to decrease photosynthesis [15]. Particulate pollution caused stoma closure in poplar leaves; this may restrict CO₂ influx to the cells and chloroplasts, and finally affects photosynthetic activity [16]. Considering the stoma results of this study, it is thought that the cause of the increase of open stoma density as it moves away from the source of pollution may be related to the decrease of the emissions causing pollution. Other researchers [17] reported that increased air pollution caused decreased in stoma intensity.

**Proline Contents.** Proline content of *Eucalyptus camaldulensis* leaves was decreased with increasing distance to the highway as shown in Fig. 1. The change in the groups was found to be significant according to the ANOVA test, and the proline contents of the plants at 0 and 10 meters distances were significantly different from the other distances [18] depicted that increased proline in leaves is a marker of vehicular pollution which supports our results. In another research, proline content increased in experimental areas too, which contains densely populated with vehicular activities [19]. It is a kind of amino acid and known as a non-enzymatic antioxidant. According to Mukherjee and Agrawal [20], variations in proline content with air pollution may be correlated with the presence of heavy metals which induce oxidative stress after accumulation inside the leaves. Proline is also described for its role in metal chelation and Reactive Oxygen Species (ROS) scavenging. Some workers have been published the increase in free proline content in response to various environmental stresses in plants [21]. Typical environmental stress (high and low temperature, drought, air, and soil pollution) can cause excess ROS in plant cells, which are extremely reactive and cytotoxic to all organisms.

**Total Sugar and Amino acid Contents.** The changes in the total sugar content tend to increase as they move away from the highway as seen in Fig. 2 and all of these changes were statistically significant. When the total amino acid contents are compared, it is seen that the way away from the path leads to an increasing trend. These increases
showed statistically significant differences. An increase in total sugar was observed in leaves collected from the investigated site (Fig. 2). Soluble sugar is an important constituent and source of energy for all living organisms. Plants manufacture this organic substance during photosynthesis and breakdown during respiration [22]. In this study, soluble carbohydrates in polluted leaves were reduced under pollution conditions.

Sugar is a mainly important organic compound for whole organisms as it is the resource of energy. Plants produce this material during photosynthesis and breakdown during respiration. The concentration of soluble sugar is an indicator of the physiological activity of plants and it is considered to show the sensitivity of plants against air pollution. Scientists [22] reported that the sugar content in the Eucalyptus leaves decreased in air polluted area. While the reduction in soluble sugar content in polluted stations may be associated with increased respiration and decreased CO₂ fixation due to pigment degradation. These researches also found reduced sugar content in Eucalyptus leaves at air polluted area, as our results.

In the present study, amino acid content showed the same trend as sugar content. The total amino acid concentration reached the highest values in control and the farthest plants to the highway and the variation as shown in Fig. 2. In contrast to our results [23] stated that their Eucalyptus leaf samples exhibited an increasing trend for amino acids, and they explained this situation with the degradation of proteins to amino acids and nitrate reductase activity. In addition to these, amino acid content wasn’t affected by air pollution in Eucalyptus leaves in another research.

**Heavy Metal Concentrations.** In heavy metal concentrations, it is seen that there is a decreasing tendency in heavy metals other than Cu as seen in Fig. 3. Other metals especially zinc (Zn), lead (Pb) and cadmium (Cd) significantly decreased except Cu. Lead content of Eucalyptus leaves was decreased by increasing far to the highway, and control plants did not lead in their tissues. Organometallic compounds like tetraethyl lead and zinc dialkyl dithiophosphate are often added to gasoline and lubricating oils and these heavy metals cause atmospheric pollution with auto-exhaust emission. Similar to the present results, [24] stated that lead concentrations of reference site samples were less than the tertiary road, secondary road, and primary road, and the highest lead concentration was found at primary road samples. Moreover, the Cd content of eucalyptus leaves of the present study decreased with increasing distance to highway, and eucalyptus leaves accumulated more Cd than Pb. The reasons for Cd in the atmosphere are natural and anthropogenic resources and do not have any biological function [25]. The copper (Cu) content of eucalyptus leaves had a bell curve image and the highest Cu content was found at the mid-far site (30 m distance away highway) while, the lowest content at the farthest distance. The iron (Fe) content of eucalyptus leaves showed a wavy tendency; while control plants had the highest Fe content, but the lowest Fe was established at the farthest distance to the highway. Furthermore, zinc (Zn) content had another bell curve but a contrast to Cu image. This situation may be correlated with these three metals of Zn, Cu and Fe nutrient features. The high concentration of these metals might be due to atmospheric conditions at the highway, and their trace element features might be due to at far distances. In a similar study with the present one, researches found Cu and Zn content of the plane at the rural site more than urban site [14].

![Figure 2](image_url)

**FIGURE 2**
Total sugar and amino acid contents Standart Deviations of *Eucalyptus camaldulensis* leaves at different distances to the highway (meter)
CONCLUSION

The results of this study revealed that opened stoma intensities, proline content and heavy metal concentrations (Zn, Fe, Pb and Cd) remarkably decreased with increasing distances to the highway. It was also seen as a meaningful increasing tendency of total soluble sugar and amino acid contents. In general, E. camaldulensis shows high resistance to air pollution and can be used for filtering air pollutants. It was observed higher soluble sugar and proline contents as compared to plants in control. Proline rates in polluted leaves significantly increased, suggesting the activation of protective mechanism in plants under air pollution, and the plant makes physiological adjustments to compensate for that air pollution stress.

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SEDIMENTARY CHARACTERISTICS AND PETROLEUM GEOLOGICAL SIGNIFICANCE OF NEAR-SOURCE SANDY BRAIDED RIVER DELTA: A CASE STUDY OF THE UPPER THIRD MEMBER OF SHAHEJIE FORMATION IN LAIZHOU BAY SAG, CHINA

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ABSTRACT

The near source sandy braided river delta sedimentation is an important reservoir in the Upper Third Member of Paleogene Shahejie formation (E2-S3) in Laizhou Bay Sag, Bohai Bay Basin. Based on the integrated analyses of core observation, grain size statistics, seismic data and well logging data, combined with the regional research, we found that Laizhou Bay Sag is a faulted basin in the period of the Upper Third Member of Shahejie. The provenance mainly comes from the Kendong uplift, 20 kilometers away. The core and grain size show that the reservoir is dominated by sandy sedimentation. The lithology is mainly siltstone, fine sandstone and medium sandstone. The coarse sandstone is relatively few and the conglomerate is undeveloped. Three kinds of subfacies were recognized, including braided river delta plain, braided river delta front and pro-braided river delta, which can be further divided into the following six sedimentary microfacies: upper-river distributary channel, alluvial plain, under-water distributary channel, underwater distributary interchannel, mouth bar and sheet sand. The near source sandy braided river delta is close to the hydrocarbon generating center of the faulted basin and formed a high quality trap combined with the fault system, which is a favorable reservoir for oil and gas accumulation. This provides an important research direction for oil and gas exploration in the sag.

KEYWORDS:
Near source sedimentation, sandy braided river delta, petroleum geological significance, Laizhou Bay sag

INTRODUCTION

Bohai Bay Basin is one of the continental fault lacustrine basins with the highest oil and gas reserves in eastern China. Bohai Bay is an important area for exploration in recent ten years, especially the Jiyang Bay Sag and the Laizhou Bay Sag in the southern Bohai Sea [1-2]. A number of large and medium-sized oil and gas fields have been discovered and developed. Previous studies on Laizhou Bay Sag have focused on structural evolution, combinatorial characteristics of the fault development, petroleum system and its influence on oil and gas migration and accumulation. A series of results have been achieved [3-5]. However, due to the lack of offshore drilling data and low seismic resolution, there is no systematic study on the sedimentary system, sedimentary characteristics and petroleum geological significance of the Upper Third Member of Paleogene Shahejie formation.

Combined with the regional geological background, we found that the research area is only 20 kilometer from the provenance Kendong uplift [6], which belongs to the near source sedimentation. Based on the comprehensive analysis of core, thin slice, logging and other data, it is concluded that most of the sedimentary rocks are distributed in the range of siltstone and medium sandstone. It belongs to sandy sedimentation. Based on the characteristics of "S" reflection of seismic profile and combination characteristics of logging morphology, we concluded that near source sand braided river delta sedimentation are developed in the Upper Third Member of Shahejie formation in Laizhou Bay Sag, which is different from the conventional braided river delta. Due to the characteristics of sufficient provenance supply, stable hydrodynamic development and close to the center of the lake basin, its distribution range is wide, the extension distance is far, and the overlap is developed. The sandstone can cover 45-60% of the
sag area. The thickness of single layer sandstone is more than 10 meter. The plane extension is about 5 to 15 kilometer. The matrix content is low and the physical property of reservoir is good. In addition, the reservoir is spatially close to the main source rocks in the sag and becomes a high-quality reservoir for oil and gas accumulation. Therefore, combined with the recent achievement of the oil and gas exploration, the analysis of the sedimentary characteristics with the recent achievement of the oil and gas exploration and the prospect evaluation in Laizhou Bay Sag.

REGIONAL GEOLOGICAL CHARACTERISTICS

Laizhou Bay Sag, located in the southern Bohai Sea, is a half graben basin with a fault in the northern side and an overlap in the southern side (Fig. 1). The research area is close to the sedimentary center of the hydrocarbon generation depression, which has become a favorable reservoir for oil and gas accumulation [7]. During the Paleogene period, the Bohai Bay Basin was in the second rifting episode, and the Laizhou Bay Sag began to take shape. The Weibei uplift in the south direction was relatively low, the sediment supply was weak and the transport distance was short. Kendong uplift became the main provenance area of Laizhou Bay sag. During the Paleogene period, the strike-slip fault property of the Tan-Lu fault zone was left-handed [8-10], which controlled the transport of sediments to the north-east and filled the north depression of the Sag.

Laizhou Bay Sag is a small faulted basin in Ji-yang Sag of Bohai Bay Basin [11]. The reflection characteristics of 3D seismic profile show that it has a typical forward product structure. This is the seismic response of the delta entering the slope break of the lake basin. Combined with the tectonic sedimentary background, a set of sedimentary system, which is from braided river to braided river delta to lake, was developed in the Upper Third Member of Shahejie formation. Braided rivers are distributed in the basin margin of Kendong uplift. From the basin margin to the center of the lake basin, braided river deltas and lacustrine sediments are in turn. The braided river delta in the Upper Third Member of Shahejie formation is the main sedimentary body in the sag, with wide distribution range and large thickness of sedimentary strata. Braided fluvial delta sandstone, especially the braided fluvial delta front sand body, which extends to the lake basin and adjacent to the hydrocarbon source facies, has become an important oil and gas reservoir in the sag.

RESEARCH METHODS

In this study, the macro description of the core and the microscopic research methods, such as microscopic observation and grain size analysis, are comprehensively used to analyze the characteristics of petrology and sedimentary structure. Combined with seismic interpretation data and logging data, we think that near source sandy braided river delta sedimentation was developed in the Upper Third Member of Shahejie formation in Laizhou Bay sag. The basic data include 120 meter cores from 8 wells, logging data from 10 exploration wells and 50 development wells, and grain size analysis data of 85 samples. Firstly, the sedimentary facies types and dominant reservoir microfacies types in the research area are determined by using core data, grain size statistics and seismic reflection termination characteristics. Then, by using logging data and seismic data, the characteristics of sedimentary facies profile and plane distribution in the Upper Third Member of Shahejie formation were analyzed. Finally, combined with the characteristics of fault development and recent exploration results, the potential and prospect evaluation of oil and gas accumulation in the sag were analyzed.

FIGURE 1
The tectonic unit map (a) and regional location map (b) of Laizhou Bay Sag in Bohai Bay Basin
RESULTS

Characteristics of sedimentary facies. 85 samples were systematically sampled from drilling cores. The characteristics and grain size data under thin slice microscope were calculated. The results show that the lithology is mainly fine sandstone, medium sandstone and siltstone. A small amount of coarse sandstone is developed (Fig. 2). The grain size sorting coefficient of sandstone is large and the sorting ability is poor. To sum up, the sedimentary environment is near source sandy braided river delta sedimentation. Combined with seismic data and mud logging data, three kinds of subfacies were recognized, including braided river delta plain, braided river delta front and pro-braided river delta, which can be further divided into the following six sedimentary microfacies: upper-river distributary channel, alluvial plain, underwater distributary channel, underwater distributary interchannel, mouth bar and sheet sand [13-15].

Braided river delta plain subfacies. The braided river delta plain subfacies is mainly composed of upper-river distributary channel and alluvial plain. Some wells were drilled into thin coal lines. This indicates that the reservoir is an aquatic sedimentary environment [15-17], which is a swamp deposit developed in humid climate.

(1) Upper-river distributary channel. The lithology of upper-river distributary channel is mainly gray-white, variegated coarse sandstone and medium sandstone (Fig. 3a). The average particle size of clastic particles is 0.56mm, the median particle size is 0.62 mm, and the probability accumulation curve is mainly two-stage.

The maturity of sandstone composition is relatively low. The average content of quartz is only 30.8%. It mainly contains feldspar and cuttings, with an average content of 69.2%. Most of the cuttings are igneous rocks and metamorphic rocks. Igneous rocks are mainly acid ejected rocks and neutral ejected rocks. The metamorphic rocks are mainly quartzite and metamorphic quartzite. The structural maturity is low. The separation coefficient is between 2.75 and 3.86. The shape is mainly in the shape of grinding sub-angular shape. The development of flushing surface and retained sedimentary gravel at the bottom is the result of sudden flood scouring mudstone at the edge of the channel during the flood period. The shape of logging curve is bell type.

(2) Alluvial plain. The sediments are mainly mudstone or silty mudstone. The color of mudstone is mainly purplish red, brown and other oxidation colors. Carbonaceous mudstone and coal blocks are developed in drilling cuttings. The frequent swing of the braided channel leads to the entry of thin coarse debris or gravel into the alluvial plain, which shows a medium tooth shape in the logging curve.

The braided river delta front subfacies. The braided river delta front subfacies is the main sedimentary body of the Upper Third Member of Lai-zhou Bay Sag. The underwater distributary channel microfacies sandstone reservoir is the most favorable section for oil and gas accumulation in the sag and is the key target layer for oil and gas exploration in recent years [18-19].

(1) Underwater distributary channel. Underwater distributary channel is the underwater extension of the braided channel with a wide range of development and a large sedimentary thickness. It is the main sedimentary body of the front subfacies. The scouring and undercutting of the river is still strong (Fig. 3b). Its lithology is mainly gray or gray medium sandstone and fine sandstone (Fig. 3c). The average particle size is 0.21mm. The median granularity is 0.19mm. Its particle size is smaller than that of braided channel. The probability cumulative curve is still two-stage.

The rock composition is still dominated by feldspar and cuttings. Its composition maturity is low. The separation coefficient is between 1.76 and 2.96. The structural maturity is low. Plant debris can be seen. Trough crisscross bedding, plate crisscross bedding and wavy crisscross bedding are developed (Fig. 3d). The mudstone tearing block is formed by rapid sedimentation and swinging erosion (Fig. 3e). The underwater distributary channel is the main...
body of progressive reflection in the upper slope break zone of seismic profile. The logging curves are mainly of the medium and high amplitude box type or the bell type.

(2) Estuary bar. The estuary bar sedimentation is formed by the continuous extension of channel sandstone to the lake basin and the transformation of lake waves. Therefore, it shows the characteristics of reverse grain sequence (Fig. 3F), and the estuary bar deposits are mainly gray fine sandstone and siltstone. The average particle size is 0.12mm. The median granularity is 0.08mm. On the probability accumulation curve, due to the double action of the river and lake waves, the grain size response has a phenomenon of two leaping secondary population.

The distribution range of estuary bar is relatively limited and only exists in the front of the early underwater distributary channel in the Upper Third Member of Shahejie formation. Wavy bedding and massive bedding can be seen in sedimentary structures. The logging curve is funnel shape or toothed funnel shape.

(3) Sheet sand. Sheet sand is formed by the rapid rise of the lake level and the further enhancement of the transportation and transformation of the front edge sand by the action of lake waves. However, due to the strong water energy of the near-source braided channel, sheet sand is very rare in the research area. Sheet sand sedimentation is mainly dark gray siltstone with an average particle size of 0.015mm and a median grain size of 0.012mm. The logging curve is finger-shaped.

(4) Underwater distributary channel. The lithology of the underwater distributary channel is dominated by mudstone and silty mudstone. The fossil stems of plants can be seen from Fig. 3G. When the underwater distributary channel swings, it will erode the previously sedimentary interchannel mudstone (Fig. 3b). The logging curve is flat.

Pre-braided river delta subfacies. Pre-braided river delta subfacies is located below the surface of the wave base and the water energy is weak. The sediments are mainly dark gray, gray-black mudstone and silty mudstone. Due to the strong hydrodynamic force of the near-source braided river delta, the strong fracture in the Upper Third Member of Shahejie formation and the large dip angle of the slope break zone, a small amount of gravity flow sedimentation will be developed in the slope break zone (Fig. 3h). The sandstone bodies deposited by gravity flow are the most likely to form lithologic reservoirs because they are closest to the source rocks in the lake basin [20-25].

![FIGURE 3](image-url)
Core and thin slice photo of near source sand braided river delta
Notes: (a) Upper-river distributary channel variegated coarse sandstone, massive bedding, 2100.7 m, Well KL-1; (b) Underwater distributary channel oil-bearing grayish brown medium sandstone, flushing surface, strong undercut of the river, 2182.7 m, Well KL-1; (c) Underwater distributary channel dark gray fine sandstone, massive bedding, 2181.4 m, Well KL-4; (d) Underwater distributary channel brown-gray fine siltstone, wavy bedding, 2159.6 m, Well KL-2; (e) Underwater distributary channel brown-gray oil-immersed fine sandstone, positive rhythm, development of mudstone tear block, top gray siltstone, lenticular bedding, 2205.3 m, Well KL-5; (f) Estuary bar siltstone-fine sandstone, reverse rhythm, carbonaceous bands and fine fractures, 2184.5 m, Well KL-4; (g) Underwater distributary channel gray-black mudstone, visible plant stem fossils, 2150.6 m, Well KL-3; (h) Pre-braided river delta subfacies gray-black silty mudstone, gray-white fine sand mass, deformation bedding, 2160.5 m, Well KL-6.
In recent years, most of the oil and gas reservoirs discovered in Laizhou Bay Sag are concentrated in the Upper Third Member of Paleogene Shahejie formation. Therefore, the study on the characteristics and distribution of sedimentary facies in the Upper Third Member of Shahejie formation is of great significance for oil and gas exploration and evaluation. Based on logging data and seismic data, the cross-well profile (Fig. 4) and the plane distribution characteristics of near-source sandy braided river delta in the Upper Third Member of Shahejie formation in Laizhou Bay sag are drawn (Fig. 5).

It can be seen from Fig. 4 that the near-source sandy braided river delta is pushed into the lake basin as a whole in the form of progradation and aggradation. Only in the middle of the Upper Third Member of the Shahejie formation, retrogradation sedimentation developed briefly. The microfacies is dominated by underwater distributary channel sedimentation, followed by estuary bar. The estuary bar is developed in the underwater distributary channel.
near the front of the lake basin. According to the logging interpretation results, the thickness of the single layer sandstone can reach 13m. The deposited sandstone extends to the lake basin up to 15km. It can be seen from Fig. 5 that the near source sandy braided river delta sedimentation were widely developed in the Laizhou Bay Sag during the Upper Third Member of the Shahejie period. Due to the frequent channel swing, the sandstone can cover 45 to 60% of the sag area on the plane. The underwater distributary channel and estuary bar sedimentation of the front subfacies are influenced by the waves of the river and the lake basin. The content of sandstone miscellaneous base is low and the reservoir performance is superior.

The sandstone reservoir deposited in the near source sandy braided river delta is spatially close to the hydrocarbon source facies in the sag, so it has become a favorable reservoir for oil and gas accumulation. When anticline, fault nose structure and fault block traps are formed due to structural fluctuation or fault sealing, structural oil and gas reservoirs and lithologic structural reservoirs are easy to form. This is the focus of recent exploration in Laizhou Bay Sag, and a number of large and medium-sized oil and gas fields have been discovered (Fig. 6). However, due to the intense F1 activity of the synsedimentary fault in the Paleogene period, the near-source sandy braided river delta front sandstone was induced to retransport and develop gravity flow sedimentation to form a series of lithologic oil and gas reservoirs. The front braided river delta subfacies near the front subfacies will be the potential area for prospect evaluation of Laizhou Bay sag.

CONCLUSIONS

(1) The transportation distance is short and the reservoir is mainly deposited by sandstone. Three kinds of subfacies were recognized, including braided river delta plain, braided river delta front and pro-braided river delta, which can be further divided into the following six sedimentary microfacies: upper-river distributary channel, alluvial plain, underwater distributary channel, underwater distributary interchannel, mouth bar and sheet sand.

(2) The dominant reservoirs in the Upper Third Member of Shahejie in Laizhou Bay Sag are mainly distributed in the sandstones deposited in the underwater distributary channel of the braided river delta front subfacies, followed by the estuary bar. The thickness of the single layer is 13m. The deposited sandstone extends to the lake basin up to 15km. The plane distribution range can reach 60% of the sag area.

(3) The sandstone reservoir deposited in the near source sandy braided river delta is spatially close to the hydrocarbon source facies in the sag. When anticline, fault nose structure and fault block traps are formed due to structural fluctuation or fault sealing, combined with the high quality reservoir of the braided river delta front, structural oil and gas reservoirs and lithologic structural reservoirs are easy to form. However, in the pre-braided river delta subfacies, the gravity flow sedimentation is easy to form lithologic oil and gas reservoirs. This provides a direction for oil and gas prospect exploration in Laizhou Bay Sag.

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A DYNAMIC GRADIENT ANALYSIS OF LANDSCAPE PATTERNS OF THE ZHENGZHOU URBAN AREA IN RESPONSE TO RAPID URBANIZATION

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ABSTRACT

To better understand landscape pattern dynamics in a rapidly urbanizing area, this paper analyzes land use patterns and land cover data of Zhengzhou, Henan, China, using data from three Landsat TM images taken in 1988, 2001, and 2014. By combining gradient analysis and landscape pattern analysis, and by using multiple landscape on both class and landscape scales, we quantified the spatial dynamics of the landscape patterns of the study area. Our results revealed dynamic changes in the landscape patterns of the Zhengzhou area. We found that the urban landscape had expanded, and unused land had decreased from 1988 to 2014. Indexes of patch density, spread and landscape diversity showed regularity in the gradient belt near the administrative center, and their peak values expanded over time. We also found that gradient changes of urban outskirts areas in 2014 were significantly different from those in 1988 and 2001, indicating that the urban construction has resulted in considerable ecological disturb. Finally, we discuss the impact of urbanization processes on the urban core and suburban areas at different time points. We hope that these results may be beneficial for the regional land use policy-making and sustainable development planning.

KEYWORDS:
Urbanization, landscape pattern, gradient analysis, buffer zone, Zhengzhou City

INTRODUCTION

The degree and speed of urbanization has a direct impact on regional landscapes as well as, on the ecology, biodiversity [1], and human quality of in urban areas. The relationships between spatial patterns of urbanization and ecological processes are an important research topic for urban and landscape ecologist [2-4]. After more than 60 years of development, Zhengzhou, the capital of Henan province, has become an important transportation hub, and the largest city in the Central Henan urban agglomeration. The spatial development of rapidly urbanizing areas is different from those of traditional urban areas. Due to rapid urbanization, the central urban spaces of many densely populated areas have rapidly expanded. Such expansion is affected by many causal factors, and the speed, intensity, and shape of urban spatial expansion generally occurs in discrete stages.

Landscape pattern metrics are used to aggregate quantitative information regarding landscape space, and widely used in landscape ecology. Gradient analysis, a technique adapted from vegetation science, can reveal the spatial distribution of different ecosystem features. The combination of landscape pattern metrics and gradient analysis is a powerful tool to characterize the morphology of urban areas; combined analyses of this type have been used both in China and abroad[5-8]. In this study, we use the combination of these techniques to analyze the landscape of Zhengzhou City over different historical periods. Our results may, be of interest to urban planners interested in exploring the expansion of Zhengzhou; or in developing regional land use and sustainable development policies for this area. More generally, our results may also be of interest to scholars studying the evolution of urban development.

MATERIALS AND METHODS

Survey area. Zhengzhou (34°16’N—34°58’N, 112°42’E—114°14’E) is located in the central part of northern Henan, a province of which it is the capital. The altitude of Zhengzhou ranges from 75 m in the east and 1512 m in the west. The maximum distance between the easternmost and westernmost points is 166 km, and the maximum longitudinal distance from the north to the south is 75 km. Zhengzhou has a subhumid monsoon climate and lies in a warm temperate zone. There it, experiences drought in the spring, heat and rain during the summer, extensive sun during the autumn, and snow and cold during the winter. The annual average temperature is 14.4°C, while the hottest month is July with an average of 27.3°C; the coldest month is January, with an average tempera-
ture of 0.2°C. The average rainfall in Zhengzhou is 640.9 mm, and the city experiences 220 frost-free days and 2400 hours of annual sunshine. The Zhengzhou region contains 35 small rivers that belong to the in Yellow River and Huai River basins. Zhengzhou has jurisdiction over 7 administrative regions (the Zhongyuan, Erqi, Jinshui, Zhengdong, Huiji, Shangjie, and Guancheng districts), 5 cities (Xinzheng, Dengfeng, Ximmi, Xingyang, and Gongyi), and 1 county, (Zhongmou; Figure 1).

**Research data collection.** Three Landsat thematic mapper (TM) images from satellite orbit number 126/36 taken in May 1988, August 2001, and May 2014 were selected as the research dataset. These images included high-resolution cloudless map. Atmospheric correction was carried out using the FLAASH atmospheric radiometric correction model, and geometric rectification was performed using a 1:10 million topographic map. The error control was within 0.5 pixels.

TM images were interpreted by a combination of supervised classification and visual interpretation. All land cover in the study area was classified into 6 types: construction land (including urban and rural construction), agricultural land, grass land, wood land, bodies of water (including rivers, reservoirs, and ponds, among others), and unused land. All classified results were converted to vector format. By combing historical land use and GPS survey data, we obtained a landscape map from each of the 3 time periods with a granularity of 30 m. The accuracy of the interpretation of remote sensing images was over 90%.

**Establishment of the buffer zone gradient.** Landscape patterns consist of the structure of the spatial features in any landscape. To analyze the spatial structure of Zhengzhou, we first established a buffer gradient zone in the research area. This involved taking the administrative core of Zhengzhou City as the center and setting up a ring-shaped buffer zone with radius of 5 km toward the outer edge of the city; this processes produced 20 buffer gradient zones (Figure 2), numbered 1 (the innermost) to 20 (the outermost). We then analyzed the type, number, spatial distribution, and configuration of landscape units of each buffer gradient zone using the landscape metrics method, which relates landscape characteristics to ecological processes. The analysis of the characteristics of related buffer zones can then reflect fine changes in urban landscape gradients.

**FIGURE 1**
Zhengzhou administrative division

**FIGURE 2**
Buffer zones in the study area

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**Calculation of landscape metrics.** We used FRAGSTATS version 3.3 to calculate the landscape metrics for all patch types as well as landscape level of each buffer area. The selection of landscape metrics should reflect the relationship between landscape patterns and ecological processes, and should also have clear ecological significance [9]. Metrics should also be sensitive to the gradient of urbanization in order to reflect the regulation of landscape patterns on the gradient within the buffer zone. With these selection criteria in mind, we selected the following landscape metrics (Table 1): landscape area ratio (PLAND), patch density (PD), largest patch index (LPI), shape index (LSI), Shannon's diversity index (SHDI), Shannon evenness index (SHEI) and contagion index (CONTAG). These seven indexes we used to compare differences in landscape types among the different buffer zones.

**RESULTS AND DISCUSSION**

**Overall characteristics of landscape pattern.** The most abundant landscape type was grassland, followed by agricultural land and construction land. The least abundant landscape type was bodies of water (Figure 3). With the expansion of Zhengzhou, construction has increased each year. This has resulted in an increase from 9.5% to 22.8% of all land from 1988 to 2014, a total increase by a factor of 2.4. Correspondingly, the proportion of unused land declined each year, from 15.7% in 1988 to 2.3% in 2014. The agriculture and woodland areas first increased and then decreased from 1988 to 2014. The total area of agriculture and wood land in 2014 has increased compared with that in 1988. On the whole, the area of grass land in 2014 was less than that in 1988.

<table>
<thead>
<tr>
<th>Table 1: Description of landscape metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Landscape metrics</strong></td>
</tr>
<tr>
<td>Class percent of landscape</td>
</tr>
<tr>
<td>Patch density</td>
</tr>
<tr>
<td>Largest Patch Index</td>
</tr>
<tr>
<td>landscape shape index</td>
</tr>
<tr>
<td>Patch Cohesion Index</td>
</tr>
<tr>
<td>Shannon diversity index</td>
</tr>
<tr>
<td>Shannon evenness index</td>
</tr>
<tr>
<td>Contagion index</td>
</tr>
</tbody>
</table>

**FIGURE 3**

Relative abundance of selected landscape types in the Zhengzhou area from 1988 to 2014
TABLE 2
Landscape indexes for the Zhengzhou area at three time points from 1988 to 2014

<table>
<thead>
<tr>
<th>Year</th>
<th>LSI</th>
<th>PD</th>
<th>LPI</th>
<th>SHDI</th>
<th>CONTAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>124.8081</td>
<td>7.6014</td>
<td>42.2379</td>
<td>1.3777</td>
<td>50.8766</td>
</tr>
<tr>
<td>2001</td>
<td>121.7592</td>
<td>7.9881</td>
<td>24.6153</td>
<td>1.5432</td>
<td>50.2246</td>
</tr>
<tr>
<td>2014</td>
<td>94.6378</td>
<td>4.8365</td>
<td>32.2405</td>
<td>1.3379</td>
<td>54.2600</td>
</tr>
</tbody>
</table>

Landscape metrics for the Zhengzhou region at each time point are shown in Table 2. From 1988 to 2014, the landscape shape index decreased each time point. This index reflects the complexity of the shape of all patches of the landscape; the smaller the value, the more regular the shape of the patch. Patch density increased slightly from 1988 to 2001, but significantly decreased from 2001 to 2014. However, the contagion index slightly decreased from 1988 to 2001 but increased from 2001 to 2014. Patch density and the contagion index mainly reflect the degree of landscape fragmentation; smaller patch density values and greater contagion index lower degree of landscape fragmentation. The largest patch index also showed a downward trend over these the 23 years, while the diversity index first increased and then decreased over the same period. The lowest value of the largest patch index and the largest value of the diversity index were both recorded at the 2001 time point. The largest patch index describes landscape dominance, with lower values indicating landscapes that are less dominant. Our results suggested that there was no obvious dominant landscape. The diversity index reflects the presence of many landscape patches, with more balanced distributions of landscape path types resulting in higher diversity index values. These results indicated that the distribution of landscape types was uneven in 1988 and 2014. Relatively speaking, the landscape dominance recorded at the 2001 time point is low, a balanced distribution.

Landscape-level index gradient analysis.
Changes in landscape pattern index among the gradient zones of the Zhengzhou buffer zone recorded on the landscape level at three different time points are shown in Figure 4.
Patch density first increased then decreased on buffer zones 1-5 but no consistent patterns were evident for buffer zones 6-20. The patterns of variation among zones at the three time points were not consistent. In buffer zones 1-5, the patch density increased over time, and the peak patch density area recorded in subsequent time points expanded outward to the city periphery. This showed that during the expansion of the city, small patches near the administration center were amalgamated with those farther outward or were replaced by large patches. Our results also show that the degree of landscape fragmentation decreased over the three time points. In buffer zones 6-20, although no distinct trends in patch density were evident over the three time points, the patch density values recorded in 1988 and 2001 were higher than that in 2014. This was especially true for the patch density of the 13th buffer zone, which showed a strong decline from 2001 to 2014. These data showed that the degree of patch fragmentation was high in 1988 and 2001 in the outskirts of the city. In contrast, in 2014 patches on the outskirts of the city were strongly disturbed by human activity, resulting in a reduction in the degree of patch fragmentation.

As one moved from the central zones outward, the landscape shape index is first increased and then decreased. Values of the landscape shape index close to 1 indicate that the shape of the patch is approximately circular, while lower values indicate irregular-patch shape. The patch shape index reached a peak value in 1988 in the 8th buffer zone, while the peaks in 2001 and 2014 were found in the seventh and fourth buffer zones respectively. This indicates that over time the peak position of the landscape shape index moved farther from the center of the city, which suggests that patches on the outskirts of the city tended to be regular, but as time passed urban expansion progressively broken up these natural landscapes.

Largest patch index values showed a sharp decline from the city center to areas immediately adjacent, followed by irregular upward and downward fluctuations in areas further from the center. Taken together, our results show that the city has continued to push forward as it develops, and that the degree of fragmentation caused by intensive urbanization has gradually expanded outward. The sharp decline in the largest patch index may be due to the fact that the study area is large, since areas from the city center vary in spatial and temporal factors determined by geographical environment and urban development strategy.

The Shannon diversity index and Shannon evenness index reflect the heterogeneity of landscapes. The Shannon diversity index at each time point increased from the city center to buffer zones 2-5 and fluctuated afterward. These results show that the sharpest increase in the Shannon diversity index occurred near the most urbanized areas. There, urban construction land is much more abundant than other types of land, and the heterogeneity of the landscape is therefore very different from areas further from the city center. For example at a distance of 5km from the city center, the diversity index in 1988 was greater than that in 2001, which in turn was greater than that in 2014. This shows that landscape diversity gradually decreased from
the city center outward at all time points. The peak of the diversity index was also found where the patch type was the most complex and patch fragmentation was highest. In buffer areas 4-10, the diversity index in 1988 was stable, while in 2001 the diversity fluctuated significantly in these areas, indicating that urban construction was more active at this time point. In buffer area 12-17, the diversity index in 1988 and in 2001 was relatively high, while it was much lower in 2014. This result indicated that urban construction in 2014 had strongly altered the landscape.

The contagion index showed a tendency to decline strongly from the city center to adjacent buffer zones, followed by a gradual rise with subsequent fluctuations. In 1988 the minimum value of the contagion index occurred in the second buffer zone, in 2001 it occurred in the third buffer zone, in 2014 appeared in the fourth buffer zone; thus the minimum value of the contagion index slowly increased each time point, indicating that urban centers continue to expand to form large urban patches. Moreover, the degree of aggregation and patch spread near the center of the city increased over time, while at each time point the contagion index fluctuated after reaching a relatively low value. In buffer zones 5-12, the image of 2001 remained at a low level which is obviously different from those two, indicating the patches in this buffer area have a dense pattern of various elements. In buffer zones 12-17, the fluctuating image in 2014 significantly differed from the values of the other two years. In addition, the value of the contagion index in 2001 was significantly higher, which suggests that some dominant patches appeared in these buffer areas after 2001 but before 2014.

Generally speaking, the urbanization process in Zhengzhou is accelerating year by year. The degree of disturbance on the periphery of the city is increasing year by year, and the area with high
fragmentation caused by urban development is expanding outwards. Compared with 1988 to 2001, the urban development center expanded significantly from 2001 to 2014, and the landscape diversity and sprawl increased significantly in the 12-17 buffer areas.

**Gradient analysis of construction land.** Construction land is the landscape type most sensitive to the process of urbanization, so we also analyzed difference in index values among buffer areas along the gradient specifically with respect to construction land.

Figure 5 depicts trends in construction land within the gradient zone at each time point. Our results show that the percentage of construction land decreased with distance from the city center. With steadily increasing urbanization over time, the construction land to area ratio within each buffer zone has increased to varying degrees. Between 1988 and 2001, the construction land to area ratio in buffer zones 1-3 showed the greatest increase, while buffer zones 4-9 showed a smaller increase and buffer zones 10-19 showed almost no increase.

These results show that urban construction during this period mainly occurred within 15 km of the administrative center of the city, and that areas far away from the center were stagnant. Over the period from 2001 to 2014, the land area ratio of buffer zones 1-3 and 10-19 increased significantly, indicating that urbanization in this ten-year period occurred both near the center of the city and in the suburbs.

The patch density of construction land first rose and then decreased within 20 km of the downtown area, and irregular fluctuations in patch density were observed in areas farther than 20 km from the downtown area. In 1988, peak patch density appeared 12 km from the city center, while in both 2001 and 2014 the peak appeared 18 km from the center. Here, the patch density peak decreased in magnitude and moved away from the city center over time. Moreover, fluctuations in patch density in 1988 and 2001 were similar in areas farther than 25 km from the city center. However, the 2014 results were in sharp contrast. At this time point, construction land was barely present in the 13th buffer zone but increased sharply in buffer zones 14-16.

At all time points, the largest patch index after leaving the urban core, and showed small fluctuations near its minimum value afterward. In 1988, the largest patch index dropped sharply from the urban core to the buffer zones within 2-15 km of the city center, and leveled off after 15 km. In 2001 and 2014, the largest patch index dropped sharply after 2-18 km from the city center, and leveled off after 18 km. The largest patch index of the three periods increased abruptly in the thirteenth buffer zone, especially in 2014.

![Figure 5](image_url)

**FIGURE 5**

Construction land variation in landscape indices along the buffer zones
In all time periods, the patch shape index of the buffer zone gradient showed a pattern of an increase near the urban center followed by a decrease further away. Across buffer zones 1-12, we found that the patch shape index in 1988 was greater than in 2001, which was in turn greater than that in 2014. These findings reveal that the patch shape index of the same area decreased at each subsequent time point. However, for buffer zones 13-20, the patch shape index in 2014 exceeded the values recorded for the other two periods.

Overall, the percentage of construction land in an area decreased with increasing distance from the city center. In addition, the patch density first increased then decreased, and the peaks of patch density in 1988 and 2001 were located 12 km and 18 km from the city center, respectively, while the peak in 2014 was 22 km from the center. Over the time interval of the study, the city center expanded to the 9th buffer zone, the largest patch index decreased, and the shape index increased along the urbanization gradient. In buffer zones 9-20, the percentage of construction land and largest patch index of construction land in 2001 and 2014 appeared as a small peak in the 13th buffer zone. This is because urban development requires both a main hub in the administrative center of the city, as well as sub centers in the suburbs. Thus, large patches of construction land have developed far from the city center.

CONCLUSION

The spatial and temporal evolution of the landscape of the urbanized gradient zone in the Zhengzhou administrative division has been identified by analyzing the radiant buffer zone surrounding the city center. Our results show that there is a significant correlation between the landscape pattern index and the urbanization gradient [10, 11]. During the study period, landscape diversity and patch fragmentation decreased in areas near to the urban core, while in areas farther away, landscape diversity and patch fragmentation increased over time, and the landscape index peak tended to move further from the city center. The degree of fragmentation in the central urban area decreased as the intensity of land use reached its upper limit. However, urban construction in suburban areas occurred at both the 2001 and 2014 time points. These data indicate that the degree of disturbance in the outskirts of the city is becoming serious, and that landscape fragmentation is worsening in peripheral areas. In conclusion, this study describes the development of landscape patterns in the urban area surrounding Zhengzhou, and provides a comprehensive empirical study of urban ecological processes in a rapidly urbanizing area.

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OIL AND GAS ACCUMULATION LAW OF CHANG 23 RESERVOIR IN HESHUI AREA OF ORDOS BASIN

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ABSTRACT

In order to study the oil and gas enrichment of the Chang 23 reservoir in the Yanchang Formation of the N138 block in the Heshui area of the Ordos Basin, on the basis of the fine division and comparison of the small layers, logging data, scanning electron microscopy, cast thin sections, and high pressure mercury intrusion analysis, and multi-phase seepage core experiments, etc. are used to study the characteristics of sedimentary facies and reservoir distribution characteristics. The study shows that the Chang 23 reservoir is the delta front subspecies deposit, the river channel is distributed in the southwest-northeast direction, and the underwater distributary channel is the most favorable sedimentary facies belt. The Chang 23 reservoir is mainly controlled by structure, and the sedimentation is equal to it. The impact is a typical lithology-structural reservoir.

KEYWORDS:
Ordos area, Heshui area, Chang 23 reservoir, sedimentary facies, Oil and Gas accumulation law

INTRODUCTION

Ordos Basin is a cratonic basin with abundant oil and gas resources and stable structure. The Yanchang Formation of Upper Triassic in Mesozoic has good oil and gas shows from Chang 10 to Chang 1, of which Chang 7 and Chang 4+5 have good reservoir source rocks [1-2]. The river-lake sedimentary facies system developed in the Ordos Basin. The whole Yanchang Formation formed a complete structural evolution process of the Inland Lake basin. The Chang 10-Chang 9 period was the initial expansion period of the lake basin in the Ordos Basin, the Chang 8-Chang 7 period was the peak sedimentary period of the lake basin, the Chang 6-Chang 3 period was the shrinkage period of the lake basin, and the Chang 2-Chang 1 period was the shrinkage and extinction period of the lake basin [3-4]. Influenced by tectonic uplift and denudation, the top strata of Chang 2 reservoir are mostly denuded. While the lake basin shrinks and disappears, shallow lakes, deltas and rivers remain in some areas. Good sedimentary environment lays a foundation for oil and gas enrichment. On the basis of abundant experiments and reservoirs, this paper deeply studies the reservoir characteristics of Chang 23 reservoir group in Heshui area, and then studies the reservoir enrichment law of Chang 23 reservoir group, which provides basic data and scientific basis for further exploration and development of Chang 23 reservoir.

GEOLOGICAL SURVEY

The Heshui area is located in the eastern part of Longdong area, being the southwest part in Yishan Slope of Ordos Basin. The west of Heshui area is Qingyang, with the east being Fuxian, south being Ningxian and north being Huachi and Zhidan of Shaanxi Province. Under the large regional tectonic background of the west-dipping monoclinic, the Western stratum is gentle, the eastward stratum is steeper and the slope becomes larger. Two West-East anticlines are developed in the area. Several NE-SW trending nose uplifts are developed. Chang 23 is a rock association composed of sandstone and mudstone interbeds, with an average thickness of 57.49m. There is a weathering crust on the top of Chang 23, which is affected by denudation and its top thickness fluctuates slightly. There is a section of mudstone at the bottom of Chang 23. The electrical characteristics have obvious positive anomalies on the AC curve. The GR curve has a higher amplitude and is "finger" prominent and it also has obvious characteristics of well diameter expansion, which can be used as a basis for stratigraphic division in the study area (Fig.1). According to core observation, logging data and sedimentary cycle, Chang 23 can be subdivided into three sublayers, which are Chang 23, Chang 23 and Chang 23 from bottom to top.

SEDIMENTARY FACIES AND MICROFACIES

The sedimentary environment of Chang 23 of Yanchang Formation of Upper Triassic in Ordos Basin is different in different geographic locations. The sedimentary period of Chang 23 in Heshui area is the
period of Lake Basin atrophy and extinction. According to core observation, the autochromic color of Chang 2 sandstone is mainly grey-green and its environment is underwater sedimentary environment. Its GR curve mudstone baseline is more right than that of Yan'an Formation. According to lithology, sedimentary structural characteristics, regional environment, and logging data [5-7], and comprehensive analysis shows that Chang 2 in Heshui area mainly develops delta front sedimentary subfacies, including underwater distributary channel, underwater natural dike, underwater distributary channel and other microfacies.

**Underwater distributary channel microfacies.** The microfacies of underwater distributary channel are mainly grey-green thick and fine sandstone. Parallel bedding, plate-like cross bedding and deformation structures are developed in the sandstone, which represent a great change in hydrodynamics. Carboniferous laminae and wormholes developed in channel sandstones [Fig.2(d)–(e)]. Core observation shows that the underwater distributary channel is usually composed of 2-3 sandstone sedimentary cycles, with a single cycle reaching 4-9m. The grain size of underwater distributary channel sand body is mainly suspended ($\Phi>3$) and jumping component ($2<\Phi<3$), and there is no traction component with coarse grains ($0<\Phi<2$), which indicates that the hydrodynamic force is weak or medium. The log curves of the microfacies are mainly low natural gamma ray and natural potential. The shape of the microfacies is box-shaped, and the local zigzag protrusions may be affected by the inclusion of carbonaceous laminae.

**Underwater Natural Dike.** The microfacies of underwater natural dike mainly develop at the edge of underwater distributary channel, and their lithology is mainly fine sand, silt or argillaceous siltstone. Sandstone body is thinner, usually 1-3m, and contains entrapment bedding and collapse structures [Fig.2(f)–(g)]. Logging curves show a toothed linear distribution, the curve cycle amplitude is small, and that are low natural potential and natural gamma value.

**Underwater diversion Bay.** The underwater interdistributary bays are distributed on the edge of the whole subfacies or locally in the channel. The lithology is mainly silty mudstone and mudstone, and plant stem fossils are developed [Fig.2(h)]. The sedimentary thickness is large (8-10m). The spontaneous potential and gamma ray on the log curve are of high value and have dentate distribution.

**RESERVOIR CHARACTERISTICS**

**Petrological Characteristics.** The Chang2 sandstone reservoir is mainly feldspathic lithic sandstone with a small amount of feldspathic lithic quartz sandstone (Fig.3). The size of debris particles ranges from 0.05 mm to 0.5 mm, the maturity of sandstone structure is medium and the sorting property is good.
The debris particles are mostly sub-angular-sub-circular, and the cementation type is mainly enlarged-porous. The average clastic composition of Chang2 reservoir sandstone is 87.9% of the total rock content, of which the average quartz content is 52.6%. The average total clastic content is 21.4%. The main types of clastic are metamorphic clasts and volcanic clasts. The feldspar content is 13.9%.

Physical Characteristics. Core physical property statistics show that Chang 2 reservoir in N138 area has good physical property, good correlation between reservoir porosity and permeability, and good linear relationship between pore and permeability dispersion point trend line, indicating that there is no obvious difference in reservoir pore structure (Fig.4). The average permeability is 13.445*10^-3um. Figure 5 shows that the reservoir is a porous single-porous reservoir with no high-amplitude anomaly.

Pore Space Characteristics. The porosity of reservoir is 3.8%~7.5%. The main pore type is intergranular pore (2.0%~4.0%), followed by feldspar solution pore (1.0%~2.0%) with a small amount of cuttings solution pore (0.3%~0.5%) and intergranular pore (0.5%~1.0%). A small amount of intergranular

**FIGURE 2**
Typical Sedimentary Structures of Chang 2 Layers in Heshui Area
a. fine sandstone, parallel bedding, underwater distributary channel, well B101, Chang 2/ section, buried depth 1323.24m; b. fine sandstone, plate cross bedding, underwater distributary channel, well N19-19, Chang 2/ section, buried depth 1088.8m; c. fine sandstone, deformation structure, underwater distributary channel, well N137, Chang 2/ section, buried depth 1272.15m; d. fine sandstone, carbonaceous ripple, underwater distributary channel, well N137, Chang 2/ section The depth of burial is 1254.2m; e. fine sandstone, wormhole, underwater distributary channel, well N9, Chang 2/ section, depth of burial is 1211m; f. Fine sandstone, wrapped bedding, underwater natural dike, well N65, Chang 2/ section, buried depth 1139.4m; g. fine sandstone, slump structure, underwater natural dike, well N19-19, Chang 2/ section, 1084.2m; h. mudstone, plant stem fossils, shunt bay, well N9, Chang 2/ section, buried depth 1213.5m.

**FIGURE 3**
Sandstone Types of Chang2 Reservoir in Block N138 in Heshui Area

**FIGURE 4**
The relationship between porosity and permeability of Chang2 layer in Heshui area
The measured porosity of Chang2 reservoir sandstone ranges from 12% to 17%, averaging 16.4%. Permeability is mainly distributed between 1 mD and 17 mD, with an average of 13.445 mD. And it belongs to medium-low porosity and low permeability reservoirs. The pore throat of reservoir is mainly flaky and curved, and the average width of the throat is 4.34 μm.

**ENRICHMENT LAW OF CHANG 23 RESERVOIR**

Chang 23 reservoir in Heshui area is a lithologic-structural reservoir, which is mainly influenced by structure and lithological influence is second. Chang23 reservoir is the result of the interaction of various enrichment factors [8-11].

**Tectonics.** Structural control of Chang2 reservoir in N138 block is obvious. The sedimentary period of Chang 23 in Ordos Basin is mainly the period of Lake Basin shrinkage and extinction. Reservoirs mainly distribute on the sand bodies of underwater distributary channel, and the two sides are blocked by the restriction of underwater distributary bay. Chang 23 reservoir is distributed in the southwest of northern Shaanxi slope. Generally speaking, under
the background of westward dip monoclinic structure, the reservoir is controlled by the top structure. At the structural high point, the oil test is better and the oil production is higher in production and development. There is a nearly EW-trending anticlinal structure in N138 block with a closure height of 20m and a closure area of 2.38km² (Fig.6). The anticlinal structure has an amplitude of 5-15m and an inclination angle of about 0-5 degrees. Because the upper part of the stratum is denuded, and it directly covers Jurassic mudstone and is sealed by compact sandstone or mudstone on both sides [12].

Influenced by differential compaction, Chang 2 reservoir develops a series of nose uplift structures, which basically dip slightly westward and open eastward. Local nose-shaped uplift is similar to lithologic updip pinch-out matching, which plays an important role in controlling oil well production and water cut in N138 area. The thicker the uplift is, the greater the oil saturation is, and the production test also increases [13].

**Sedimentary facies belt.** Sedimentary facies zones control the distribution of reservoir sand bodies. Chang2 reservoir has a large thickness of underwater distributary channel sand bodies, which have good physical properties and form a large area of high-quality sandstone reservoirs. It is also a high-yield area for oil and gas enrichment. The sand body of the underwater natural dike extending to the side is smaller, the oil layer thickness is smaller, the oil saturation is smaller, and the oil and gas show is general. Sedimentary facies belts distributed in the area extend from south-west to north-east, and underwater interdistributary bay deposits located at the edge of dominant sandstone block oil and gas laterally and form large-scale lenticular sandstone, which makes dominant sandstone facies belts abundant enrichment and accumulation [14-18]. Figure 7 Oil-water difference is obvious in reservoir profile, oil-water relationship in vertical profile is controlled by sedimentary facies zone. Sand body in the middle part of N22-22 well is not connected with the surrounding area. It is an isolated sand body in a certain range. Affected by the bay between upper and lower distributaries, lenticular sand bodies are formed, and oil and water show as oil layers. In the plane distribution of sedimentary microfacies, the good oil testing column mainly distributes in the sedimentary microfacies of underwater distributary channel. The oil and gas show weakly in the microfacies of underwater natural dike, and the sedimentary facies zone has obvious control effect on oil and gas enrichment.

**Heterogeneity and Mudstone Interlayer.** The stratum thickness of the chang 2r, 2i and 2s layers of N138 in Heshui area is stable, averaging about 15-20m. Interlayer heterogeneity in reservoir can be expressed by parameters such as interlayer distribution frequency and interlayer distribution density. And intralayer permeability heterogeneity can be expressed by gradient, penetration coefficient and variation coefficient. Interlayer heterogeneity is mainly measured by interlayer thickness, gradation, penetration coefficient and variation coefficient. All kinds of heterogeneity parameters indicate that the heterogeneity of Chang 2 reservoir in Heshui area is moderately weak as a whole. The mudstone barrier of 3-5 m is developed on the top of each small layer of reservoir. Its tone is mainly dark gray and its distribution is continuous, forming a series of interlaced network sealing spaces, forming a caprock to seal the underlying sandstone, which is conducive to reservoir enrichment (Fig.8). Locally overlying mudstone encloses sandstone lenses and forms so-called lithologic-structural traps [19-20]. Mudstone barrier is an important factor for reservoir enrichment in this area.

**Hydrodynamic Conditions.** Hydrodynamics also affects the accumulation of Triassic reservoirs, which is an important factor in Chang2 reservoir of N138 block in Heshui area. Local areas in the upward dip direction of the nose uplift structure, so-called higher structure, the oil content obtained by trial production is poor, even the results of drilling show that the water layer. The accumulation of oil and gas is controlled by hydrodynamics [21]. Chang 2 reservoir is located in the northern slope of Shaanxi Province, where the formation is relatively flat and the lateral pressure is insufficient to reach the threshold of large-scale migration during oil and gas filling. While the upward migration of oil and gas encounters lithological, physical or structural changes that cause slight changes in the formation, the hydrodynamic force will show a greater role at this time, and a series of structural-hydrodynamic traps or lithological-hydrodynamic traps will be formed, which is beneficial to oil and gas reservoirs enrichment (Fig.9).

**RESULTS AND CONCLUSIONS**

(1) The Chang 2 reservoir in Heshui area of Ordos Basin mainly develops delta front sedimentary system, which is divided into underwater distributary channel, underwater natural dike, underwater distributary Bay and other microfacies. The underwater distributary channel microfacies is the most favorable sedimentary facies zone, and the grain size of sandstone is mostly suspended component and jumping component, without coarser component.

(2) Chang2 sandstone reservoir is a medium-low porosity and low permeability reservoir. The rock type is mainly feldspathic lithic sandstone with a small amount of feldspathic lithic quartz sandstone. The pore space type includes intergranular pore, feldspathic karst pore, lithic karst pore and intergranular pore.
FIGURE 7
Sedimentary facies zone map of Chang2 reservoir in Heshui area
a. Profile of Chang 2 reservoir in Heshui area from Well N22-21 to Well N22-27; b. Planar distribution of sedimentary microfacies of Chang 2 reservoir in Heshui area.

FIGURE 8
Section of Chang 2 Interlayer in Heshui Area
(3) Chang 2 reservoir in Heshui area is a lithologic-structural reservoir. Structures are the key factors affecting reservoir enrichment. Sedimentary facies zone controls the reservoir enrichment area in the central region. The formation of sealing space in mudstone barrier is conducive to reservoir enrichment. Hydrodynamic force is an important condition for reservoir formation to form good traps. Therefore, good structural background, reservoir conditions and natural sealing mechanism contribute to reservoir enrichment.

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STUDY ON WATER INJECTION DEVELOPMENT OF UNCONSOLIDATED SANDSTONE RESERVOIRS IN SHENGLI OILFIELD, CHINA

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ABSTRACT

It is well known that the unconsolidated sand reservoirs (USR) has high permeability and high oil-water viscosity ratio. Continuous water injection development process will form high permeability and large pore channels in the reservoirs. At the same time, high displacement phase fluidity can easily causes oil-water interface moves fast to the bottom of production wells and reduces final recovery efficiency. In order to solve this problem, a reservoir geological model of A area has been established by analyzing the existing static and dynamic production data. Based on this, the seepage mechanism of USR has been comprehensively analyzed, and evaluates the influence of waterflooding mode (WFM) difference of depth displacement between injector producer pair effect by means of numerical analogue. And then, a new evaluation standard called sum of field oil recovery (SFOE) is introduced, which is defined as the sum of the values of anhydrous field oil recovery (AFOE) and field oil recovery (FOE). The results revealed that, the optimum injection/production ratio based on this evaluation criterion is 1.1, the optimum WFM is low injection and high production. In addition, the result of economic evaluation shows that a higher economic return gets by using this development factor in A area. The evaluation system studied in this paper can effectively increase the sweep area among USR during waterflooding development, and provide a reliable reference for the development of similar reservoirs.

KEYWORDS:
Unconsolidated sandstone, water injection development, optimization analysis, development effect, Shengli oilfield

INTRODUCTION

The types of sedimentary facie of USR are various [1-4], mainly including inland lake deposits, alluvial fan deposits, dialectic river-delta plain deposits, delta front deposits and lake-delta deposits [5-9]. The reservoirs structure is complex and often accompanied by multi-level faults. The interlayer heterogeneity of USR is very strong. In the process of waterflooding development, the clay mineral migrates and enhances conductivity of the original high permeability channel [10-14]. At the same time, the migrated clay particles swell when they encounter water, which blockages the low permeability channel [15-19], aggravates the interlayer heterogeneity, induces the tongued advance phenomenon, reduces the final conformance efficiency and reduces the ultimate water flooding recovery [20-21].

During the adjustment process of waterflooding development of USR, Zeng et al. [22] studied the formation mechanism of large pore channels in USR based on sand-filled glass plate model. Meanwhile, grey correlation method was introduced to study the effects of reservoir permeability, rock cementation degree, fluid viscosity and recovery rate on the formation of large pore channels. Physical simulation experiments showed that the sign of the formation of large pore channels was the increase of flow pressure and production. In order to plug the high permeability holes, Petro Andina Resources implemented the first polymer gel treatment in an USR In March 2008. However, sand production and the generation of wormholes resulted in rapid transit times between injector and offset producers in patterns. Based on the numerical simulation method, Mao et al. [23-26] studied the influencing factors of the formation of large channels. By introducing the concept of comprehensive coefficient, the coefficient is controlled by rhythm, multiple of permeability difference between layers, oil-water viscosity ratio, oil-water density difference, and fluid production rate. The data are in good agreement with the actual data of Ng61/4 in West Gudong Seven Area. In view of the weak cementation ability of USR formation and the serious problem of producing sandstone in China, Yao et al. [27-31] optimized the sand consolidation reagent of epoxy resin HY1 through indoor evaluation experiments, which can meet the performance
indexes of chemical sand consolidation, profile control and water shutoff in USR injection wells. Sun [28] fully considers the factors of plugging removal and safety in the process of water injection development. By studying the water injection fracture mechanism of USR and the calculation method of fracture pressure, he puts forward the calculation method of reasonable injection pressure for USR injection wells in Bohai Bay.

In this paper, aiming at the problems existing in the development of USR, such as low reservoir elastic energy, short water free oil production period and poor communication between oil-water wells. The development plan of area A in Shengli oilfield is taken as an example to optimize and adjust, so as to provide some references for the development of USR. There are three large transverse faults in area A, which divide the working area into four blocks of different sizes. The reservoir type is high pressure reservoir with bottom water fault block.

MATERIALS AND METHODS

Geology Characteristic. The study area is located in the Kenli County, Dongying City, Shandong Province. The sedimentary facies type of area A belongs to meandering river facies types, and the characteristic of depositional cycle is shallow to deep to shallow. The sedimentation type mainly is the middle-high energy platform shoal with thin layer possess low energy interbank sea.

Model of Macroporous Channels Formation in USR. With reference to Hu’s [7] Seepage model of large pore formation during waterflooding development of USR. We make the following assumptions: (1) assume that the reservoir pore space is an ideal equal diameter percolation model; (2) assume that the fluid in reservoir pore is an ideal fluid; (3) assume that the heterogeneity of reservoir rocks is neglected; (4) assume that the reservoir rock and fluid compressibility are neglected; (5) assume that the reservoir fluid satisfies Darcy’s percolation law.

The streamline of the injection-production model of five-point area pattern is uniformly distributed around the injection wells. So that the 0~45° area between injection-production wells is selected for analysis, and a two-dimensional Cartesian coordinate system is drawn. Origin I is calibrated as the injection wells, and top right corner endpoint P is the production wells. The seepage model of injection-production in five-point square well pattern is analyzed as formula (1).

\[
\arctan \frac{y}{x-a} - \arctan \frac{y}{x+a} = C
\]

And an exponential function, as shown in formula (2).

\[
y = x^a \quad (0 \leq x \leq 1, 1 \leq a \leq +\infty)
\]

Because formulas (1) and (2) have similar streamlines in two-dimensional Cartesian coordinate system, formulas (2) are used to replace the five-point injection-production inter well seepage formula [31-32]. When \(a = 1\), the streamline is the connection between two points of I and P. With the value increase of \(a\), the streamline type changes to concave function, and the extreme value gradually approaches the positive direction of the x-axis. The streamline length under different values of \(a\) are \((L_a)\).

\[
L_a = \int_0^1 \sqrt{1 + \left( \frac{dx}{dx} \right)^2} \, dx = \int_0^1 \sqrt{1 + a^2 x^{2(a-1)}} \, dx
\]

The values \(a\) are 1, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 respectively. The injection-production streamlines of five-point area pattern are drawn and the graphs are shown in Fig. 1.

![FIGURE 1](image)

Streamline distribution in reservoirs
In the seepage process at this time, the bottom hole pressure of water injection wells is defined as \( P_i \); the bottom hole pressure of production wells is defined as \( P_w \). When \( a = 1 \), the corresponding streamline length is \( L_{ai} \), the corresponding flow velocity is \( V_{ai} \), and the corresponding streamline length is \( L_a \) and the corresponding flow velocity is \( V_a \) when the value of \( a \) is any value \( a_i \). In this case, according to Darcy's seepage formula, if the production pressure difference remains unchanged, the flow velocity in any streamline is inversely proportional to the length of streamline between injection and production wells. Then the velocity under a streamline is (\( V_a \)):

\[
V_a = \frac{L_{ai}}{L_a} V_{ai} \tag{4}
\]

Assuming that the total number of injection-production streamlines is \( n \), the water production rate of production wells is (\( V \)):

\[
V = \sum_{i=1}^{n} V_{ai} = \sum_{i=1}^{n} \frac{L_{ai}}{L_a} V_{ai} \tag{5}
\]

On the basis of formula (5), the velocity under a streamline is (\( V_a \)):

\[
V_a = \frac{V}{L_a \sum_{i=1}^{n} L_{ai}} \tag{6}
\]

For a certain time \( t \), the water production rate of production wells is expressed by \( V(t) \), then the inner flow velocity of the streamline at that time is (\( V_a(t) \)):

\[
V_a(t) = \frac{V(t)}{L_a \sum_{i=1}^{n} L_{ai}} \tag{7}
\]

Referring to the formula of pipe flow along the pipeline, the following equations are obtained.

\[
V_a(t) = \frac{V(t)}{L_a \sum_{i=1}^{n} L_{ai}} = \left( \frac{P_i - P_w}{\gamma} \right) \frac{\gamma}{8\mu} R^2_a(t) \tag{8}
\]

Where \( \gamma \) is permeability of fluids in pipelines, \( R_a(t) \) is pipe diameter of arbitrary seepage channel, \( \mu \) is viscosity of fluid in pipeline.

The diameter of any seepage passage is (\( R(t) \)):

\[
R(t) = \sqrt{\frac{2\mu V(t)}{(P_i - P_w)} \frac{\gamma}{L_a \sum_{i=1}^{n} L_{ai}}} \tag{9}
\]

The pipe diameter at any time of arbitrary streamline is (\( R(t_i) \)):

\[
R(t_i) = \sqrt{\frac{2\mu V(t_i)}{(P_j - P_w)} \frac{\gamma}{L_a \sum_{i=1}^{n} L_{ai}}} \tag{10}
\]

Optimizing Water Injection Mode. Different characteristic of oil bearing strata, especially permeability contrast can arise fingering of the water oil front during waterflooding stage in USR. Adjustment of liquid production profile and water shutoff can plug aquifers to a certain extent and improve sweep coefficient, thus enhancing oil recovery. However, the injection of chemical organic reagents in water shutoff progress will damage the reservoir, plugging the low permeability layer, making the fluid production rate of oil wells greatly reduced. In view of this situation, low salinity water injection is proposed to improve oil recovery [33-35]. The main mechanism of this method is wetting transition, which changes rock from oil wetting to water wetting, improves pore-throat connectivity and enhances pore-throat channel conductivity. In addition, injected low salinity water can reduce the rock surface tension, and the reduction of surface tension can reduce the seepage resistance, that is, reduce the injection pressure difference and reduce the energy consumption of water injection.

Because area \( A \) belongs to unsaturated reservoir, some dissolved gas will overflow during natural elastic displacement. When there is a certain degree of gas saturation in the reservoir, water-mixed gas flooding can achieve higher oil recovery than water injection alone, and also can ensure higher single well production and oil recovery speed. However, the trap structure in area \( A \) is relatively complete and the connectivity of edge water is poor. When natural elastic displacement is used to develop reservoirs, the pressure of reservoirs will drop too fast. As a result, a large amount of oil spills in the gas, and the viscosity of crude oil increases, thus reducing the production capacity of a single well. In this paper, a numerical model for area \( A \) is established. The model consists of 30 production wells and the fluid production of a single well is set at 100 t/d. The contrast development effect of pressure-retainng water injection and elastic development are shown in Fig. 2.

The result shown in Fig. 2 indicate that the higher oil recovery rate can be obtained by pressure-retainng water injection in the early stage of development, and the oil recovery rate is slightly lower than that of elastic recovery rate in the later stage of development. As for the pressure-retainng water injection process can maintain a relatively high bottom-hole flow pressure, it can effectively suppress a large number of medium-chain hydrocarbons spilling from the bottom of the well because of low pressure. At the same time, with the increase of reservoir pressure, the gas ratio in the reservoir increases accordingly, the fluidity increases in the reservoir seepage process, and the oil recovery rate maintains a relatively high value. With the continuous development of water injection, for the case of the high fluidity of displacement phase, the oil-water interface will move...
quickly to the bottom of production wells, resulting in the continuous increase of water cut and the continuous decline of oil recovery rate.

**Structural Relief between Injector Producer Pair.** In the process of reservoir waterflooding development, the difference of depth displacement between injector producer pair will cause the difference of seepage pressure in displacement process, which will change the production performance data of single well and affect the final FOE of reservoir. In this case, the optimization of the difference of structural relief between injector producer pair should focused. According to the difference of injector producer pair horizon elevation, the simulation of the development effect of high injection-low production (HILP) and low injection-high production (LIHP) can be finished. Fig. 3 shows the time-varying data of recovery under two development modes.

![Graph](image1)

**FIGURE 2**
Oil recovery rate comparison of area A under two water injection modes

![Graph](image2)

**FIGURE 3**
Elevation recovery of different injection-production structures varies with time

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Effect of different injection-production elevation in area A of Shengli Oilfield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Items</td>
<td>Well number</td>
</tr>
<tr>
<td>LIHP</td>
<td>24</td>
</tr>
<tr>
<td>HILP</td>
<td>6</td>
</tr>
</tbody>
</table>
Fig. 3 shows that the (LIHP) phase can achieve greater recovery than the (HILP) phase. Because in the process of (HILP), the elevation difference can increase the drawdown pressure between injector producer pair and the driving force in Darcy seepage process, which results in too fast water breakthrough at the bottom of production wells and decrease the final FOE. However, the process of low injection and high recovery can ensure that bottom water reaches the upper oil-bearing area, increase the swept area among water flooding process, and then improve the FOE among water flooding process. Table 1 shows effect of water injection and oil increase at different structural elevations.

Table 1 shows that the process of LIHP possess a better production efficiency, and the oil increment ratio is 90.23%. At the same time, the process of LIHP can get a higher ratio of response well, the ratio of response well in the area is 83.33%.

**Optimum Injection/Production Ratio.** Injection/production ratio is a comprehensive index to characterize the injection/production balance in the process of oilfield waterflooding development and to reflect the relationship between fluid productions, water injection and formation pressure. At the same time, it is an important guarantee to maintain reasonable formation pressure, so that the oilfield has strong liquid and oil production capacity, reduce inefficient energy consumption and achieve higher oil recovery [26]. As for this USR, the recovery efficiency of pressure-retaining water injection is good. In view of the current production situation, it is necessary to maintain a reasonable pressure and use a reasonable injection/production ratio to improve the pressure recovery rate. Fig. 4 shows pressure recovery rate varies under different injection/production ratio:

Calibration of five injection/production ratios for 40 years of simulated reservoir development by using numerical simulation method. Define the anhydrous field oil recovery (AFOE) is when the water cut of produced liquid is equal to the initial water cut of reservoir [28]. Define sum of field oil recovery (SFOE) as the sum of AFOE and FOE. Table 2 shows the AFOE and FOE under different injection/production ratios:

<table>
<thead>
<tr>
<th>Injection/production ratio</th>
<th>AFOE/%</th>
<th>FOE/%</th>
<th>SFOE/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8:1</td>
<td>5.67</td>
<td>21.52</td>
<td>27.19</td>
</tr>
<tr>
<td>0.9:1</td>
<td>6.25</td>
<td>25.99</td>
<td>32.24</td>
</tr>
<tr>
<td>1.0:1</td>
<td>6.37</td>
<td>29.54</td>
<td>35.91</td>
</tr>
<tr>
<td>1.1:1</td>
<td>6.83</td>
<td>29.26</td>
<td>36.09</td>
</tr>
<tr>
<td>1.2:1</td>
<td>6.62</td>
<td>28.21</td>
<td>34.83</td>
</tr>
</tbody>
</table>

**TABLE 2**

**The AFOE, FOE and FOE under different Injection/production ratios**

**FIGURE 4**

Pressure recovery rate under different injection/production ratio
Table 2 shows that when 1:1 injection/production ratio is used to develop reservoirs, one can obtain the maximum FOE, that is, the maximum FOE can be obtained by pressure-retaining water injection. The FOE is lower under low injection/production ratio because it is difficult to maintain reservoir pressure stability at low injection/production ratio, which results in the decrease of dissolved gas content in reservoir fluid, the increase of seepage resistance and the decrease of bottom hole flowing pressure, thereby reducing FOE. When the Injection/production ratio is 1:1, the AFOE and the SFOE is the highest. Considering that the oil field is in the stage of insufficient formation energy supply, overpressure water injection can achieve better development effect, so the injection/production ratio is calibrated to be 1.1:1 under the evaluating system of SFOE.

RESULTS

Comparison of Development Parameters of Two Standards. Under the criterion of SFOE evaluation system in this paper, the optimum injection/production ratio is 1.1:1, while the optimum injection/production ratio is 1:1 through the criterion of FOE evaluation system. According to the existing area A data, five-point area well pattern is selected to simulate the development of reservoirs for 40 years under two injection/production ratios. Fig. 5 shows the change of FOE under different injection/production ratio in the whole area.

Analyzing the data in Fig. 5, under the criteria of SFOE evaluation, greater recovery can be obtained in the initial stage, and the increment of recovery value gradually decreases in the later stage of the development process, until it lower than the FOE obtained under the criteria of final recovery evaluation.

Contrastive Evaluation of Final Income. Considering that the injector producer distance has not changed under the evaluation of the new standard, it is indicated that the well pattern density has not changed, that is to say, the investment cost of the two development schemes is the same. The interest rate of fixed deposit of the calibrated bank is 2.0%, the conversion rate of the calibrated commodity is 0.95, the calibrated tax rate is 36%, and the selling price of the calibrated crude oil is 60 dollars/barrel. The economic evaluation is carried out by the method of future value of block output. Table 3 shows the future value of sales revenue under the two standards.

Table 3 show that higher oil recovery can be achieved in the reservoir development process under the original standard. However, it is well known that the initial recovery degree of the oilfield has a great impact on the final economic benefits. At the same time, it can get a larger cumulative future value income by the new evaluation criteria proposed in this paper, which shows that the new criteria proposed in this paper have better feasibility.

![Graph showing the change of recovery in the whole area](image)

**FIGURE 5**
The change of recovery in the whole area

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulated income statement with different criteria</td>
</tr>
<tr>
<td>Criterion</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Criterion of FOE</td>
</tr>
<tr>
<td>Criterion of SFOE</td>
</tr>
</tbody>
</table>

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Field Experiment Analysis. Area A of Shengli oilfield has 41 wells, of which 8 are water injection wells, 33 are oil production wells, and the pattern of well distribution is inverse nine-point area well pattern. The oil production rate of production wells is generally low (8.3%). Besides, the recovery degree of area A of Shengli oilfield was low. As for this case, basing on SFOE evaluation criterion, area A of Shengli oilfield has been developed by pressure-retaining water injection since 2016. Low concentration brine is used as injection source to induce wetting reversal. By 2018, reservoir recovery has been improved. Specific features are as follows. Firstly, the increase rate of reservoir water cut decreased from 3.7% in 2016 to 1.6% in 2018. Secondly, reservoir water drive efficiency is improved, and the water drive curve is biased to the production axis. Finally, the recovery rate is increased by 6.8%, and the effect of unit development is effectively improved.

CONCLUSIONS

(1) In view of the development process of USR, pressure-retaining water flooding can maintain formation seepage well, inhibit gas and light hydrocarbon molecular spillover, and ensure that the fluidity of displaced phase is not lost.

(2) Different from polymer flooding process, HILP in USR water flooding process fail to plug the large pore channels in water injection profile, while gravity displacement and continuous water line scouring will result in the establishment of dominant channels, resulting in the oil displacement effect of HILP is inferior to that of LIHP.

(3) For the optimization process of injection/production ratio, using the evaluation criterion of SFOE, the injection/production ratio of 1:1:1 can increase the seepage driving force to a certain extent, and then increase the production of single well. But when the injection/production ratio is too large, the displacement phase intrudes too fast, and the oil-water front will move too fast to the bottom of the well, which will lead to the final recovery reduction.

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IMPLEMENTATION OF HIGH-SPEED AND HIGH-PRECISION SOBEL ALGORITHM BASED ON IMPROVED COORDINATE ROTATION DIGITAL COMPUTER USING IN ENERGY SEISMIC EXPLORATION

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ABSTRACT

Seismic exploration is one of the important exploration methods in engineering geophysical prospecting. It is very important to make good seismic interpretation for the analysis of engineering geophysical exploration results. Edge detection can directly reflect the edge of seismic profile strata, which has a significant effect on improving the seismic data interpretation. On the premise of guaranteed edge identification accuracy of seismic profile strata, in order to improve the edge identification speed of seismic profile strata, a high-speed and high-precision Sobel algorithm based on optimized Coordinate Rotation Digital Computer (CORDIC) is proposed. Through the parallel data-handling method, it uses the pipelined CORDIC algorithm which extends data bits, covers all angles and disassembles multiple-bit addition or subtraction operations into single-bit addition or subtraction operations on the ZYNQ platform. It improves the efficiency of Sobel while ensuring calculation accuracy. The results of experiments show that compared with the traditional acceleration method, the method proposed in this research can increase the efficiency by 115.74 percent with the operation accuracy ensured.

KEYWORDS:
Seismic exploration, seismic interpretation, geological structure, edge detection, hardware accelerator

INTRODUCTION

Digital image processing technology plays an important role [1-3]. With the development of digital image processing technology, various geophysical methods and equipment have a new development foundation. Digital image processing technology plays an increasingly important role in engineering geophysical exploration [4-6]. Seismic method is a widespread method in engineering geophysical exploration. The seismic method, which mainly judges the geological structure through characteristics of vibration generated by seismic signal source generated by artificial in the strata, are mainly divided into reflection wave method, refraction wave method and transmitted wave method [7]. Seismic images are easily affected by refraction wave, the complexity of underground structures and noise, so that, Seismic images often do not reflect the geological structure clearly and intuitively. In order to highlight the key information in seismic images more intuitively and accurately, noise reduction and feature extraction, which is important research directions of seismic data interpretation, are carried out in seismic images [8]. At present, edge detection is widely used in seismic data interpretation because of its brief principle and good interpretation results [9-10].

When the image processing technology was still in its nascence, most researchers implemented the Sobel algorithm and its variants in a software-only way [11]. In order to improve the computing speed of Sobel algorithm, Lyu et al. [12-14] implemented Sobel algorithm on Field—Programmable Gate Array (FPGA), which can effectively improve the computing speed.

Singh et al. [15-17] made an equivalent substitution of the original calculation formula of gradient value, replacing the formula which required complex operations such as the extraction of root and the square with the formula that only required simple operations like addition, subtraction and absolute value operation. This method effectively improves the speed of Sobel.

The method proposed by Singh, S. et al effectively improved the speed of Sobel, but the calculation accuracy decreased significantly [16]. To improve the operation speed of the Sobel algorithm on the premise of guaranteed calculation precision, the CORDIC algorithm is used in this research to realize the operation of the gradient value, and the formula of complex operation such as the extraction of root is decomposed into a formula with only shift and addition [18-22]. Using this method, Sobel can reach a computing speed similar to that achieved by Singh, S. et al, but with the computing accuracy greatly improved.

In order to further improve the speed of Sobel, this research further optimized the key path of the CORDIC algorithm. This method disassembles the
addition or subtraction of multiple bits into the addition or subtraction of single bits through the pipeline. This method can effectively improve the computing speed.

**MATERIALS AND METHODS**

On the premise of ensuring the edge identification accuracy of seismic profile strata, in order to improve the edge identification speed of seismic profile strata, a high-speed and high-precision Sobel algorithm based on optimized CORDIC is proposed. In this research, ZYNQ is used to implement high-speed and high-precision Sobel algorithm. When ZYNQ is used to implement Sobel algorithm, the computing speed is limited by the gradient value formula with square and extraction of root. In this research, CORDIC algorithm of vector module in circular system is selected to decompose the formula of gradient value into pipeline operations of shift, addition and subtraction. Considering that CORDIC algorithm is used to realize the calculation of gradient value, only the value of the horizontal and vertical coordinates need to be obtained in real time during iterative calculation, and the angle value does not need to be obtained. Therefore, angle calculation part of CORDIC algorithm is optimized to reduce the consumption of resources. In order to further improve the operation speed of Sobel algorithm, critical path of Sobel algorithm is optimized. The multi-bit addition and subtraction operation is decomposed into pipeline operation of single-bit addition and subtraction. Finally, a high-speed and high-precision Sobel algorithm proposed in this research can meet the requirements of geological structure information extraction.

![Image](image-url)

**FIGURE 1**

Basic data of Sobel algorithm

**The sobel algorithm.** The Sobel operator is the edge detection operator of the first derivative, and there is a $3 \times 3$ table of gradient convolution in the $x$ direction and $y$ direction, respectively. When the pixel points of $3 \times 3$ in the image are convolved in the convolution table of $x$ direction and $y$ direction, respectively, the horizontal gradient and vertical gradient of the image can be obtained. The gradient in the horizontal direction and the gradient in the vertical direction are obtained through (1). The gradient value can be obtained through (2). The gradient value is compared with the preset threshold value to determine whether it is the edge part of the image.

The image data to be processed are shown in Fig. 1(a), the gradient operator in the horizontal direction is shown in Fig. 1(b), and the gradient operator in the vertical direction is shown in Fig. 1(c).

The calculation formulas of horizontal gradient $D_x$ and the vertical gradient $D_y$ are shown in (1):

\[
\begin{align*}
D_x &= (D_{02} + D_{22} + 2D_{12}) - (D_{00} + D_{20} + 2D_{10}) \\
D_y &= (D_{00} + D_{02} + 2D_{01}) - (D_{20} + D_{22} + 2D_{21})
\end{align*}
\]

(1)

The gradient value can be obtained after the respective gradient value of the horizontal direction and the vertical direction is obtained. The formula to obtain the gradient value is shown in (2):

\[
D = \sqrt{D_x^2 + D_y^2}
\]

(2)

The gradient value of each pixel of the image is calculated by (2). After the preset threshold value and the final gradient value are compared, whether the pixel point is an edge point can be determined. Singh, S. et al replaced (2) which entailed extraction of root operation with (3) which required mere operations of the absolute value and addition, and hence greatly improved the operation speed [16]; however, this method will greatly reduce the calculation accuracy.

\[
D = |D_x| + |D_y|
\]

(3)

In order to ensure the calculation accuracy and improve the operation speed, this research chooses to use the optimized CORDIC algorithm to carry out the operation of (2). Through this method, the computing accuracy is greatly improved and the computing speed is increased. The theory and implementation of the CORDIC algorithm are described below.

**CORDIC algorithm principle.** The CORDIC algorithm is an algorithm that transforms complex computation into simple computation, and transforms complex computation into iterative operation requiring only shift, addition and subtraction. In the circular coordinate system, calculation of extraction of root and arctangent can be realized when the vector mode is selected. The complete iterative formula of the vector mode is shown as follows:

\[
\begin{align*}
&x_{i+1} = x_i - 2^{-i} \delta_i y_i \\
y_{i+1} = y_i + 2^{-i} \delta_i x_i \\
z_{i+1} = z_i - \delta_i \theta_i \\
\delta_i = -sgn y_i \\
\theta_i = \arctan(2^{-i})
\end{align*}
\]

(4)

After $N$ times of iterations, $y_N$ will approach 0, and the values of $x_N$ and $z_N$ are shown in (5):

\[
\begin{align*}
x_N &= k \sqrt{x_0^2 + y_0^2} \\
z_N &= z_0 + \tan^{-1}(y_0/x_0) \\
\theta_i &= \arctan(2^{-i}) \\
k &= \prod_{i=0}^{N-1} \cos^{-1} \theta_i
\end{align*}
\]

(5)
We set the value of \( z_0 \) as 0, the value of \( x_0 \) as \( D_x \), the value of \( y_0 \) as \( D_y \), and multiply the value of \( x_N \) by 1/k to get the following formula:

\[
x_N = \sqrt{D_x^2 + D_y^2} \\
y_N = \tan^{-1}(D_x/D_y)
\]

(6)

Through the above derivation, the rotation mode of the circular system can realize the calculation of sine and cosine. The vector mode of the circular system can be calculated by (2). As shown in (4), (5) and (6), we set the value of \( z_0 \) as 0, the value of \( x_0 \) as \((1/k)D_x\), and the value of \( y_0 \) as \((1/k)D_y\), after \( N \) times of iterations, \( y_N \) will approach 0, and \( x_N \) will approach \( D_y \) in (2).

After comprehensive consideration, this research finally chooses to use the vector mode under the circumference system to do the calculation of (2).

**Optimization of CORDIC algorithm in vector mode.** In order to improve the overall performance of the system, the CORDIC algorithm in the vector mode is optimized. In the end, under the premise of ensuring the calculation accuracy of the CORDIC algorithm, the computing speed is greatly improved.

(1) Extension of Coverage Angle. As shown in (7), when the CORDIC algorithm is implemented, there is a specific rule for its rotation angle, and the angle size of each time is the arctangent of \( 2^{-1} \). The angle covered by the CORDIC algorithm is only [-99.827°, 99.827°], which cannot cover all angles.

\[
\begin{align*}
\theta_i &= \arctan(2^{-1}) \\
\Pi_{i=1}^{n-1} \theta_i &\approx 99.827°
\end{align*}
\]

(7)

In order to reduce the times of iterations, this research further processed the input data. We compare \( |D_x| \) with \( |D_y| \), and if \( |D_x| \) is larger, the \( |D_x| \) and \( |D_y| \) values remain the same; and if \( |D_y| \) is larger, the \( |D_x| \) and \( |D_y| \) values are interchanged. After the above operation, the input data quadrant will be limited to \([0°, 45°]\), so the first-order iteration can be reduced. After the above processing, (6) will become (8).

\[
\begin{align*}
x_N &= \sqrt{|D_x|^2 + |D_y|^2} \\
y_N &= \tan^{-1}(|D_x|/|D_y|) \\
|D_x| &> |D_y|
\end{align*}
\]

(8)

(2) Data bit spread. In 1992, Hu et al. [22] pointed out that the number of iterations and data bit width of the CORDIC algorithm had a great impact on the quantization error of calculation. Hu et al. pointed out that the quantization error consists of approximate errors and rounding errors. The approximate error is the quantization error caused by quantization of the CORDIC rotation angle determined by a finite number of rotation angles, which is determined by the maximum vector modulus value and the number of iterations. The rounding error is the error caused by insufficient data bits in calculation, which is determined by the bit width of data.

For each bit width of the data, the rounding error will be reduced by 1/2 [22]. When the CORDIC algorithm is used to implement (2), the operational data bits are extended by 3 digits to greatly reduce the rounding error under the premise of ensuring a large number of iterations.

(3) Implementation of CORDIC algorithm after optimization. In the circular coordinate system, when you use the vector mode, you can directly calculate the gradient based on the input \( D_x \) and \( D_y \). The hardware structure of the gradient value operation module is shown in Fig. 2. The gradient value operation module mainly consists of three parts: the preprocessing part, the CORDIC iteration part and the post-processing part.

The preprocessing part mainly implements three functions. The first function is to adjust the angle of the input data to \([0°, 45°]\). The second function is to modify the data in advance to compensate for the final result. The third function is to extend the data bit, increase the input data by 3 bits, reduce the rounding error to 1/8, improve the accuracy of the operation.

After the data is preprocessed, it will enter the CORDIC iteration part, and the iteration part will adopt the pipeline mode of level 15. According to (4) and (2), to solve \( D \), we only need to obtain the values of \( D_x \) and \( D_y \), and carry out the operation related to \( D_x \) and \( D_y \). Therefore, the correlation operation of \( z_i \) is omitted to save some resources. The specific implementation method is shown in Fig. 3. There are two shift registers and two addition or subtraction in each line of the iteration section. The sign control signal is Sign, which is determined by \( y_i \). According to (4), when \( y_i \) is positive, the adder at \( x_i \) and the subtraction at \( y_i \) are selected. When \( y_i \) is negative,
the subtraction is selected at \( x_i \) and the adder is selected at \( y_i \).

After the data is iterated through the part, it enters the post-processing part. In the post-processing part, the signal is cached for one beat and part of the data is obtained for processing, and then \( x_{15} \) [26:3], namely \( D \) in (2), can be obtained. In order to improve data throughput and maximize working frequency, the pipeline structure is adopted in this research.

In order to improve the operation speed of the CORDIC algorithm, this research optimized the critical path of the CORDIC iterative pipeline. In this research, the multi-bit addition or subtraction operation in the pipeline is decomposed, and the 27-bit addition and subtraction operations are decomposed into the pipeline operation of single-bit addition or subtraction. After the above operation, the combined logic time delay between \( D \) triggers in the CORDIC pipeline will be greatly reduced, and the operation speed of the system will be greatly increased. The specific implementation method is shown in Fig. 4. \( x_i \) and \( y_i \) are multi-bit data requiring addition or subtraction. \( x_i(m), m \in \{0,1,2,\ldots, 26\} \) is the \( m \) bit of \( x_i \). \( y_i(n), n \in \{0,1,2,\ldots, 26\} \) is the \( n \) bit of \( y_i \). \( x_i(i)d, i \in \{0,1,2,\ldots, 26\} \) represents the \( x_i \) signal delayed by i clock cycles. \( y_i(j)d, j \in \{0,1,2,\ldots, 26\} \) represents the \( y_i \) signal with a delay of j clock cycles. \( c(a), a \in \{0,1,2,\ldots, 26\} \) represents the carry result of the addition or subtraction of the bits of \( x_i \) and \( y_i \). \( \text{sum}_e(b), b \in \{0,1,2,\ldots, 26\} \) represents the sum or difference of the b bits of \( x_i \) and \( y_i \) after addition or subtraction. \( \text{sum}_e(e), e \in \{0,1,2,\ldots, 26\} \) is the sum or difference of the e bits in front of \( x_i \) and \( y_i \).

RESULTS

An engineering geophysical exploration project in Tongling city, Anhui province, China, needs to explore the geological stratification and overburden thickness of a certain place, in order to facilitate the construction of the project. In this project, seismic exploration was carried out by using sparker method, and seismic section map was obtained by seismic reflection wave method. Seismic wave reflection method is mainly used to identify the layer interface by the wave impedance difference between two layers of media. However, the seismic section image was obtained by seismic wave reflection method in this project, it cannot directly show the boundary of strata. Therefore, it is necessary to use edge detection to show visually stratigraphic boundaries. Edge detection of seismic section was implemented on xc7z020clg400-1 chip using Verilog HDL language. In addition, MATLAB R2015b is also used for the simulation test of this design.

The precision simulation was carried out. The method presented in this research was compared with the method proposed by Singh et al. [16], which replaced equation (2) with equation (3). Figure 5 shows the error using Singh et al.’s method, the maximum error is close to 50%. After using the optimization algorithm in this research, the error is within 2x10^-9, and the error is greatly reduced, Figure 6 shows result.

After precision simulation was carried out. An image was processed by the method presented in this research and by the method of Singh, S., Lyu et al. respectively [14, 16], the results of the two methods were compared. Figure 7(a) shows the seismic section image from an engineering geophysical exploration project in Tongling, Anhui province. The main purpose of this seismic interpretation is to explore the geological stratification and overburden thickness. Due to the influence of reflection wave, the complexity of underground structure and noise, the seismic profile obtained in this project cannot clearly and intuitively reflect the geological stratification and overburden thickness. In order to highlight the key information of seismic profile, image edge detection technology was used.
Notes: Figure (a) is Original diagram, the yellow box is the partial identification results of the stratigraphic edge of the seismic section. Figure (b) shows high-precision and low-speed algorithm processing figure, the yellow box is a partial recognition result image of the stratum edge of the seismic section using high-precision and low-speed algorithm. Figure (c) shows low-precision and high-speed algorithm processing figure, the yellow box is a partial recognition result image of the stratum edge of the seismic section using low-precision and high-speed algorithm. Figure (d) shows improved algorithm processing figure. The yellow box is a partial identification result image of the stratigraphic edge of the seismic section of the optimized algorithm.

Singh, S. et al.’s method was used for edge detection of image. Figure 7(c) shows the processed results. The boundaries of strata are not obvious and there are many false edges [16]. This method cannot detect the strata edges well, accuracy is low.

Improved algorithm was used for edge detection of image, Figure 7(d) shows the processed results. On the premise of guaranteed the computing speed, the image edge can be well identified. The boundary edge of stratum is more obvious, false edges are suppressed. The regions with small structural differences in seismic profiles are clearly delineated. This method can guide the tracing and calibration of strata boundary.

Lyu et al.’s [14] method was used for edge detection of image. Figure 7(b) shows the processed results. This method is carried out by calling IP cores of square and extraction of a root, and has high precision. The method also highlights the stratigraphic boundary of seismic profile. Comparing Figure 7(b) with Figure 7(d), the method proposed in this research can realize edge detection well. On the premise of improving the speed of operation. The accuracy is similar to that of Lyu et al. [14].
Compared Figure (d) with Figure (a), Figure (d) has a higher contrast, the stratigraphic boundaries are more obvious. Compared Figure (d) with Figure (c), the stratigraphic boundaries are more obvious in Figure (d). In Figure (c), there are many false edges, the four boundaries in the yellow box are mixed together and not easily distinguishable. In figure (d), the four boundaries in the yellow box are obvious. Recognition effect of Figure (b) is similar to that of Figure (d), compared with the high-precision algorithm, the processing speed of the optimization algorithm is increased by 115.74%.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High Precision Algorithm</th>
<th>The Approximate Algorithm</th>
<th>Improved Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{	ext{op}}$ (MHz)</td>
<td>214.592</td>
<td>271.003</td>
<td>462.963</td>
</tr>
</tbody>
</table>

After precision simulation and actual testing of pictures, the highest working frequency was tested. High-precision edge detection was performed with the highest working frequency of 214.592MHz. The method proposed by Singh, S. et al. [16] was used for low-precision edge detection with a maximum operating frequency of 271.003MHz. The improved algorithm proposed in this research achieved a maximum frequency of 462.963MHz. On the premise of guaranteeing the operation accuracy, the speed of the relatively high-precision algorithm is increased by 115.74% and that of the approximate algorithm is increased by 70.83%, as shown in Table 1.

**CONCLUSIONS**

In this study, a high-speed and high-precision Sobel algorithm is implemented on ZYNQ platform using the optimized CORDIC algorithm. On the premise of ensuring the accuracy of stratum edge identification of seismic profile, the optimized Sobel algorithm greatly improves the identification speed. Compared with the traditional high-speed and low-precision Sobel algorithm, the speed of optimized Sobel algorithm is increased by 70.83%. Compared with the traditional low-speed and high-precision Sobel algorithm, the speed of optimized Sobel algorithm is increased by 115.74%. Optimized Sobel algorithm is suitable for the edge identification system of seismic profile strata which requires high accuracy and speed.

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**REFERENCES**


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QUICK TO UPSCALE, SLOW TO UPGRADE: DELAYS IN F-T REACTOR AND COAL GASIFIER DEVELOPMENT IN CHINA’S COAL-TO-LIQUID INDUSTRY

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ABSTRACT

Since 2011, the single plant scale of China’s coal-to-liquid industry has increased from 1 Mt/a to 4 Mt/a, but the sizes of gasifiers and Fischer-Tropsch (F-T) reactors have not been scaled up. A large number of parallel lines have increased investments and operational risks, resulting in the inability of new plants to achieve economies of scale. This paper calculates the static economies of scale for a coal-to-liquid plant without realizing the scale-up of key equipment and the effect, including the technological progress, of the realization of stepwise scale-up of equipment. The delays in the research and development of F-T reactors and gasifiers in China’s current coal-to-liquid industry have led to losses of $184.8 million per year. If a 4 kt/d gasifier and 1 Mt/a reactor are deployed in a new 4 Mt/a coal-to-liquid plant during China’s 13th and 14th Five-Year Plans, the plant break-even point can be maximally reduced by $11/barrel compared with the first batch of million-ton plants that entered production before 2015. This paper also provides the optimal scale-up frontier for key equipment in China’s coal-to-liquid industry. It is recommended to complete scale-ups of the 6 kt/d and 8 kt/d gasifiers during the deployment of 5 Mt/a and 8 Mt/a scale coal-to-liquid plants, and scale-up of the 1 Mt/a FT reactor is recommended during the deployment of 7 Mt/a plants. The deployment of a coal-to-liquid plant based on the aforementioned optimal scale-up frontier can maximally save an amortized cost of 776 million dollars per year for a coal-to-liquid industry with a total capacity of 45 Mt/a. Finally, the paper proposes improvement of the development plan of the coal chemical industry by increasing policy consistency, developing detailed technical development roadmaps and avoiding repeated deployment of same-quality small-scale plants.

KEYWORDS: Coal-to-liquid, Fischer-Tropsch (F-T) reactor, coal gasifier, scale-up, optimal frontier

INTRODUCTION

Coal-to-liquid (CTL) technology takes advantage of China’s abundant coal resources by obtaining liquid fuels and reducing China’s reliance on imported oil, which has long been an important part of China’s energy security strategy. Due to the current tensions between China and the United States, energy security has become more important to China, and CTL technology may play a more important role in the development of China’s energy industry in the next fifteen years. Although the feasibility of commercial-scale CTL technology has been validated in several demonstration plants in China, issues still remain regarding the economic feasibility of CTL technology. In 2014, international oil prices dropped, and a large number of CTL plants suffered serious losses. These losses indicate that there is still a gap between the competitiveness of the CTL industry and that of the petroleum refining industry, and the independent operation capacities of the plants need to be strengthened. The CTL industry must further reduce costs to successfully shift from demonstration to commercialization.

The first way to help reduce the costs of the CTL process is the upsizing of plants to achieve static economies of scale[1]. Static economies of scale are not associated with technological advancements and only indicate that a larger-scale plant requires lower unit investments and fewer unit workers. When the process involved in a technology is more complex and the investment is more intensive, there is a greater potential to reduce costs by scale-up, which is reflected well in the nuclear [1], chemical [2], photovoltaic [3], and other energy sectors [4]. The amortization of fixed assets and financial expenses of CTL accounts for more than half of the unit cost of the products [5,6], while raw material costs account for up to one-third [7] or even lower [6]. Scale-up can achieve a significant decrease in the unit investment costs, thus effectively reducing the unit cost of CTL products. In addition, the average global scale of the refineries sharing the liquid fuel market with the CTL industry is 7.5 Mt/a, which is
four times as much as the current average scale of China’s CTL industry. Thus, there is ample room to increase the current scale of CTL facilities.

The second way to reduce the cost of CTL plants is from learning. Although learning also includes more skilled workers and improved organizational management, in this paper, learning mainly refers to advances in production technology. The potential to reduce process costs through learning is directly proportional to the number of key conversion steps involved in the process [8]. From this point of view, the CTL process, which contains a large number of core conversion steps, possesses great advantages. The most widely deployed indirect coal liquefaction process currently consists of four core conversion steps or subsystems (see Figure 1): air separation, gasification (including coal preparation), water-gas shift (WGS, including gas purification), and synthesis (including oil product upgrading). Via a coal preparation system, the coal is processed into pulverized coal or coal-water slurry, which is then gasified in a gasifier with the oxygen obtained by the air separation system. For the resulting syngas, the ratio of C and H is adjusted by a water-gas shifting device, and then the product is sent to a sour water stripping system to carry out desulfurization and decarburation. The purified syngas is sent to a Fischer-Tropsch (F-T) reactor for synthesis, and the mixed product obtained undergoes separation and upgrading to yield products such as liquid petroleum gas (LPG), naphtha, gasoline, diesel, and F-T wax. Part of the hydrogen recovered during the synthesis is used for the product upgrading, and part is returned to the synthesis step. The number of core conversion steps of CTL is 2 to 4 times that of petroleum refining [3]. Assuming that the probability of improvement of each core conversion step in both industries and the resulting cost reduction are the same, then theoretically, for every 10% increase in efficiency in the refining industry, the efficiency of the CTL process may increase up to 21% (1.1^2-1)−46.4% (1.1^4-1).

The scale-up of key equipment such as gasifiers and F-T reactors is an effective technical means to reduce the investment costs of CTL plants. Since the investment in syngas and F-T synthesis steps (including WGS and syngas purification units) accounts for 51%–85% of the total equipment investment, the scale-up of the equipment in these two steps can significantly reduce the cost per unit product. Furthermore, the scale-up of equipment can reduce the number of parallel lines in the process (Figure 1). For example, due to the use of 2 kt/d gasifiers, the ShenhuaNingmei indirect liquefaction plant, with a capacity of 4 Mt/a, requires the deployments of 28 gasification lines. This requirement not only greatly increases the purchase and installation costs of major equipment, auxiliary equipment, pipelines, and meters but also increases the probability of failure and the operational risk. Scale-up of the gasifier and F-T reactor can considerably reduce the number of parallel lines, the complexity of the process, and investment and operating costs.

Although industry and policy makers recognize the importance of reducing costs through economies of scale, there is still a lack of basis for decision-making on the scale of CTL plants to achieve economies of scale. For example, the "No-

FIGURE 1
Schematic diagram of indirect coal liquefaction, where parallel lines need to be designed for F-T reactors and gasifiers.
tice on Strengthening the Construction and Management of Coal Chemical Plants and Promoting the Healthy Development of the Industry” recommended that the scale of the proposed new CTL plant be above 3 Mt/a. In the “Notice on Regulating the Orderly Development of the Coal Chemical Industry”, the regulators believed that a plant scale of 1 Mt/a could reach economies of scale. In 2014, certain industry insiders suggested that small-scale plant of 0.16–0.5 Mt/a showed unique advantages in terms of economy, flexibility, and technical risk reduction [8]. Similar confusion also occurred in the United States. According to a report by the National Energy Technology Laboratory [10,11], a 0.5 Mt/a CTL plant could achieve an internal rate of return of 12%, but in 2008, a study by RAND[11] concluded that to achieve economies of scale, a CTL plant must have a capacity of 1.5–4 Mt/a. In view of the aforementioned confusion, we need to calculate the economic scale of China’s CTL plants based on changes on a technical level and price level within the foreseeable range of the future.

On the other hand, if we only focus on increasing plant scale and do not focus on the research and development (R&D) of key equipment, the reduction in the unit investment cost by static economics of scale will be offset by the diseconomies of scale from operation risks and repeated purchases caused by the increase in the number of parallel lines. According to calculations by Chandan and Rubin [12], when plant capacity is scaled up 2.5 times from 0.5 Mt/a, the break-even point can be reduced by 12.8%; if the size of the key equipment is not simultaneously scaled up, a 2.5 times scale-up of a 2.5 Mt/a plant can only achieve a 5.2% cost reduction. Regrettably, although the scale of planning for China’s CTL plants is increasing, we have not observed any plans for further scale-up of F-T reactors. In the “13th Five-Year Plan for Deep Coal Processing Demonstration”, only R&D and demonstration of gasifiers of 4 kt/d (pulverized coal gasifier) and 3 kt/d (coal-water slurry gasifier) were mentioned, and the scale up at this level showed very limited benefits for CTL plants of 4 Mt/a and above in reducing investment costs. Therefore, the plan for capacity up-scaling of CTL plants should consider the scale-up of key equipment to effectively achieve economies of scale.

To explore a technical development roadmap that includes the scale-ups of the plant capacity and key equipment, this paper aims to answer the following questions:

1. With regard to current equipment sizes, what CTL plant capacity can achieve economy of scale?

2. What is the optimal time to scale up the key equipment of a CTL plant? At what plant scale can the maximal cost be saved when the key equipment is scaled up to the next level?

3. What is the optimal deployment path for a country to develop its own CTL technology by gradually scaling up the key equipment and plant scale?

BACKGROUND

Scale-up level of F-T reactor in China’s CTL industry. The reactors currently used in commercial-scale F-T synthesis plants mainly include multitubular fixed-bed reactors that pass syngas multiple times, circulating fluid-bed systems, in which the fine catalyst particles trapped in the syngas circulate in a riser reactor at a very high speed, and slurry reactors, in which the syngas is in a bubble column in contact with fine catalysts suspended in liquid[23]. Due to structural differences, the scale-up of these different reactors is not synchronous (see Figure 5(b)). To date, the largest single size multitubular fixed-bed reactor was installed in the 7 Mt/a Shell Pearl gas-to-liquid (GTL) plant in Qatar in 2010 with a single reactor production capacity of 0.3 Mt/a. The maximum single production capacity of the existing circulating fluid-beds is 1 Mt/a, and this system was used in the upgrades of the Sasol II and Sasol III plants (each with a total production capacity of 3 Mt/a) in 1999. The maximum single production capacity of a slurry reactor is 0.85 Mt/a, which was used in the 1.7 Mt/a Sasol ORXY GTL plant in Qatar. In China, the current commercial-scale reactors are all slurry reactors and are only available in sizes of 0.16 Mt/a and 0.5 Mt/a.

By integrating the scale of deployment at different sizes, the scale-up process for F-T reactors is divided into three waves (Figure 2(a)). For a long time after 1955, the F-T reactors deployed in commercial-scale plants were only fixed-bed reactors, with a single production capacity of 0.075 Mt/a in the Sasol I plant. The first wave of scale-up occurred as the Sasol II and III plants began production from 1980 to 1995. In this wave of the scale-up process, medium-sized (0.2–0.4 Mt/a) reactors dominated, accounting for 91.7% of the reactor deployment. The second wave of scale-up occurred from 1999 to 2009. During this period, deployments of larger-size (0.4–0.6 Mt/a) reactors rapidly increased and replaced a large number of medium-sized reactors. During the same period, reactors of 0.8 Mt/a or higher were deployed on a large scale, triggering a third-wave scale-up of the reactors. As of 2009, in regions of the world outside China, reactors with sizes of 0.4–0.6 Mt/a accounted for 20.1% of the total deployment, while reactors with a scale of 0.8 Mt/a or greater accounted for 52.05%. In addition, since Shell selected a conservative fixed-bed reactor for the large-scale Pearl GTL plant in 2010, the total number of medium-scale reactors deployed returned to the same
level as before 1999. Despite this trend, the size of fixed-bed reactor used in this new plant was double that used in the Shell Bintulu GTL plant in 1993 and four times that of the fixed-bed reactor in the Sasol I plant in 1955 (Figure 2(b)).

![Figure 2](image)

(a) Scale-up of the F-T reactors in the global and Chinese CTL industries after World War II and (b) changes in the sizes of the F-T reactors deployed in the world and China relative to the plant scale.

The scale-up of the slurry-bed reactors in China’s CTL industry lags behind that of the world and also lags behind the increase in the plant scale (Figure 2(a) and (b)). In other regions of the world, small-scale slurry-bed reactors below 0.2 Mt/a were not developed after 1993. In 1993, Sasol added a 0.125 Mt/a slurry reactor at one of its plants for experimental and transitional purposes, so only one reactor of this size was built, and then a direct scale-up to 0.85 Mt/a was carried out in the ORXY plant. Three slurry-bed plants of 0.16 Mt/a were built in China between 2006 and 2009, and a large number of similar-scale plants entered the planning and preliminary work phases at that time. Without the intervention of industry regulators, China’s CTL industry was locked in a low-level industrial development path using F-T reactors below 0.2 Mt/a. After three years of redeployment in 2009-2011, the slurry-bed reactors used in the new CTL plant had all reached 0.5 Mt/a, which was equivalent to the average of the second-wave scale-up. Unfortunately, in the following seven years of development, the CTL plants were scaled-up, but the F-T reactors were not simultaneously scaled-up. We compared the scale-up times of the FT reactor of Sasol with that of China’s CTL industry: to achieve economy of scale, Sasol used two 0.85 Mt/a slurry-bed reactors in the 1.7 Mt/a plant; in contrast, China still used a 0.5 Mt/a slurry-bed reactor in 4 Mt/a plants, the same as that in 1 Mt/a plants. We believe that to achieve economies of scale in large-scale CTL plants, further scale-up of the F-T reactors must be considered.

**Scale-up of gasifiers in China’s CTL industry.** Unlike F-T reactors, which are mainly used for synthetic fuels, coal gasifiers are widely used in coal chemical industries such as coal gas, coal to olefins, and synthetic ammonia. There are many gasifiers that can be used for indirect liquefaction, such as Shell pulverized coal gasification, GSP (GazCombinatSchwarzvePumpe) pulverized coal gasification, HT-L coal gasification, multinozzle water-coal slurry gasification, Texaco coal slurry gasification, other technologies of entrained bed routes, and pulverized coal moving-bed pressurized gasification routes, including Lurgi pressurized gasification and British Gas/Lurgi (BGL) pressurized gasification technology [13]. The choice of a specific technology is mainly based on the nature of the raw coal[24].

Integrating the scale of deployment at different sizes shows that the scale-up process of gasifiers in China’s CTL industry is divided into three waves (Figure 3(a)). The first wave occurred between 2003 and 2010. The total scale of indirect liquefaction plants deployed during this period was lower than 0.18 Mt/a, and only one or two gasifiers with a coal throughput of 1800-2200 t/d could meet the demands of syngas production. At this point, the gasification of CTL plants had generally achieved economies of scale[25]. However, when the CTL plant deployed in China reached 1 Mt/a in 2011, the existing sizes of gasifiers could not achieve economies of scale. Even considering the largest 2000–2500 t/d pulverized coal gasifier at that time, 7–9 of these gasifiers were needed for the aforementioned liquefaction plants. Therefore, since 2013, the second wave of scale-up of gasifiers appeared in the CTL industry; during this period, larger-size (3000-3200 t/d) gasifiers were used in two new plants. The application of the world’s largest 4000 t/d gasifier in 2017 marked the beginning of the third wave of scale-up. From the perspective of single size, China’s CTL industry is at the frontier of the development and application of gasifiers.

However, the deployment of gasifiers in Chi-
na’s CTL industry still lags behind the plant scale-up (Figure 3(b)). First, the current scale-up size of gasifiers is still far from achieving economies of scale. The 4 Mt/a plant built in the second-wave scale-up needs 28 (including spare gasifiers) 2000 t/d gasifiers, at least 15 4000 t/d gasifiers, and more than 20 3000 t/d gasifiers. Second, due to the sensitivity of the gasifier efficiency to coal quality, it is difficult to use the existing gasifiers after scale-up of other plants. Although the sizes of the gasifiers increased to 3000–4000 t/d by the second and third waves of scale-up, the gasifiers sized 2000–2200 t/d are still dominant. As of 2018, the 2000 t/d gasifier accounted for 78.23% of the deployment scale in the CTL industry. Only when scale-up is achieved on different types of gasifiers will the average single size of the gasifiers in China’s CTL industry change significantly.

(a)

(b)

FIGURE 3
(a) Scale-up of the gasifiers in China’s CTL industry and (b) changes in the size of coal gasifiers deployed in China relative to CTL plant scale.

CALCULATION METHOD

This paper mainly investigated the impact of static economies of scale and technological progress on the decline in unit investment costs. The unit investment cost is the total plant investment (TPV) divided by the plant production capacity. The calculation includes the unit cost saved by the scale-up of the plant (static economies of scale), the decrease in the average installation cost of multiple identical trains of equipment (learning during the installation), and the reduction in unit investment costs caused by the scale-up of single pieces of equipment. The learning methods not covered by this paper include an increase in worker proficiency, an enhancement of management organization, an increase in the efficiency caused by improvements in the equipment and processes without scaling up the size of the plants and equipment, and an increase in the efficiency achieved by R&D of efficient catalysts.

The total plant investment was the total plant cost (TPC) plus the interest during construction (IDC). The IDC was set to 10% of the TPC[26,27]. The TPC consisted of two parts, the direct costs and indirect costs. The indirect costs included investment costs of office buildings and public facilities other than those inside the main battery limit (ISBL) and were set at 32% of the direct costs [28]. The direct costs were calculated as follows:

\[
C^d = \sum E + \sum (f^{in} \times E) \times f^{bop}
\]

Where \(E\) = cost of equipment purchased, including piping, electrical, and instrumentation
\(f^{in}\) = multiplying factor for installation costs, set at 0.39
\(f^{bop}\) = multiplying factor for balance of plant (BOP) costs, set at 1.155[29].

In this paper, the scale-up of the reactor and the gasifier was mainly investigated as the technological advancement factors. In the calculations, we considered each gasifier and its coal blending and transferring equipment as a gasification train, and thus, the direct cost of the gasification process referred to the sum of the delivered-equipment cost[29] and installation cost of each train. The installation cost of each train was the coefficient of the delivered-equipment cost, which was set at 0.39. Considering the learning role, the actual total cost of installing multiple identical lines could be lower than the sum of the independent installation costs. This paper used a scaling exponent of 0.9 to estimate the total post-installation cost of multiple identical process trains[14].

\[
c_{\text{multi-trains}}^{\text{in}} = E \times f^{\text{in}} \times (n)^{f}
\]

where \(n\) is the number of lines and \(f\) is the scaling factor.

The direct cost of the F-T reactor was also based on the same learning coefficient and installation cost proportional coefficient. A hydrogen separation system, a water separation system, and a product upgrading system closely related to F-T synthesis were incorporated into the F-T synthesis train, which was treated as a whole in calculating the direct cost. The delivered-equipment costs of the two systems were calculated according to the corresponding scale-up coefficients (Table 1). The
air separation system, the WGS system, and the sour water stripping system were treated as separate devices to calculate the overall direct cost rather than several parallel trains. Based on the actual investment data (Table 2) of China’s CTL plants collected by the author, the equipment costs of different sizes were calculated using the cost scaling factor (CSF) of each system[14,28,29,30]:

\[ E_i = E_{i,\text{ref}} \times \left( \frac{S_i}{S_{i,\text{ref}}} \right)^{k(i)}, i = 1, 2, \ldots, 4 \]  

Where \( E_{i,\text{ref}} \) = delivered cost of the reference equipment
\( S_i \) = scale of the estimated equipment
\( S_{i,\text{ref}} \) = scale of the reference equipment
\( k(i) \) = cost scaling factor (CSF); see Table 1

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Scale-up coefficients of equipment for four key process steps.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Major subsystems and units</td>
</tr>
<tr>
<td>1</td>
<td>Air separation system</td>
</tr>
<tr>
<td></td>
<td>ASU</td>
</tr>
<tr>
<td>2</td>
<td>Gasification</td>
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<tr>
<td></td>
<td>Coal Preparation</td>
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<tr>
<td></td>
<td>Gasifier</td>
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<tr>
<td>3</td>
<td>WGS &amp; gas cleanup</td>
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<td></td>
<td>WGS unit</td>
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<td></td>
<td>Rectisol</td>
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<td></td>
<td>Claus</td>
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<tr>
<td>4</td>
<td>Synthesis system</td>
</tr>
<tr>
<td></td>
<td>F-T reactor</td>
</tr>
<tr>
<td></td>
<td>Refine &amp; Upgrade</td>
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<td></td>
<td>PSA</td>
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</table>

The complete parameters of the operating costs are shown in Table 3. To conservatively reflect the external cost of water for a CTL plant, we used the largest value of the estimated values (5.6-13.45 tons of water consumption/ton of oil products) from the literature [10,31,32] to estimate the water consumption. Based on the actual consumption of the CTL plants already in operation, we estimated the cost of catalyst to be 237.5 Yuan/ton [7], which is close to the estimated results of foreign technical disclosure data[33]. Nevertheless, certain reports have claimed that the cost of indirect liquefaction catalysts can be less than 100 Yuan/ton of oil [10]. Based on the reported data[20], we used a scale-up coefficient of 0.25 [34] to estimate the operational labor costs and the direct supervisory costs for facilities of different sizes. The financial expenses here were mainly the interest of the borrowed capital. The borrowed capital was estimated at 55% of the initial investment, and the annual interest rate was 5% [14]. To simplify the calculations, the proceeds from the sale of byproducts, power generation, and steam were not considered.

**OPTIMAL TIME FOR SCALE-UP OF KEY EQUIPMENT: SCALE-UP FRONTIER**

Scale-up frontier of the F-T reactor: To explore the impact of F-T reactor scale-up on investment costs and operational capacity, we first divided the reactors into four sizes of 0.16 Mt/a, 0.5 Mt/a, 0.75 Mt/a, and 1 Mt/a. The first two sizes of reactor

<table>
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<tr>
<th>TABLE 2</th>
<th>Data and sources for the equipment estimation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>Process</td>
</tr>
<tr>
<td>2013</td>
<td>ASU</td>
</tr>
<tr>
<td>2014</td>
<td>Gasifier</td>
</tr>
<tr>
<td>2014</td>
<td>Gasifier</td>
</tr>
<tr>
<td>2014</td>
<td>WGS, gas cleanup&amp; PSA</td>
</tr>
<tr>
<td>2014</td>
<td>F-T reactor</td>
</tr>
<tr>
<td>2010</td>
<td>F-T reactor</td>
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</table>

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Estimation of costs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Scaling factor</td>
</tr>
<tr>
<td>Feed coal consumption, ton/ton fuel output</td>
<td>3.33</td>
</tr>
<tr>
<td>Power coal consumption, ton/ton fuel output</td>
<td>1.43</td>
</tr>
<tr>
<td>Price of coal, Yuan/ton</td>
<td>150</td>
</tr>
<tr>
<td>Cost of catalyst, Yuan/ton fuel output</td>
<td>237.5</td>
</tr>
<tr>
<td>Water consumption, ton/ton fuel output</td>
<td>13.45</td>
</tr>
<tr>
<td>Price of water, Yuan/ton</td>
<td>5</td>
</tr>
<tr>
<td>Operating labor for 4 Mt/a capacity, person</td>
<td>0.25</td>
</tr>
<tr>
<td>Direct supervisory labor, % operating labor</td>
<td>15</td>
</tr>
<tr>
<td>Average unit wage, million Yuan/year</td>
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</tr>
<tr>
<td>Maintenance costs, % total plant cost</td>
<td>11</td>
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<tr>
<td>Administration costs, % operating labor cost</td>
<td>25</td>
</tr>
<tr>
<td>Total borrowed capital, % total plant investment</td>
<td>55</td>
</tr>
<tr>
<td>Financial costs, % total borrowed capital</td>
<td>5</td>
</tr>
</tbody>
</table>
have been deployed in China; the estimation of the larger reactors was based on a study by Sie and Krishna [13]. According to the researchers’ calculations, for a single reactor with a weight limit of 900 tons, the theoretical limit of converting syngas into heavy products by a non-homogeneous bubble bed is 1 Mt/a[14]. Moreover, for China’s current equipment manufacturing industry, there is no real obstacle to the design and manufacture of 0.75-1 Mt/a reactors.

More importantly, the feasibility of this size of slurry-bed reactor has been validated in the ORXY plant. This paper did not consider the scale-up of larger-size reactors, as this paper was based on current reactor manufacturing, transportation, and lifting capabilities. The deployment of larger-size reactors needs further development of the aforementioned matching capabilities. According to Xue et al.[15], further scale-up of a slurry reactor to 1.5 Mt/a can result in a single reactor weight of 4,849 t, and the single weight is as high as 6,172 t for a scale-up to 2 Mt/a, which approaches the limits of the existing lifting capacities[15].

We used the scaled-up marginal return(MRSU) to calculate the optimal scale-up time for an F-T reactor at a given CTL plant size. We first calculated the unit investment cost for CTL plants at different scales (1–10 Mt/a) using the four sizes of reactors described above, i.e., the purchase and installation equipment costs of the reactor divided by the plant capacity. Subsequently, we subtracted the unit-size investment cost of a reactor at one size from that of the reactor at the next larger size and obtained the amount of investment saved when the reactor is scaled up to the next level at different plant scales, i.e., the scaled-up marginal return curve (see Figure 4(b) and (d)). The equation is as follows:

$$MR_{SU} = \frac{\Delta E_{i} + \Delta E_{i+1} + \Delta E_{i+2} - S_{i}}{S_{i}}; \ i = 1, 2, 3, 4$$

where $S_{i}$ refers to the size of the F-T reactor, $S_{i}$ = 0.16 Mt/a, 0.5 Mt/a, 0.75 Mt/a, and 1 Mt/a.

To minimize the technical risk, we assumed that the R&D and deployment of the reactors are scaled up stepwise and in order; the size of the first plant constructed in the CTL industry and the initial size of the F-T reactor are both 0.16 Mt/a. Under ideal conditions, when the CTL plant is to be further scaled up to 1 Mt/a, the scale-up of the reactor to the next larger size (0.5 Mt/a) can save a unit investment cost of 550 million Yuan (Figure 4(b)). However, an immediate scale-up does not always lead to additional returns. When a CTL plant is scaled up to 2 Mt/a, the scale-up of the reactor from 0.5 Mt/a to 0.75 Mt/a does not result in additional cost savings. Only when the plant scale reaches 3

FIGURE 4
(a) (top left): Ideal scale-up frontier for the F-T reactor, (b) (bottom left) and (d) (bottom right): scaled-up marginal returns of the F-T reactor, and (c) (top right): realistic scale-up frontier of the FT reactor.
Mt/a can the aforementioned scale-up bring about a reduction of approximately 100 million Yuan for the unit-size reactor investment costs. Therefore, scale-up from 0.5 Mt/a to 0.75 Mt/a should be achieved on a 3 Mt/a CTL plant. According to the idea of maximizing the local scaled-up marginal return, we plotted the optimal path for the stepwise scale-up of F-T reactor with increasing plant scale, i.e., the ideal scale-up frontier (Figure 4(a)).

However, in the actual development of China’s CTL industry, the scale-up of F-T reactors has not been carried out in accordance with the ideal scale-up frontier. As we discussed in the previous section, the scale-up of the reactor lags behind that of the plant scale. Although the reactor was scaled up from 0.16 Mt/a to 0.5 Mt/a in time at a plant scale of 1 Mt/a, China’s CTL industry missed the optimal time for 3 Mt/a reactors in the process of scaling up to the next larger size. China has directly

**FIGURE 5**
(a) (top left): Ideal scale-up frontier for the gasifier, (b) (bottom left) and (d) (bottom right): scaled-up marginal return of the gasifier, and (c) (upper right) realistic scale-up frontier of the gasifier.

**FIGURE 6**
(a) (left chart): Break-even point of the plant under different scale-up paths and (b) (right chart): cumulative savings of the investment cost for the CTL industry with different deployment paths compared with the industry under static economies of scale.
developed a liquefaction plant at a scale of 4 Mt/a, and at this scale, the marginal return of further scaling up a 0.5 Mt/a F-T reactor is zero. According to the scaled-up marginal return curve, the next beneficial scale for further scale-up is 6 Mt/a. Similarly, scale-up from 0.75 Mt/a to 1 Mt/a should be postponed until the plant scale reaches 7 Mt/a. According to this result, we plotted a suboptimal scale-up path that was more in line with the development status of China’s CTL industry, i.e., a realistic scale-up curve (Figure 4(c)).

Scale-up frontier of the gasifier. To explore the impact of gasifier scale-up on investment costs and operational capacity, we divided the gasifiers into four levels: 2 kt/d, 4 kt/d, 6 kt/d, and 8 kt/d. The 2 kt/d gasifier has been widely deployed in the CTL industry. One 4 kt/d gasifier has been used as a backup gasifier in a CTL plant that is in operation for demonstration and improvement, and the larger-size gasifiers are still in the R&D stage. We included the 6–8 kt/d gasifiers into the calculation range because first, there is ample room for the scale-up of the gasifiers in theory, although the technical paths of different gasifiers are quite different and cannot be generalized. In terms of the most widely used entrained bed technologies, the approaches of scale-up include increasing the number of nozzles, scaling up and improving the nozzles [16,17], adjusting the length and diameter of the vessel, and correspondingly increasing the axial velocity of the jet [18]. Second, the existing industrial level in China can fully meet the demands for design and manufacture of larger gasifiers. Finally, the current largest 4 kt/d gasifier has a total weight of approximately 400 tons, and the weight after a one fold scale-up is still far below the limit of the current construction, transportation, and lifting capacities of China’s equipment manufacturing industry. For the convenience of the calculations, we assumed that the scale-up starts from a 2 kt/d gasifier and the larger sizes are multiples of 2 kt/d. As with the F-T reactor, we assumed the principle of stepwise scale-up and used the scaled-up marginal return curve to determine the optimal scale-up path (Figure 5(b) and (d)). The scaled-up marginal return equation is shown in Equation 4, where $S_g$ refers to the size of gasifier, and $S_g = 2$ kt/d, 4 kt/d, 6 kt/d, and 8 kt/d.

First, we discuss the ideal scale-up curve (Figure 5(a)). Although the scale-up of gasifiers from 2 kt/d to 4 kt/d at a scale of 1 Mt/a can reduce the investment costs per unit gasifier by approximately 160 million Yuan, the aforementioned scale-up used directly on a plant with a scale of 2 Mt/a can certainly produce more returns (Figure 5(b)). Subsequently, each time the plant scale is scaled up by 1 Mt/a, the gasifier is scaled up by one level, and optimal scale-up can be achieved.

However, in reality, China’s CTL began to develop 4 kt/d gasifiers after the 4 Mt/a plant was put into operation. Assuming that this size of gasifier is used in two 4 Mt/a scale reserve plants during the 13th Five-Year Plan period, the optimal implementation time for the next level of scale-up (6 kt/d gasifier) should occur before the deployment of the 5 Mt/a CTL plant. An earlier scale-up, such as a 6 kt/d gasifier being used in a 4 Mt/a plant, does not considerably reduce the gasifier unit investment (Figure 5(d)). Similarly, 8 kt/d gasifier scale-up should be deployed on 8 Mt/a plants to achieve the local optimization. Based on the above results, we plotted the realistic scale-up frontier of the gasifier (Figure 5(c)).

RESULT

We estimated the break-even points by simply scaling up the sizes of and scaling up the F-T reactors and gasifiers in CTL plants based on different frontiers and evaluated the static economies of scale and the financial impact of technological advancements according to different strategies. The calculations show that the static economies of scale of the CTL industry are significant (Figure 5(a)). In China’s CTL industry, if only the plant size is scaled up in the future, and the sizes of the FT reactors and gasifiers remain at the current levels of 0.5 Mt/a and 2000 t/d, respectively, the unit cost will still be reduced by increasing the plant size due to the static economies of scale. When the plant scale increases from 1 Mt/a to 4 Mt/a, the plant break-even point will decrease from 66 USD/barrel to 59 USD/barrel. However, the static economies of scale from the further scale-up of the production capacity to 8 Mt/a are very limited, only a reduction of US$2/barrel compared with that of the 4 Mt/a scale.

Second, scale-up of the F-T reactors and gasifiers and other technological advancement methods can obtain economies of scale in CTL plants. If a CTL plant is deployed according to the realistic frontier (Figure 6(a), orange line) (that is, 4000 t/d gasifiers are deployed in two 4 Mt/a reserve plants during the 13th Five-Year Plan period, and the existing 0.5 Mt/a FT reactors are used), a break-even point of $57/barrel can be achieved, equivalent to the profitability at a scale of 8 Mt/a in static economies of scale. Compared with that of the 1 Mt/a plant, the break-even point of the CTL plant under these setting decreases by 13.64%, of which the static economies of scale contribute 10.6% and the scale-up of gasifiers contributes 3.04%. If an 8000 t/d gasifier and a 1 Mt/a reactor are deployed in an 8 Mt/a plant in the future, the break-even point can be further reduced to $53/barrel, which is 10.17% lower compared with that of the current 4 Mt/ plant. Considering that the feasibility of the 1 Mt/a F-T reactor has been validated, the challenge to achieving cost reduction of the aforementioned magnitude
is the scale-up of the gasifier.

Furthermore, for countries that want to develop their own CTL technology, the ideal scale-up frontier is a more noteworthy curve (Figure 6(a), purple line). If 4000 t/d gasifiers are deployed in a 2 Mt/a plant with 0.5 Mt/a reactor, the break-even point of the plant can reach $60.2/barrel, close to the profitability of a 4 Mt/a CTL plant under static economies of scale. If both the gasifier and the FT reactor can be scaled up to 8000 t/d and 1 Mt/a in a 4 Mt/a CTL plant, respectively, the plant break-even point can be reduced to $55/barrel, which is 16.67% lower than that of the 1 Mt/a plant. The scale-ups of the gasifier and the F-T reactor contribute a total of 6.06%.

In addition, if countries attempting to develop their own CTL technology can obtain a stable source of raw coal or directly develop GTL technology, then it is not necessary to simultaneously test multiple gasifier technologies to adapt to different coal qualities. Under this circumstance, the CTL industry does not need to build multiple intermediate-scale homogeneous demonstration plants in parallel. At the 1 Mt/a and 2 Mt/a scales, China built 3 plants in different regions; according to the 13th Five-Year Plan, 3 plants at a scale of 4 Mt/a will be built. This plan is determined by the history of the early development of China’s CTL industry. Different entities must develop different gasification and synthesis processes to adapt to different coal qualities. When the raw materials are stable, a suitable gasification–synthesis process should be selected, and on average, only one transition plant should be built to achieve stepwise scale-up of the equipment.

Finally, we simulated a plant deployment path with a long-term industry size comparable to China’s future potential size (assuming 45 Mt/a), according to the technology upgrade path indicated by the optimal scale-up front (Figure 6(b)). The results show that CTL industries (three 1 Mt/a plants, three 2 Mt/a plants, three 4 Mt/a plants, and three 8 Mt/a plants) that are gradually scaled up according to the realistic scale-up frontier will eventually save 776 million US dollars per year compared with the simple increase of plant scale (the number and type of plants are the same as the former). Our optimal deployment path (one 1 Mt/a plant, one 2 Mt/a plant, two 3 Mt/a plants, one 4 Mt/a plant, and four 8 Mt/a plants) will help save $1.104 billion Yuan annually after deployment is complete through the entire 45 Mt/a CTL industry.

CONCLUSIONS

The conclusions of this paper include the following three points:

1. Our research shows that the scale-up of China’s gasifiers and F-T reactors lags behind the increase in the plant scale and has resulted in losses of $184.8 million per year. To achieve economies of scale, the size of the gasifier for the new 2 Mt/a scale CTL plant should be doubled compared with the current 2000 t/d; the new 4 Mt/a scale CTL plant should be postponed until the 8,000 t/d gasifier and 1 Mt/a F-T reactor are technically mature.

2. We recognize that there are two main reasons for the lag in the scale up of key equipment. The current lag in the deployment of larger-size of F-T reactors occurred because the benefits of further scale-up are not significant. Currently, the average size of the domestic F-T reactor is 0.5 Mt/a. Even if this size is doubled to 1 Mt/a, the unit investment cost can be reduced by no more than 3%. At the same time, due to the process characteristics of the slurry reactor, the production capacity of the existing 0.5 Mt/a F-T reactor can be further increased to 0.75 Mt/a by adjusting the reaction temperature and pressure without relying on the scale-up of vessel [19]. More importantly, due to strong asset specificity, the F-T reactor cannot be used in industries other than the CTL industry. Therefore, there is a high probability that the F-T reactor will remain at the current size of 0.5 Mt/a in the future.

3. The scale-up of gasifiers is subject to technology and experience. Although commercial gasifiers with independent intellectual property rights have been deployed on a large scale in China, China’s current R&D capabilities regarding gasifiers still lag behind those of advanced countries. The current diseconomies of scale of gasifiers are the main equipment bottleneck for the development of ultra large-scale gasification plants, such as CTL and coal-to-gas, greatly affecting the operating efficiency and investment efficiency of the entire process. The successful scale-up of gasifiers broadly impact the development of China’s modern coal chemical industry and help China’s equipment suppliers become global leaders in manufacturing coal chemical equipment.

2. This paper reveals the value of waiting options for investment after technology matures: if investors wait to invest until after scale-up of key equipment, the investment amortization can be significantly reduced. However, the actual policy environment facing the industry has weakened the value of waiting options: in the past, the approval policies for coal chemical plants were discontinuous and excluded new entrants, so investors feared that if they did not “enter the industry” in the early stages, they would have no chance to do so in later periods. We believe that this is the policy reason for repeat construction of homogeneous small-scale plants in the past.

Under the background of this policy, the long-term technical development strategy of certain CTL plant investors is to “enter the industry” under the locally satisfactory technical level, while accumulating experience through plant operation and
waiting for improvement in the process and further scale-up of key equipment. Once the technology is mature, operators can use the improved equipment for new plants or replace the equipment that has not achieved economies of scale for a long period of time. A similar strategy has been used in the Sasol II and III plants: after 20 years of production, the original twenty 0.325 Mt/a Synthol reactors in Sasol were replaced with four large-size SAS reactors (Figure 2(a)).

3. Our research shows that China’s CTL industry has established excessive homogeneous transition plants while gradually scaling up the plant size, which unnecessarily increases the investment cost of the entire industry to achieve economies of scale. Many industry studies have shown that the “formative phase” of the industry, a large number of small-scale experiments can help to form leading technologies and promote industrial development [20]. However, in the three 0.16–0.18 Mt/a plants of the early days of the development of China’s CTL industry (2003–2009), all the processes came from the same technology supplier. A homogeneous application plant does not promote learning among technology suppliers. Instead, this condition may allow developers to be satisfied with the relatively high market share and reluctant to upgrade technology, so the technology is locked on a low-level path [21]. As a positive example of controlling experimental costs, Sasol only converted the main vessel of the original circulating fluid-bed system into a 125,000-ton slurry reactor when validating the industrial-scale slurry reactor technology. After the validation was successful, Sasol immediately installed a slurry reactor of 850,000 tons on the ORXY plant.

From the perspective of regulators, repeated construction of transition plants do more harm than good; small-scale and low-level CTL plants that do not realize economies of scale not only cause industrial risks but can also waste resources and cause environmental pollution since the byproducts (light hydrocarbons) and emissions (such as oxides) cannot be processed more economically [22]. Industry regulators should encourage different technologies to compete in the early stages of technological development and prevent the early commercial diffusion of premature technology. After the emergence of leading technologies, equipment scale-up and economies of scale in the industry are achieved by developing a deployment plan for economic demonstration plants.

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ABSTRACT

Removing the pollutants from oilfield wastewater and reinjecting the wastewater to the formation are conducive to the protection of the local environment. Silicate is a kind of common pollutant of oilfield wastewater. And the accurate prediction of its solubility is beneficial to the formulation of wastewater treatment schemes. To predict the silicic acid solubility in varying temperature, multi-component aqueous solutions, a prediction model applicable to such solution is proposed in this paper based on Pitzer electrolyte solution theory and in consideration of the long-range and short-range Coulomb electrostatic interaction and dispersion interaction between cations and anions. For Na\(^+\)-K\(^+\)-Ca\(^{2+}\)-Mg\(^{2+}\)-Cl\(^-\)-SO\(_4^{2-}\)-HCO\(_3^-\)-F\(^-\)-SiO\(_3^{2-}\) solution, the prediction model is used to calculate the silicic acid solubility at different temperature and ion concentration, then the range analysis is used to analyze the factors affecting the solubility of silicic acid. The result indicates that temperature, Ca\(^{2+}\) and SO\(_4^{2-}\) are the main factors, with the range R of 11.1, 7.8 and 6.9, respectively. The effects of these factors on the enthalpy change, potential energy and single point energy in silicate reaction have been studied by using quantum chemistry method. The research results agree with the range analysis results, which further checks the correctness of the prediction model. Therefore, this prediction model can be used to predict the silicate solubility in the temperature-varying multi-component silicate solution.

KEYWORDS:
Oilfield Wastewater, Wastewater Treatment, Silicate Solubility, Pitzer Model, Electrolyte Solution

INTRODUCTION

Water-flooding will produce a large amount of wastewater (mainly oilfield produced water) [1]. In order to make full use of water resources, oilfield reinject the wastewater back to the formation after wastewater is treated to be qualified [2]. Silicate is a common pollutant in wastewater for conventional water-flooding oilfields [3]. Therefore, the accurate prediction of its solubility is beneficial to the formulation of wastewater treatment schemes [4]. A reasonable wastewater treatment process plan can make silicate content in wastewater unsaturated and slow down silicate precipitation, so as to avoid blocking formation pores and extend oilfield production cycle [2-3]. In addition, the prediction of silicate solubility also has positive significance for improving thermal energy utilization rate [5] and silicate production [6]. Therefore, the studies of silicate solubility have drawn attentions of a large number of researchers [7].

Silicate solution is electrolyte solution in nature. Debye-Hückel [8] deduced a DH model based on Poisson equation to calculate the solubility of low-concentration strong electrolyte solution. Based on the DH model, an expanded DH model was established by adding ion strength correction term to expand the range of application to ion strength from 10\(^{-2.3}\) to 10\(^{-1}\) [9]. Since then, several improved DH models, such as Bromley model [10], Stokes-Robinson hydration model [11], Bjerrum ion association model [12], and Lee-Wheaton Conductance model [13], were established, but there was no essential change in theory. In order to overcome the limited application range of DH models, Pitzer [14] proposed the total excess free energy theory of solution based on the statistical mechanics theory and the radial distribution function of ions in solution, and obtained the model that could calculate the solubility of high-concentration electrolyte solution. So far, Pitzer electrolyte theory has become one of the most widely used electrolyte solution theories in the world [15]. Considering the uneven ion distribution in electrolyte solution, Chen [16-17] introduced a local theoretical model and proposed the NRTL model, which was proven a success and became the default model for calculation of electrolyte solution in AspenPlus software [18]. As a result, this model has been widely used.

When applying the electrolyte solution models mentioned above, two points, acquisition of chemical equilibrium constants and acquisition of model coefficients, shall be noticed. The chemical equilibrium constants can be calculated by lnK\(_{\text{eq}}\)=ΔG/RT [19]. However, it is difficult to accurately describe
the silicate reaction equation due to the polymerization of silicate in solution [20-21]. Therefore, the silicic acid in this paper refers to the general ones, including H$_3$SiO$_4^-$, H$_2$SiO$_3^-$, HSiO$_3^-$ etc. For the solution of equilibrium constants of silicate reaction, researchers often use empirical models, such as the three-parameter reaction equilibrium constant model of amorphous silica and quartz obtained by Fleming and Crerar [22] through experiments, the four-parameter reaction equilibrium constant model of amorphous silica, quartz and cristobalite obtained by Gunnarsson and Arnórsson [23], and the three-parameter reaction equilibrium constant model of Na$_2$SiO$_3$ and Na$_2$SiO$_3$-9H$_2$O obtained by Zeng [6,24] by combining OLI software database.

The coefficients in the electrolyte solution model need to be obtained by experimental regression. For silicate solution, Zeng [6, 24] obtained the silicic acid solubility in Na$_2$SiO$_3$/Na$_2$SiO$_3$-9H$_2$O-NaCl/ KCl solution under different temperatures (288K-353K) and concentrations (0.1mol/L-3.5mol/L) through experiments, acquired the coefficients in Bromley-Zemaitis model [25], and calculated the silicic acid solubility in Na$^+$-K$^+$-Cl$^-$ and Na$^+$-K$^+$-Cl$^-$-OH$^-$ solution, which showed good results, with an average relative deviation less than 4.1%. Park and Englezos [19] obtained the silicic acid solubility in Na$_2$SiO$_3$/NaCl/ KCl solution at the concentration of 0.04 mol/L-4.02 mol/L and the temperature of 298 K, got the parameters of Na$_2$SiO$_3$ in Pitzer model ($\beta^{0.0}=0.5777$, $\beta^{0.1}=2.8965$, $C^p=0.0097$), and calculated the solubility of Na$_2$SiO$_3$ in pure water and NaOH solution, with a standard deviation less than 0.0559.

However, the studies on silicate solution mainly focus on silicic acid-monsalpast/bisalt solution [6, 26-27], and there are few studies on the temperature-varying multi-component silicate solution. Therefore, a silicic acid solubility prediction model applicable to the temperature-varying multi-component silicate solution (Na$^+$-K$^+$-Ca$^{2+}$-Mg$^{2+}$-Cl$^-$-SO$_4^{2-}$-HCO$_3^-$-F$^-$-SiO$_3^{2-}$) is established in this paper. This model is based on Pitzer electrolyte solution theory and considers the long-range and short-range Coulomb electrostatic interaction and dispersion interaction between cations and anions in solution. It can be used for predicting the silicic acid solubility in oilfield development, geothermal utilization, silicate production and other industries.

\[ \text{Na}_2\text{SiO}_3 \overset{\text{E}}{=} 2\text{Na}^+ + \text{SiO}_3^{2-} \]  
\[ K_{\text{Na}_2\text{SiO}_3} = (\alpha_{\text{Na}^+})^2(\alpha_{\text{SiO}_3^{2-}}) \]  
\[ = (m_{\text{Na}^+} \gamma_{\text{Na}^+})^2(m_{\text{SiO}_3^{2-}} \gamma_{\text{SiO}_3^{2-}}) \]  

**Pitzer Electrolyte Mode.** In order to use Pitzer model more conveniently, Harvie & Wear et al. re-organized the Pitzer model and called it HW model [28].

\[ \ln(y_{\text{Cl}^-}) = \sum_{i} m_{\text{Cl}^-} (2\text{B}_{\text{Cl}^-} + Z_{\text{Cl}^-}) + \sum_{i} m_{\text{Cl}^-} (2\text{B}_{\text{Cl}^-} + Z_{\text{Cl}^-}) + \sum_{i} m_{\text{Cl}^-} (2\text{B}_{\text{Cl}^-} + Z_{\text{Cl}^-}) + \sum m_{\text{Cl}^-} \gamma_{\text{Cl}^-} \]  
\[ \ln(y_{\text{Na}^+}) = \sum_{i} m_{\text{Na}^+} \gamma_{\text{Na}^+} \]  
\[ \ln(y_{\text{SiO}_3^{2-}}) = \sum m_{\text{SiO}_3^{2-}} \gamma_{\text{SiO}_3^{2-}} \]

In which, \( F \) can be represented by Equation (6).

\[ F = -A^0[I^1/2 + (1+1.2I^1/2)] + 2\ln([I^1/2 + 1/2]) + \sum m_{\text{Cl}^-} m_{\text{Cl}^-} \Phi'_{\text{Cl}^-} + \sum m_{\text{Cl}^-} m_{\text{Cl}^-} \Phi'_{\text{Cl}^-} \]

\[ B_{\text{Ca}^2+} B_{\text{Ca}^2+} \Phi_{\text{Ca}^2+} \Phi_{\text{Ca}^2+} \] in Equation (3) and (4) can be represented by Equation (7), (8) and (10) [29].

\[ B_{\text{Ca}^2+} = \beta_{\text{Ca}^2+} + \beta_{\text{Ca}^2+}^0 g(a_{\text{Ca}^2+} I^1/2) + \beta_{\text{Ca}^2+} g(a_{\text{Ca}^2+} I^1/2) \]

\[ B_{\text{Ca}^2+} = \beta_{\text{Ca}^2+}^0 g(a_{\text{Ca}^2+} I^1/2) + \beta_{\text{Ca}^2+} g(a_{\text{Ca}^2+} I^1/2) / I \]

\[ \Phi_{\text{Ca}^2+} = \Phi_{\text{Ca}^2+} + \Phi_{\text{Ca}^2+} \]

The parameters in Equation (7)-(10) can be obtained by Equation (11)-(17) [30].

\[ g(x) = 2[1 - (1 + x) e^{-x}] / x^2 \]

\[ g'(x) = -2[1 - (1 + x + x^2 / 2) e^{-x}] / x^2 \]

\[ x_{\text{y}} = 6Z_{\text{y}} Z_{\text{y}} A^0 I^1/2 \]

\[ (x_{\text{y}})' \theta_y = \frac{(Z_{\text{y}} I^1 / 4)(J(x_y) - J(x_y)) / 2 - J(x_y)}{2} \]

\[ (x_{\text{y}})' \theta_y = \frac{(Z_{\text{y}} I^1 / 4)(J(x_y) - J(x_y)) / 2 - J(x_y)}{2} \]

\[ J(x) = x[4 + C_x x^{-C_x} \exp(-C_x x^{-C_x})]^{-1} \]

Equation (18) is obtained by substituting Equation (6) into (3).

\[ \ln(y_{\text{Cl}^-}) = \frac{Z_{\text{Cl}^-}}{Z_{\text{Cl}^-}} A^0[I^1/2 + (1+1.2I^1/2)] + 2\ln([I^1/2 + 1/2]) / 2 \]

9432
According to Pitzer electrolyte theory, the 1st term on the right of Equation (18) represents the Coulomb long-range interaction of anion (X) in solution. The 2nd term represents the Coulomb short-range attraction and dispersion interaction of X with cations. The 3rd term represents the Coulomb short-range repulsion of X with other anions. The 4th term represents the electrostatic interaction of X with cation and anion. The 5th term indicates the electrostatic interaction of X with neutral molecules. The 6th term represents the dispersion interaction between cations and anions, between cations, and between anions in the electrolyte solution.

The order of magnitude of parameters, \( V_{\text{CAK}} \) and \( C_{\text{CK}} \), in Pitzer model is \( 10^{-1}-10^{-2} \) [15]. When the salinity in aqueous solution is low [31], the interaction between three ions, i.e., the 4th, 5th and 6th terms of Equation (18), can be ignored relative to the long-range interaction of ions. In addition, when there are no other neutral molecules in the aqueous solution, the contribution of the 7th term of Equation (18) is 0. Therefore, a Petzer model applicable to low-concentration silicate solution can be obtained, as shown in Equation (19).

By combining Equation (19) and Equation (20), it can be seen by combining Equation (19) and Equation (20) that silicic acid concentration is related to the Coulomb long-range interaction, short-range interaction and dispersion interaction between cations and anions. In Equation (19), the 1st term on the right is a function of ionic strength \( I^{1/2} \). For the terms representing the Coulomb short-range attraction and dispersion interaction (the 2nd term), the parameter \( \Phi_{\text{KD}} \) and \( \Phi_{\text{KD}} \) can be correlated to \( I^{1/2} \) to make it as the function of \( I^{1/2} \). By combining Equations (8), (10), (12) and (15), the interaction between cations and anions in solution can be correlated as the function of \( \exp(-I^{1/2})/I^{1/2} \). Within a relatively small temperature range (273.15K-373.15K), the effect of temperature is approximately linear [32-33]. Therefore, the prediction model of silicic acid solubility can be obtained by adding the temperature correlation term before each ion interaction term, as shown in Equation (21).

\[
\text{See notation for the meanings of symbols in each equation.}
\]

Solution of the Silicic Acid Solubility Prediction Model. First published by Legendre in 1805, the least square method is one of the major "observation combination" tools [34]. By minimizing the square of error and finding the best function matching of data, the least square method can easily obtain unknown data and minimize the sum of squares of errors between the obtained data and the actual data [35]. Therefore, to calculate the unknown coefficients in Equation (21), the least square method is used in this paper to solve the silicate solubility prediction model.

RESULTS AND DISCUSSIONS

Demonstration of the Silicic Acid Solubility Prediction Model. The wastewater from conventional water-flooding oilfields is mainly produced water. At this time, the wastewater composition is consistent with the formation water. With data derived from Reference [31], the coefficients of the silicic acid solubility prediction model were calculated by the least square method. After calculation, the coefficient \( C_{\phi} \) of long-range interaction term is -0.00027, and the other coefficients are shown in Table 1. Error analysis is carried out with relative error analysis method [36], with the results shown in Figure 1.

According to the error analysis results, the average relative error of silicic acid solubility prediction model is less than 4.0%, and the maximum relative error less than 8.4%, so it can be used to predict the temperature-varying low-concentration silicate solubility.

Analysis of Factors Affecting Silicic Acid Solubility. In order to further demonstrate the prediction model, the factors affecting silicic acid solubility and the reasons were studied. In terms of studying those factors, an experimental scheme for different temperatures and ion concentrations was constructed by L6(4^3) orthogonal table [37] (Table 2). Then, the silicic acid solubility prediction model was used to predict the silicic acid solubility. Finally, based on the predicted results, the sum of experimental index (\( K_{ij} \)) at Level \( j \) under Factor \( I \), the average value \( \bar{K}_{ij} \) and the range \( R \) were obtained by range analysis [38] to analyze the major factors affecting the silicic acid solubility.
TABLE 1

<table>
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<th>par m</th>
<th>Interacting ion</th>
<th>par m</th>
<th>Interacting ion</th>
<th>par m</th>
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<td>Na⁺</td>
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FIGURE 1

Relative Error between Calculated Results and Experimental Results of Silicic Acid Solubility

TABLE 2

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<th>Mg²⁺, mg/L</th>
<th>Cl⁻, mg/L</th>
<th>SO₄²⁻, mg/L</th>
<th>HCO₃⁻, mg/L</th>
<th>F⁻, mg/L</th>
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</table>

The range analysis was used to analyze the effect of each factor on the silicic acid solubility.
It can be seen from Table 3 that temperature is the main factor affecting the silicic acid solubility, with the range R value of 11.1. Following that are Ca\(^{2+}\) and SO\(_4^{2-}\), with the R value of 7.8 and 6.9, respectively. The laws of and reasons for the effect of temperature, Ca\(^{2+}\) and SO\(_4^{2-}\), on the silicic acid solubility were further studied in Section 3.3 and 3.4.

**Effect of Temperature on Silicic Acid Solubility.** 10 groups of formation water samples with different ion strength (Appendix table 1 No. 1-10 Water Samples) were selected from the lowest to the highest strength for investigating the changes in silicic acid solubility of these water samples at different temperatures, with the calculation results shown in Figure 2.

Figure 2 shows that the silicic acid solubility in all water samples increases with the rising temperature. In the prediction model (Equation 21), the effect of temperature is approximately considered as a linear effect, so the linear changes of solubility with temperature changes in Figure 2 agree with the predicted results.

---

**TABLE 3**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Temp. K</th>
<th>Na(^{+}), mg/L</th>
<th>K(^{+}), mg/L</th>
<th>Ca(^{2+}), mg/L</th>
<th>Mg(^{2+}), mg/L</th>
<th>Cl(^{-}), mg/L</th>
<th>SO(_4^{2-}), mg/L</th>
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<td>38.5</td>
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**FIGURE 2**

Effect of Temperature on Silicic Acid Solubility

![Figure 2](image-url)
The silicic acid in formation water comes from the dissolution of silicate minerals and the electrolysis of their products, for example, the hydrolysis of quartz (SiO$_2$) when reacting with water and the ionization reaction of its product (H$_3$SiO$_4^-$) in Figure 3. To this end, the enthalpy changes of above reactions at different temperatures (283.15K, 298.15K, 323.15K and 353.15K) were calculated by Gaussian09 (B3LYP/6-311G (d, p)// B3LYP/6-311G (d, p)) [41-42]. Through calculation, the enthalpy changes of the hydrolysis reaction of quartz are 0.09105 Hartree, 0.09107 Hartree, 0.09110 Hartree and 0.09113 Hartree, respectively. The enthalpy changes of ionization reaction of H$_3$SiO$_4^-$ are 0.26299 Hartree, 0.26323 Hartree, 0.26332 Hartree and 0.26344 Hartree, respectively. Both are endothermic reactions. Therefore, increasing temperature can facilitate the dissolution and ionization reactions and improve the silicic acid solubility [39-40]. The results are also consistent with the phenomena of the experiment of Rimstidt and Azaroual [32-33].

**Effect of Cations and Anions on Silicic Acid Solubility.** (1) Effect of Cations on Silicic Acid Solubility. To figure out the reason why cations affect the silicic acid solubility, the stability of the reaction products of cations with silicic acid needs to be studied. The more stable the structure of the reaction product is, the more difficult the product is to reversely hydrolyze into silicic acid and cations [43-44], so that the silicic acid concentration can be effectively and stably reduced. H$_3$SiO$_4^-$ and H$_2$SiO$_4^{2-}$ are the common forms of silicic acid, which can interact with Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ in solution. The single point energy of these products was calculated by using the Gaussian09 (B3LYP/6-311G (d, p)//B3LYP/6-311G (d, p)), with the results shown in Figure 4.

**FIGURE 3**
Hydrolysis Reaction of Quartz and Ionization Reaction of Its Product

**FIGURE 4**
Single Point Energy of the Reaction Products of Silicic Acid with Cations

Note: The numbers in red (unit: a.u.) represent the single point energy.
It can be seen from Figure 4 that the single point energy of $H_2SiO_4^-$ and $H_2SiO_4^{2-}$ is similar, and the single point energy of their products generated by reacting with Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ is also similar. Among them, the single point energy of Ca$^{2+}$L $H_2SiO_4^-$ and Ca$^{2+}$L $H_2SiO_4^{2-}$ is the smallest, so the reaction product of Ca$^{2+}$ with silicic acid is more stable, which agrees with the conclusion of orthogonal analysis that Ca$^{2+}$ is the mainly cation among them, the single point energy of orthogonal analysis that Ca$^{2+}$ is the mainly cation more stable, which agrees with the conclusion of the experimental phenomena are consistent with the results of orthogonal analysis and quantum chemistry analysis regarding the effect of cations on the silicic acid solubility.

In order to observe the effect of cations on the silicic acid solubility more directly, 5 groups of water samples with different anion strength (Appendix table 1 No. 11-15 Water Samples) were selected from the lowest to the highest strength. The water samples were used for investigating the effect of different concentrations of cations on the silicic acid solubility with the prediction model. The calculation results shown in Figure 5.

Figure 5 shows that the silicic acid solubility declines gradually with the rising concentration of cations, which is similar to the results of the experiments of Scheiber [26], Azaroual [33] and Chen [45]. Therefore, the prediction model can correctly reflect the effect of cations on the silicic acid solubility.

(2) Effect of $SO_4^{2-}$ on the Silicic Acid Solubility. The effect of $SO_4^{2-}$ on the silicic acid solubility is mainly reflected by its competition with silicic acid to attract the cations in the solution. Take $H_2SiO_4^{2-}$ for example, the potential energy of SO$^{4-}$ and $H_2SiO_4^{2-}$ at different distances from Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ was calculated by Gussian09 (B3LYP/6-311G (d, p)// B3LYP/6-311G (d, p)). The larger the potential energy is, the more unstable the material structure is, i.e., the weaker the ability of SO$^{4-}$/$H_2SiO_4^{2-}$ to associate with cations. The calculation results are shown in Figure 6.
According to the calculation results of potential energy, the potential energy of the reaction products of SO$_4^{2-}$ with Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ is lower as compared with H$_2$SiO$_4^{2-}$, which means that the product structure of SO$_4^{2-}$ is more stable. In addition, when SO$_4^{2-}$ is interacting with cations, the inflection points of potential energy changes are always larger than those of H$_2$SiO$_4^{2-}$, which means that SO$_4^{2-}$ can interact with cations at a longer distance and generate products with stable structure. Therefore, SO$_4^{2-}$ can capture cations over a longer distance and generate products with more stable structure as compared with H$_2$SiO$_4^{2-}$.

10 groups of formation water samples with different cation strength (Appendix table 1 No. 11-20 Water Samples) were selected from the lowest to the highest strength for predicting the effect of SO$_4^{2-}$ content changes on the silicic acid solubility. The calculation results shown in Figure 7.

As revealed by Figure 7, the silicic acid solubility increases gradually with the rising concentration of SO$_4^{2-}$, which is consistent with the analysis results of quantum chemistry theory and with the results of the experiments of Chen [45] and Marshall [46]. Therefore, the prediction model can correctly reflect the effect of SO$_4^{2-}$ on the silicic acid solubility.
CONCLUSIONS

To achieve effective utilization of oilfield wastewater resources (mainly oilfield produced water), oil field often reinject the wastewater back to the formation after wastewater is treated to be qualified. Silicate is a kind of common pollutant of oilfield wastewater. The accurate prediction of silicate solubility is related to the rational formulation of wastewater treatment process. However, the studies on silicate solution mainly focus on silicic acid-monosalt/bisalt solution, and there are few studies on silicate solution mainly focus on silicic acid-monosalt/bisalt solution, and there are few studies on the temperature-varying multi-component silicate solution. Therefore, a silicic acid solubility prediction model applicable to such solution is proposed in this paper based on Pitzer electrolyte solution theory and in consideration of the long-range and short-range Coulomb electrostatic interaction and dispersion interaction between cations and anions. Through experimental analysis, it is found that this model is sufficiently accurate, with an average error less than 4%, and the maximum error less than 8.4%.

For Na\(^+\)-K\(^+\)-Ca\(^{2+}\)-Mg\(^{2+}\)-Cl\(^-\)-SO\(_4^{2-}\)-HCO\(_3^-\)-F\(^-\)-SiO\(_3^{2-}\) solution, the range analysis results indicate that temperature, Ca\(^{2+}\) and SO\(_4^{2-}\) are the main factors affecting the silicic acid solubility, with the range R of 11.1, 7.8 and 6.9, respectively. Quantum chemistry method was used to study the change of enthalpy change, potential energy and single point energy in the silicate reaction. The results reveal that the enthalpy changes of hydrolysis reaction of quartz and ionization reaction of H\(_2\)SiO\(_4^-\) are greater than 0 at the temperatures of 283.15K, 298.15K, 323.15K and 353.15K, which agrees with the prediction model that the temperature rise can facilitate the silicate dissolution. Compared with Na\(^+\), K\(^+\), and Mg\(^{2+}\), the single point energy of the reaction products of H\(_3\)SiO\(_4^-\) and H\(_2\)SiO\(_4^{2-}\) with Ca\(^{2+}\) is the smallest. The potential energy of the reaction of SO\(_4^{2-}\) with Na\(^+\), K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) is less than that of H\(_3\)SiO\(_4^-\) with the cations, and reaches the smallest at a longer distance. These results conform to the range analysis results, which further checks the correctness of the prediction model.

Therefore, the prediction model can be used to predict the silicate solubility in the temperature-varying multi-component wastewater. This is conducive to improving the treatment effect of oilfield wastewater and reducing the impact on the local environment. In addition, this model is extensively applicable, as it can be expanded to predict the solubility of other inorganic substances in wastewater by proper calculation of the coefficients in it.

ACKNOWLEDGEMENTS

The authors would like to thank the referee for useful and helpful comments and suggestions.

NOTATION

- \(K_p\) solubility product constant
- \(A^\circ\) Debye-Huckel constant
- \(Z\) absolute value of ionic charge
- \(m\) molality of salt, mol/kg H\(_2\)O
- \(I\) ionic strength mol/kg H\(_2\)O
- \(T\) absolute temperature, K
- \(B_{ij}, C_{ij}\) binary ion-ion interaction parameter

Greek letters:
- \(\alpha\) activity
- \(\gamma\) activity coefficient
- \(\Phi_{ij}, \lambda_{ij}\) binary ion-ion interaction parameter
\( \psi_{ij}, \psi_{ij} \) ternary ion-ion difference parameter
\( \beta_{ij}^{(0)}, \beta_{ij}^{(1)}, \beta_{ij}^{(2)} \) Pitzer’s binary parameters
\( \phi_{ij}, \phi_{ij}^{(1)}, \phi_{ij}^{(2)} \) Pitzer’s mixing parameters

**Subscripts**

- \( M, C \) cation
- \( X, A, A' \) anion
- \( N \) molecular specie
- \( i, j \) any species

**REFERENCES**


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THE CHARACTERISTICS OF GAS ADSORPTION IN MICROPORES AND ITS INFLUENCE ON COAL AND GAS OUTBURST

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ABSTRACT

In order to study the relationship between the power source of coal and gas outburst and the adsorption in micropores, low temperature nitrogen adsorption experiment was carried out in the laboratory. The nonlinear fitting method was used to analysis the adsorption data to determine the relative pressure, at which the micropore filling phenomenon had been completed. The density of methane under the environmental conditions of 298.15 K and 0.1 MPa was calculated with the equation of SRK, equal to 0.00065g/cm³. In the absence of gas outburst, the pressure generated in gas desorption process is 0.1731–0.2741 MPa in theory, which is greater than the standard atmospheric pressure of 0.1 MPa. By analyzing the characteristics of gas flow in coal pores, the equation for the gas outburst pressure was proposed by us. In addition, the characteristic energy of adsorption and differential adsorption work were calculated based on the micropore filling theory. The characteristic energy of adsorption is 5.08–5.84 kJ/mol, which is greater than the differential adsorption work 3.70–3.91 kJ/mol. For the occurrence of gas outburst, the coal has at least an energy exchange with the external environment of the adsorption characteristic energy.

KEYWORDS:
Coal and gas outburst, adsorption, micropore filling, pressure, characteristic energy of adsorption, differential adsorption work

INTRODUCTION

More than 175 years passed since the first coal and gas outburst in record, which took place in 1843 in France [1]. However, it needs numerous efforts for us to identify this phenomenon to ensure safety of the miners working underground. At present, the research has carried out over the years by scientists [2–6], as well as the experience gathered in coal mines [7–8], make it possible to identify the factors [9–11] and interrelations occurring among them – that are of great importance as far as the researched topic is concerned. A lot of suggestions for the prediction and prevention of coal and gas outburst have been put forward [12–15]. Coal is a kind of porous solid and gas can be easily adsorbed in its pores [16]. The coal and gas outburst is related to the gas adsorption. However, the research on the power source of coal and gas outburst is rarely reported.

The majority of methane in coal seams is adsorbed in pores. To avoid the methane explosion in the laboratory, we used the nitrogen to replace the methane. In this paper, a series of low temperature nitrogen adsorption experiment were conducted and the experimental data were recorded. The nonlinear fitting method was firstly used to deal with the experimental data, aiming to find out the maximum relative pressure corresponding to the micropore filling. Secondly, the methane movement in pores was analyzed and the pressure generated in gas desorption was calculated. Thirdly, the characteristic energy of adsorption and adsorption potential were calculated based on the micropore filling theory.

MATERIALS AND METHODS

Experimental instrument. The adsorption experimental instrument (Figure 1) is ASAP2020M manufactured by Micromeritics in the United States. It is with a variety of models, such as Langmuir, BET, t-plot, HK and DFT. The ASAP2020M can measure a lot of parameters about pores, such as the specific surface area, pore size distribution, diameter of pore and the total volume of pores.

The accuracy of the parameters is as follows:
(1) The specific surface area is 0.0005 m²/g or more;
(2) The pore size is 3.5–5000 Å (nitrogen adsorption);
(3) The resolution of micropore is 0.2 Å;
(4) The minimum volume of pore is 0.0001 cc/g.
Experimental procedures. The experimental procedures of ASAP2020M consist of three major steps, including: sample preparation, sample degassing and sample analysis.

Sample preparation. Five coal samples were collected from Shazijing mine (from Gui Zhou), Yuanzigou mine (from Shaan Xi), Xiangshan mine (from He Nan), Shenjiazhuang mine (from He Bei), and Zude mine (from Yun Nan). The coal was collected by seam drilling method and the collection process was completed within 3 minutes to ensure the accuracy of various gas test indicators of coal. All of them were unaltered. The collected coal was stored in the tank and sent to the laboratory. The coal samples were ground to a particle size of 0.20~0.25 mm by ball milling. Before the experiment, the sample tube is first cleaned and labelled prior to the preparation of samples and dried in an oven at 383.15 K for at least 2 hours to reduce the effect of impurities on the samples. Then, the sample is loaded into the empty sample tube with a paper slot. The powder of coal sample must be prevented from sticking to the tube wall during the loading process, and all samples should be poured into the sample tube as much as possible.

The sample tube containing the coal sample is weighed again, and after that, it is attached to the degassing station port. The heating pack is placed and fixed with a metal clip, and then the sample is degassed.

The sample tube and sample must be dried before experiment and the mass must be weighed more than 3 times and take its average value.

Sample degassing. Degassing is carried out in a unit, which is independent of the analysis unit and allows both samples to be degassed and analyzed simultaneously. The time of degassing was greater than 6 hours at the temperature of 378.15 K to guarantee the water and gas in coal samples were removed completely. During the degassing process of coal, it would not be considered complete until the pressure is less than 1 Pa/min.

Sample analysis. Place the sample tube at the analysis station and place the Dewar containing the specified amount of liquid nitrogen on the instrument. During the analysis, the isothermal insulation sleeve must be placed on the neck of the sample, so that the error of the test result is not caused because the temperature of the sample tube is changed during the analysis. The use of isothermal insulation sleeve greatly improves the accuracy of analysis, and avoids errors due to temperature changes.

RELIABILITY OF EXPERIMENTAL INSTRUMENT

When the experiment is performed for the first time or the instrument is stopped for a period of time or the adsorption curve is abnormal, the instrument is inspected with the standard samples provided by the instrument manufacturer. The adsorption experiment cannot be carried out until the test results meet the standard parameters.

Two sets of experiment on single-walled carbon nanotube xfs05 and multi-walled carbon nanotube xfm28 were conducted for investigating the reliability of instrument. The information about them is listed in Table 1.

<table>
<thead>
<tr>
<th>Number</th>
<th>Sample</th>
<th>Mass of sample after degassing /g</th>
<th>Pressure range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>xfs05</td>
<td>0.1021</td>
<td>7.698×10⁻³ ~ 9.47 ×10⁻³</td>
</tr>
<tr>
<td>2</td>
<td>xfs05</td>
<td>0.0946</td>
<td>7.301×10⁻³ ~ 1.08 ×10⁻²</td>
</tr>
<tr>
<td>3</td>
<td>xfm28</td>
<td>0.4540</td>
<td>0.009 831–0.988 677</td>
</tr>
<tr>
<td>4</td>
<td>xfm28</td>
<td>0.3559</td>
<td>0.000 0249–0.987 701</td>
</tr>
</tbody>
</table>

FIGURE 1
ASAP2020M

The results were shown in Figure 2 and Figure 3, respectively. The adsorption isotherms of two experiments on one kind of carbon nanotube almost coincided. Furthermore, the specific surface area and pore size of nanotubes obtained from the experimental instrument were close to the values given in the specification. It indicated that the reliability of the experimental instrument was credible.
RESULTS AND DISCUSSIONS

Adsorption amount of nitrogen on coal samples. Figure 4 showed the experimental adsorption isotherms. The five determined adsorption isotherms followed the similar changing pattern with increasing significantly at the beginning, gently in the middle, and exponentially at the end. In Figure 4, the adsorption isotherms with the relative pressure less than 0.01 were enlarged, and the experimental data in this part were given in Table 2.

The nonlinear fitting method was adopted to analysis the data in the low relative pressure. Comparing the results of different fitting functions, we found that the function of \( f(x) = ax^b \) (where \( a \) and \( b \) are parameters, \( x \) represents the relative pressure \( p/p_0 \)) could well meet the trend of experimental data. The fitting curves were shown in Figure 5, and the parameters of functions were listed in Table 3.

The slopes of the fitting curves were larger at the beginning, and then decreased gradually what reflected the adsorption rate. The nitrogen molecules can get into micropores rapidly under the adsorption potential. With the molecules accumulated in micropores, the diameters of micropores would become smaller and smaller. When the diameters of micropores were less than that of nitrogen molecule, the nitrogen molecules would be hard to get into the micropores.
The correlation coefficients of fitting functions were over 0.99. The $f(x)$ was a continuous function and its second-order derivative value was greater than zero. With an incremental increasing step of 0.0001 from zero, the coordinates of inflection point of the function were obtained. The error between the $n$th step and the $(n+1)$th step was less than 0.00001. In Table 2, all the coordinates of inflection points were close to the origin point and we knew the inflection point was the demarcation point between the convex function and the concave function, so we concluded that the inflection point was the demarcation point between the micropore filling and multilayer adsorption.

**Gas pressure.** The Dubinin-Radushkevich (D-R) equation is put in linear form for testing purposes (equation (1)).

\[
\ln W = \ln W_0 - D \ln^2 \left( \frac{p_0}{p} \right)
\]

(1)

Where $W_0$ is the total volume of the micropore system, $W$ is the volume of nitrogen filled in micropores at $p/p_0$, $p$ is the equilibrium pressure and $p_0$ is the saturation vapor pressure at the temperature of 298.15 K.

A plot of $\ln W$ against $\ln^2 \left( \frac{p_0}{p} \right)$ yields a straight line of slope $D$ and intercept $\ln W_0$.

The $W_0$ and $W$ are related by the following expression.

\[
\theta = \frac{W}{W_0}
\]

(2)

$\theta$ is a parameter which can describe the degree of micropore filling.

The data before the inflection point was processed with the equation (1), and the parameters were listed in Table 3.

The correlation coefficients were over 0.99, what reflected the micropore filling had occurred in coal samples. The total volume of micropores was 0.1614–0.3029 cm$^3$/g. The fractional coverages of micropore filling got by equation (2) were 0.58–0.98. Among that, the fractional coverage of sample 3 was the highest and that of sample 2 was the smallest, but both of them were over 0.50. It indicated that most of the space of micropores was filled with nitrogen. The difference in fractional coverage of micropore filling had a certain relationship with the pore size distribution. The diameter of nitrogen molecule is 0.304 nm. If the pore size is larger than 0.304 nm, the nitrogen molecules could be adsorbed in it easily. Otherwise, the nitrogen molecules would be hard to get into pores. In addition, it was also related to the number of optimal pore size that was related to the type of adsorbed gas. The greater the number of optimal pores contains in coal, the more nitrogen molecules can be adsorbed.

The relationship between methane and nitrogen maximum sorption capacity was particularly close: on a volume basis, the maximum adsorption capacity of nitrogen is 0.52 times that of methane [17]. Based on this, the adsorption capacity of methane can be obtained.

During the coal mining process, the stress state of the entire coal seam will have a change. Before the mining, the coal samples were stored for more than one year, the stress state of coal samples was relatively uniform. During the coal mining process, changes in the stress state were related to the mining process. The mining process was conducted in the direction of the coal seam. The stress state of the coal samples changes with the mining process. The mining process was divided into several stages, each stage had different stress states. The stress state of the coal samples in each stage was different. The stress state of the coal samples in each stage was different. The stress state of the coal samples in each stage was different. The stress state of the coal samples in each stage was different.
the coal body is mined, the entire coal body is in a state of balanced force, and there is no danger of coal and gas outburst. After the coal body is mined, the state of the coal body is changed from an equilibrium state to a non-equilibrium state, as shown in Figure 6. As the pressure on the mining side decreases, the pore size of the coal will gradually become larger. At this time, the effect of the adsorption potential is reduced, and the methane molecules in the liquid can break free from the binding of the potential field. The process is shown in Figure 7.

The gas desorption rate on average in different pore sizes is shown in Figure 8. The stage of 0–T1 indicates the desorption rate in macropore. Due to the capillary condensation in the macropores, the desorption rate of the gas decreases with time as the desorption increases. In the stage of T1–T2, the methane is desorbed with layer by layer. With the exchange of external energy, the methane molecules adsorbed on the inner surface of the pore are gradually desorbed, and the desorption rate is relatively flat. The stage of T2–T3 shows the desorption rate of methane in the micropores. When the diameter of micropore tends to large, the rate of desorption is increasing, and after sufficient energy exchange with the outside environment, gas desorption can be completed in a short period of time, and in this process, the pressure larger than environment generates.

In order to study the pressure generated by gas desorption in the micropores, the density of methane under the environmental conditions (298.15 K, 0.1 MPa) is needed to be calculated with the equation of SRK.

![FIGURE 6](image)

*FIGURE 6*

Force analysis of coal

![FIGURE 7](image)

*FIGURE 7*

Desorption process in micropore
\[ p = \frac{RT}{V_m - b} - \frac{a(T)}{V_m(V_m + b)} \] (3)

Where:
\[ a = \frac{4.5724 R^2 T_1^2}{p_c}, \quad b = \frac{0.8664 R T_1}{p_c}, \]
\[ \alpha(T) = \left[ 1 + k(1 - T/T_0^{0.5}) \right]^2; \]
\[ k = 0.480 + 1.574 w - 0.176 w^2; \]
\[ T_0 = \frac{T_1}{T_1}; \]
\[ w \text{ is eccentric factor}; \]
\[ T_1 \text{ is contrast temperature, K}; \]
\[ T_c \text{ is critical temperature of single component, K}; \]
\[ p_c \text{ is critical pressure of one-component, MPa}. \]

The SRK equation of state is expressed by the compression factor \( Z \), and the equation is organized as:
\[ Z^3 - Z^2 - (B^2 + B - A)Z - AB = 0 \] (4)

Where
\[ A = \frac{a(T)p_c}{RT}, \quad B = \frac{b p_c}{RT} \]

For a single component, its density can be expressed by
\[ \rho = \frac{p M}{ZRT} \] (5)

\( \rho \) is density of single component, g/cm³;
\( M \) is molar mass, g/mol.

The critical properties of methane are shown in Table 4.

The density of methane at 298.15 K was calculated to be 0.000 65 g/cm³ and the density at 190.56 K was 0.001 01 g/cm³. According to the law of conservation of mass, the mass of methane adsorbed in the pores is equal to the mass of methane after desorption. With the equation of SRK, the density of methane at 190.56 K is 0.001 01 g/cm³, and their masses are 0.001 01*volume.

\[ p = \frac{nRT}{V} = \frac{m}{M} \frac{RT}{V} \] (6)

The pore volume distribution of the five coal samples is shown in Table 5.

### Table 4

<table>
<thead>
<tr>
<th>molecular</th>
<th>( T_c /K )</th>
<th>( p_c/\text{MPa} )</th>
<th>eccentric factor</th>
<th>molar mass /g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₄</td>
<td>190.56</td>
<td>4.599 2</td>
<td>0.011 42</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table 5

<table>
<thead>
<tr>
<th>Pore type</th>
<th>size/nm</th>
<th>Micro pore</th>
<th>Small pore</th>
<th>Medium pore</th>
<th>Macropore</th>
<th>In total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>volume /ml·g⁻¹</td>
<td>proportion %</td>
<td>volume /ml·g⁻¹</td>
<td>proportion %</td>
<td>volume /ml·g⁻¹</td>
<td>proportion %</td>
</tr>
<tr>
<td>sample 1</td>
<td>0.168 9</td>
<td>95.15</td>
<td>0.007 8</td>
<td>0.000 2</td>
<td>0.000 6</td>
<td>0.1775</td>
</tr>
<tr>
<td>sample 2</td>
<td>0.288 4</td>
<td>95.34</td>
<td>0.010 9</td>
<td>0.002 3</td>
<td>0.000 9</td>
<td>0.3025</td>
</tr>
<tr>
<td>sample 3</td>
<td>0.307 9</td>
<td>94.74</td>
<td>0.0141</td>
<td>0.002 7</td>
<td>0.000 3</td>
<td>0.325</td>
</tr>
<tr>
<td>sample 4</td>
<td>0.260 5</td>
<td>95.39</td>
<td>0.010 7</td>
<td>0.0011</td>
<td>0.0008</td>
<td>0.2731</td>
</tr>
<tr>
<td>sample 5</td>
<td>0.232 4</td>
<td>98.98</td>
<td>0.001 4</td>
<td>0.000 6</td>
<td>0.000 4</td>
<td>0.234 8</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Coal</th>
<th>Volume of N₂ /cm³/g</th>
<th>Volume of CH₄ /cm³/g</th>
<th>Mass of CH₄ /g</th>
<th>Gas volume after desorption</th>
<th>Pressure/MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>0.144 8</td>
<td>0.278 5</td>
<td>0.000 281</td>
<td>0.432 7</td>
<td>0.245 3</td>
</tr>
<tr>
<td>sample 2</td>
<td>0.174 0</td>
<td>0.334 6</td>
<td>0.000 338</td>
<td>0.519 9</td>
<td>0.173 1</td>
</tr>
<tr>
<td>sample 3</td>
<td>0.296 1</td>
<td>0.569 4</td>
<td>0.000 575</td>
<td>0.884 8</td>
<td>0.274 1</td>
</tr>
<tr>
<td>sample 4</td>
<td>0.176 2</td>
<td>0.338 8</td>
<td>0.000 342</td>
<td>0.526 4</td>
<td>0.194 0</td>
</tr>
<tr>
<td>sample 5</td>
<td>0.211 6</td>
<td>0.406 9</td>
<td>0.000 411</td>
<td>0.632 3</td>
<td>0.271 2</td>
</tr>
</tbody>
</table>
It is calculated in Table 6 that the pressure generated in gas desorption process can reach to 0.1731~0.2741 MPa, which is greater than the standard atmospheric pressure of 0.1 MPa. As the degree of coal metamorphism increases, the number of micropores in the coal increases, while the number of macropores and mesopores decreases. The pore structure of coal [18] is shown in Figure 9. After desorption of methane in the dead end and interconnected pores, the gas, along the pores, get into the passing hole. If one end of the passing hole is blocked, the desorbed gas will gradually accumulate, making the pressure increase. When the pressure rises to a certain extent, the weak zones in the pore structure of the coal are destroyed, and the plurality of passing holes is mutually penetrated, causing more gas accumulating. The aggregated gas affects the structure of the surrounding coal body by means of penetration or diffusion, and changes the mechanical properties of the coal body to make it loose. When the coal mining face advances to a certain distance from the gas gathering zones, the coal and gas outburst will occur at the condition of gas pressure greater than the frictional resistance of front coal body. The strength of coal and gas outburst is related to the gas pressure and amount. Actually, the magnitude of the pressure during the coal and gas outburst should be calculated by equation (7).

\[
p = \frac{RT \sum V}{\nu M}
\]  

Where \( V \) is the volume of gas accumulation, less than the total pore volume of \( \sum V \). \( N \) is the number of micropores. The value of \( V \) is difficult to obtain. Our follow-up work will be on it.

**Characteristic energy of adsorption.** According to the change of energy in the adsorption process, the micropore filling rate can also be expressed as [19].

\[
\theta = \exp \left( \frac{-A}{E} \right)
\]  

(8)

Where \( A \) is different adsorption work, kJ/mol; \( E \) is characteristic energy of adsorption, kJ/mol; \( n \) is characteristic coefficient, and its range is 1~3. In this paper, \( n \) is equal to 2.

The differential adsorption work \( A \) can be defined according to the loss of Gibbs free energy \( (\Delta G = -RT \ln \theta) \) [20,21]. It can be expressed with the equation (8).

\[
A = RT \ln \left( \frac{P_e}{P} \right) = 2.303RT \ln \left( \frac{P_e}{P} \right)
\]  

(9)

According to the equation (2) and equation (8), we can get equation (10).

\[
W = W_e \exp \left( -\left( \frac{A}{E} \right)^n \right)
\]  

(10)

Equation (10) takes the logarithm on both sides and becomes equation (11).

\[
\log W = \log W_e - \left( \frac{0.434}{E} \right) A^n
\]  

(11)

The characteristic energy of adsorption, \( E \), is calculated from the equation (1), (9) and (11) using the following expression

\[
E = RT \left( \frac{2.303 A^n}{D} \right)^{\frac{1}{n}}
\]  

(12)

The characteristic energy of adsorption, \( E \), is a measure of the mean value of the adsorption potential. The characteristic energies of adsorption of 5 kinds of coal samples are 5.15, 5.22, 5.84, 5.15 and 5.08 kJ/mol, respectively. The characteristic energy of adsorption is greater, resulting that the adsorption amount of coal samples is more.
The fact that the values of $E$ and $A$ are in close correlation. In Table 7, it shows that the ratio $E/A$ is almost constant. The ratios of sample 1, sample 2, sample 4, and sample 5 are nearly 1.35. The ratio of sample 3 is 1.52, over 1.35, what is caused by the value of $D$. According to the equation (12), the value of $E$ is only related to $D$. The value of $D$ we obtained in calculation is greater than its real value, resulting that the value of $E$ is larger than 1.35.

### CONCLUSIONS

For coal, the relative pressure corresponding to the completion of micropore filling is slightly larger than zero. In gas desorption process, the pressure generated in theory is 0.1731–0.2741 MPa, which is greater than the standard atmospheric pressure of 0.1MPa. By analyzing the characteristics of gas flow in coal pores, the equation for the gas outburst pressure is proposed. The strength of gas outburst is positively correlated with the pressure and the amount of methane. Since the coal is relatively soft and there exist weak zones between adjacent pores, when the pressure reaches to a certain extent, the limitation of the weak zone can be broken, and the pores are connected to each other, eventually forming a large passage for releasing gas.

The differential adsorption work of the five coal samples, that is, the adsorption potential is $3.70–3.91 \text{ kJ/mol}$, less than the characteristic energies of adsorption $5.08–5.84 \text{ kJ/mol}$. If the gas adsorbed in the micropores is completely desorbed, at least the characteristic energy of adsorption needs to be given to overcome the binding of the adsorption potential and the resistance loss in the process of molecular motion.

### ACKNOWLEDGEMENTS

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### REFERENCES


### TABLE 7

<table>
<thead>
<tr>
<th>Coal</th>
<th>$E$ kJ/mol</th>
<th>$A$ kJ/mol</th>
<th>$E/A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>5.15</td>
<td>3.81</td>
<td>1.35</td>
</tr>
<tr>
<td>sample 2</td>
<td>5.22</td>
<td>3.86</td>
<td>1.35</td>
</tr>
<tr>
<td>sample 3</td>
<td>5.84</td>
<td>3.83</td>
<td>1.52</td>
</tr>
<tr>
<td>sample 4</td>
<td>5.15</td>
<td>3.91</td>
<td>1.32</td>
</tr>
<tr>
<td>sample 5</td>
<td>5.08</td>
<td>3.70</td>
<td>1.37</td>
</tr>
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IMAGE-BASED DETECTION OF NEEDLE-LIKE PARTICLE AGGLOMERATION WITH A CASE STUDY ON L-GLUTAMIC ACID CRYSTALLIZATION

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ABSTRACT

Particle agglomeration commonly associated with crystallization processes has been increasingly studied for assessing the crystallization quality. An effective image analysis method is proposed for the agglomeration detection of needle-like particles during the crystallization process. The proposed method mainly consists of image pre-processing, primary sieving, re-segmentation, and particle classification. Firstly, the online captured images are efficiently pre-processed to reduce the influence from uneven illumination. Secondly, a primary sieving algorithm is established for discriminating candidate agglomerates, based on a shape feature. Thirdly, a re-segmentation algorithm is developed to extract the potential primary particles from the candidate agglomerates. In the end, a shape identification algorithm is given to recognize pseudo agglomerates from the candidate agglomerates based on two texture features. Thus, the agglomeration degree could be effectively assessed during the crystallization process. Experimental results on monitoring the cooling crystallization of β-form L-glutamic acid well demonstrate the effectiveness of the proposed image analysis method.

KEYWORDS:
Particle agglomeration, crystallization detection, agglomerate identification, image processing, feature analysis

INTRODUCTION

Crystallization is an important operation step for chemical and pharmaceutical industries. In order to monitor crystallization processes, it is necessary to develop advanced techniques (e.g. process analytical technology, PAT) for estimating the process information of crystal growth [1-4]. Recently, the online image analysis method becomes a new and effective tool for the analysis of crystal size distribution (CSD) and morphology. It is likely known that crystallization imaging is able to provide realistic and visual information on particle morphology. Therefore, online imaging systems have been applied into the monitoring of the crystallization process [1]. Accordingly, some image analysis methods in previous works [5-11] have seen effective progress for crystallization monitoring.

It is generally known that several crystal morphologies have been related to difficulties in dissolution rate, precipitation, milling and grinding process etc. [12-15]. In particular, agglomeration is an undesired crystal shape which has a significant effect on the final product quality and downstream processing, so the detection of agglomeration has attracted considerable attention of many researchers. However, only a few publications have reported the related issues about the agglomeration estimation of crystals [16-18], especially by using online automated image analysis instead of human assessment. In the early literate [19], crystalline product properties visually were quantified by image analysis, and the description of agglomerates for crystal images were evaluated with principal component analysis. Lately, Terdenge et al. [20] used image analysis technique and discriminant factorial analysis to determine the agglomeration degree distribution, which was a quantitative characterization for describing the quality of crystalline product. To characterize needle-like crystals, Ochsenbein et al. [21] developed a novel technique based on machine learning method to characterize both the size distribution of the agglomerates and the primary particles. Recently, Heisel et al. [22] proposed a novel online image measurement method combined with artificial neural networks to quantify agglomeration in the crystallization process. However, as a matter of fact, there could be some pseudo agglomerates in the crystallization images [21], due to the location problem of particles in the imaging. These pseudo agglomerates definitely affect image measurement accuracy of CSD and assessment of the agglomeration degree.

Hence to suppress the above mentioned problem, an automatic imaging analysis is introduced to available identify the agglomerate based on an online imaging system. The image preprocessing includes image enhancement and image segmentation. Secondly, the agglomeration identification has three
stages. In the first stage, candidate agglomerated particles are selected by a shape feature. In the second stage, the candidate agglomerated particles are re-segmented further by a line-based re-segmentation method. The last stage is the agglomeration identification by particle classification. Finally, the percentage of agglomeration as the agglomeration degree [23] as a key parameter of the crystallization process evaluation is calculated by the measurement for agglomeration.

The rest of the paper is structured as follows: First of all, the image preprocessing methods are described and discussed. Afterwards, a valid agglomeration recognition method is given for the measurement of agglomerated particles. Experimental results are shown to demonstrate the effectiveness of the propose method for β-form LGA. Finally, some conclusions are drawn.

**IMAGE PREPROCESSING**

For a non-invasive microscopic imaging system, image enhancement is used to deal with poor visibility and low contrast of captured images. Then the multi-scale retinex algorithm [24] is adopted to strengthen the particle regions and facilitate the subsequent segmentation.

Subsequently, the segmentation threshold $t^*$ is computed by using the Otsu thresholding function [25] as:

$$t^* = \underset{t}{\arg \max} \left( \sigma^2(t) \right)$$

where $\sigma^2(t)$ is the variance between two gray level classes with the threshold value of $t \in [1, L]$ and $L$ is the maximum gray level.

Finally, the segmented image is obtained by:

$$q(x, y) = \begin{cases} 
0, & f(x, y) < t^* \\
1, & f(x, y) \geq t^*
\end{cases}$$

After the segmentation, morphological region filling is performed to fill up all the holes inside the segmented image $q(x, y)$, such that the resulting binary image only remains all the particle regions while the image background is removed.

**AGGLOMERATION RECOGNITION**

To detect the agglomeration in captured images, an agglomeration recognition algorithm is proposed to procure accurate recognition including the following three stages based on the needle-like features in the following subsections in Fig.1.

**Primary sieving.** The needle-like particles are generally classified into three shapes: integral particles, misshapen particles and agglomerates in captured images. It is known that the integral crystals are likely convex while misshapen particles are and the candidate agglomerates are both non-convex in the captured images. Convexity index is a good descriptor for distinguishing between unagglomerated particles (integral particles and misshapen particles) and candidate agglomerates. The convexity $\alpha'$ is defined as:

$$\alpha' = \frac{S_p}{S_c}$$

where $S_p$ is the particle area, and $S_c$ is the area of the convex hull.

**Flowchart of the proposed method for agglomeration recognition**

**TABLE 1**

<table>
<thead>
<tr>
<th>Type</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integral particles</td>
<td>$\alpha' &gt; 0.95$</td>
</tr>
<tr>
<td>Misshapen particles</td>
<td>$0.75 &lt; \alpha' \leq 0.95$</td>
</tr>
<tr>
<td>Candidate agglomerates</td>
<td>$\alpha' \leq 0.75$</td>
</tr>
</tbody>
</table>

The candidate agglomerates are sieved preliminarily by the simple linear classification method according to convexity index. The central value $\mu_i$ in the $i$-th class for the training vector $a_i$ of convexity index is calculated by:

$$\mu_i = \arg \min_{\mu} \sum_{j=1,2,3} ||a_j - \mu||^2, \ i = 1,2,3$$

The threshold value $t_k (k=1,2)$ for the following classification is determined by:

$$\begin{align*}
  t_1 &= \frac{\mu_1 + \mu_2}{2} \\
  t_2 &= \frac{\mu_2 + \mu_3}{2}
\end{align*}$$
In this study, about 60 particles are randomly chosen as the training vector $\sigma$, of convexity index to compute the threshold values, and the classification criterion is listed as shown in Table 1. Note that the classification criterion can be improved with experimental particle shapes.

Re-segmentation. Regular needle-like crystals have the characteristics as shown in Fig.2: the long edges of particles are linear; two long sides of a particle are parallel or nearly parallel to each other. In order to further recognize pseudo agglomerates, the candidate agglomerates are re-segmented with the important features in this study. By virtue of the analysis on needle-like particles in Fig.2, it is proposed to use the line features to segment the primary particles in these candidate agglomerates. A valid line-based re-segmentation algorithm is therefore given as illustrated in Fig.3, after using the Canny edge detection.

**FIGURE 2**
Focused and defocused particles

Step 1. A toolbox algorithm with Hough transform [25] is used to detect the lines in the edges of the agglomerates.

Step 2. Collinear lines are determined and connected based on the line parameters (the slope and intercept), if line endpoints are located in the concave corners defined by the corner detection method [26].

Step 3. Cluster the parallel lines based on the determining parameters: the slopes and the distance between the two lines.

Step 4. Close every two clustering parallel lines to make a complete square, which is approximately a particle shape.

**Particle classification.** It is seen as Fig.2, the defocused particle is more blurred than the focused particle, because of different distances from the camera lens focus of the online imaging system. Therefore, for example, if a candidate agglomerate includes both focused and defocused particles, then it is considered as a pseudo agglomerate. The texture features [25] are considered to describe the surface roughness of particles. Compared with the focused particles, the defocused particles have different characteristics even though they may have similar shapes. Herein the texture features based on gray-level co-occurrence matrix (GLCM) [25] are adopted to discriminate different types. Two irrelevant features are determined for texture analysis, i.e. entropy and inverse difference moment.

1. Entropy $f^{Ex}$ is defined as:

$$f^{Ex} = -\sum_{i=0}^{L-1} \sum_{j=0}^{L-1} P(i, j \mid \Delta x, \Delta y) \log_{2} P(i, j \mid \Delta x, \Delta y)$$

2. Inverse difference moment $f^{Id}$ is defined as:

$$f^{Id} = \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} \left[ 1/(1+(i-j)^2) \right] P(i, j \mid \Delta x, \Delta y)$$

where $P(i, j \mid \Delta x, \Delta y)$ is the probability of the gray level pairs $i$ and $j$ in an image,

$$P(i, j \mid \Delta x, \Delta y) = \frac{G(i, j \mid \Delta x, \Delta y)}{N}$$

where the total number $N$ is equal to the sum of the elements of $G(i, j \mid \Delta x, \Delta y)$, $\Delta x$ is the horizontal pixel offset and $\Delta y$ is the vertical pixel offset.

The two-dimensional feature vector $f$ for a particle is defined as:

$$f = [f^{Ex}, f^{Id}]$$  \hspace{1cm} (9)

To guarantee effective classification of texture feature vectors, a simple classification method is adopted. Denote by the training data set $F^* = \{ f^*_p \mid p = 1, 2, ..., m \}$, and the number of particles $m$. Compute the two cluster centroids $\mu^*_c \ (c=1,2)$ with

$$\mu^* = \arg \min_{\mu} \sum_{p} ||f^*_p - \mu||^2$$  \hspace{1cm} (10)
The distance based on the Euclidean distance between \( f_p \) and the cluster centroids \( \mu_i \) is defined as

\[
D_{p,i} = \sqrt{(f_p - \mu_i)^2} \tag{11}
\]

The classification rule is given by:

\[
p \in \begin{cases} 
  \text{class}_1 & \text{if } D_{p,2} \geq D_{p,1} \\
  \text{class}_2 & \text{if } D_{p,2} < D_{p,1} 
\end{cases} \tag{12}
\]

RESULTS AND DISCUSSION

A seeded cooling crystallization experiment for LGA was performed to verify the effectiveness of the proposed image analysis method for detecting needle-like particle agglomeration with the non-invasive imaging system in Ref [26]. Based on the proposed method, the particles are preprocessed and re-segmented into individual particles as shown in Fig.4. Fig.4 (a) represents one of the captured gray images. Fig.4 (b)-(c) show the results of the enhancement and the segmentation. Fig.4 (d) shows the candidate agglomerate recognized by the preliminary sieving with convexity as listed in the figure. Fig.4 (e) shows the line identification result of the candidate agglomerate. Subsequently, Fig.4 (f) shows the candidate agglomerate is segmented by using the proposed re-segmentation algorithm. After the agglomerate was re-segmented, the texture features of the individual particles were calculated respectively. Based on the proposed particle classification, no pseudo agglomerate was identified in the image.

To further show the effectiveness of the proposed method, another image taken from the crystallization process is processed for identifying the pseudo agglomerate as shown in Fig.5 (a). It is seen that an overlapping particle consists of a blurred appearance of defocused particle and a clear appearance of focused particle, and it is re-segmented in Fig.5 (b). Combined with the proposed particle classification, the pseudo agglomerate was automatically identified, which was segmented into two individual particles.

Correspondingly, the number of the agglomerated particles was efficiently counted after the above image processes. Table 2 shows the detection results for the two different cases shown in Fig.4 (a) and Fig.5 (a), respectively, for true agglomerates and pseudo agglomerates. It can be seen that the proposed image measurement method can give precise
assessment on the agglomeration degree [23] in Table 2. The agglomeration degree is evaluated by:

\[ p_d = \frac{N_{dp}}{N_p} \times 100\% \]  \hspace{1cm} (13)

where \( N_{dp} \) is the number of primary particles involved in the agglomerates, and \( N_p \) is the total number of valid particles counted in the image.

![Image 1](image1.png)

**FIGURE 5**
Re-segmented result for a crystal image.
(a) Original image; (b) Re-segmentation result.

<table>
<thead>
<tr>
<th>Image no.</th>
<th>Agglomerate number</th>
<th>Agglomeration degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig.4 (a)</td>
<td>1</td>
<td>67%</td>
</tr>
<tr>
<td>Fig.5 (a)</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

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SPATIO-TEMPORAL VARIATION OF PRECIPITATION AND DROUGHT IN SOUTHWEST GUIZHOU AUTONOMOUS PREFECTURE OF CHINA DURING 1961-2015

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ABSTRACT

Precipitation is one of the most important meteorological variables which can impact the occurrence of drought. Analyze the spatio-temporal variation of precipitation and drought is very important for drought resistance and the agricultural production, while such work are rare reported in Southwest Guizhou Autonomous Prefecture (SGAP). Thus, eight meteorological stations located within the SGAP were selected, and the integrated approach (linear regression analysis, moving average, geostatistical analyst and Mann–Kendall trend detection test) was used to assess the spatio-temporal variation of precipitation and drought. The results revealed that the precipitation in Wangmo (WM) and Xingren (XR) stations showed an increasing trend with values of 8.269 and 20.29 mm/year. While Anlong (AL), Checheng (CH), Pu’an (PA), Qinglong (QL), Xingyi (XY), and Zhenfeng (ZF) stations showed decreasing trends of -2.419, -1.848, -3.681, -3.779, -1.815, and -1.758 mm/year. The spring precipitation in the central and southwest was significantly lower on the spatial scale. The spring drought in SGAP has occurred frequently due to the precipitation decline year by year and the uneven spatial and temporal distribution, which impacted the regional drought and agricultural production. The results of this study will provide theoretical guidance for drought resistance and agricultural production in SGAP of China.

KEYWORDS:
Precipitation, drought, SGAP, linear regression analysis, geostatistical analyst, Mann–Kendall trend detection test

INTRODUCTION

Climate change, one of the most significant issues facing the world [1], the effects of climate change are already perceptible in diverse places and with different intensities all over the world [2, 3]. Socio-economic sectors, from energy [4], to services [5], ecosystems [6] and water resources [7], will face unequivocal increase in vulnerability due to changes in climate [2]. Precipitation, is strongly affected by the climate change, which leading to the uneven distribution of precipitation resources in spatial and temporal, and caused a series of extreme weather events, such as storms, heat waves, floods, and droughts have been abruptly and acutely felt due to climate change [8]. Among these, drought is an extreme meteorological event characterized by below normal precipitation over a period of months to years. While precipitation is one of the most important meteorological variables which can impact the occurrence of drought. Associated with the uneven distribution of precipitation, the occurrence and durations of drought show an increasing tendency, and the impact is becoming more and more severe. In China, the agricultural production in most areas depends on precipitation, especially in the karst areas of southwest China. Rainfed agriculture dominates the food grain and cash crops production chain of southwest China, any abrupt change in climate variables, particularly variation of precipitation serious threats to agricultural and environmental security of southwest China. The amount and distribution of precipitation play crucial roles in the occurrence of drought in the southwest China [9]. In recent years, due to the impact of climate change, the shortage of precipitation resources is severe in southwest China, which caused seasonal drought frequently perennial, and has significantly affected the growth and development of agricultural in China, and it have posed a great threat to food production and human security [10].

Southwest Guizhou Autonomous Prefecture (SGAP) of China is located in the southwest of China, which has grievously suffered from drought, main spring drought in recent decades. SGAP has an agro-based economy and most of the state falls in the seasonal drought area. SGAP is a major produce area of flue-cured tobacco in China, and the economic value of flue-cured tobacco production plays an important role in agricultural production in the whole state [11]. The precipitation is essential to the growth of tobacco, but due to the uneven
distribution of precipitation resources, the precipitation in tobacco growth period is often concentrated on the early and mid-growth period. While different degrees of water supply and demand in SGAP's major tobacco areas, especially in the transition period (spring) of flue-cured tobacco, which caused a huge impact on the growth of flue-cured tobacco and obstructed the normal growth and development of flue-cured tobacco [11]. Moreover, the SGAP characterized by large karst landforms is plagued by massive drought, the soil porosity is large, and water retention capacity is poor, and the drought is often occurred, which seriously affects the normal growth of flue-cured tobacco and results in the unstimulability of the yield and quality of flue-cured tobacco further [12]. It is obvious that the drought has become a major factor restricting the yield and quality of tobacco leaves, and the economic losses caused by droughts far exceed those by other natural disasters. So far, there is basically no systematic analysis of the variation of precipitation and drought in the most of China's major flue-cured tobacco planting areas, the spatio-temporal variation of precipitation and drought was not unequivocal, and this have a great impact on water saving, drought resistance and flue-cured tobacco production. Therefore, it is urgent to analyze the spatio-temporal variation of precipitation and drought in agricultural planting areas, and to provide theoretical guidance for regional flue-cured tobacco production and rational agricultural water management.

Meteorologists, hydrologist and other scientists who researched the spatio-temporal variation of precipitation and drought have developed several methods to quantify the precipitation, and assess the drought. Methods like linear regression analysis and the slope [13, 14], Morlet wavelet analysis [15, 16], Moving average [17, 18], Geostatistical analyst [19, 20], Mann-Kendall (MK) trend detection test [21, 22]. Among the above-mentioned indicators, the linear regression analysis, moving average, and the geostatistical analyst could used to further analyzed the level of increase or decrease of precipitation, could explained the spatial and temporal variation of precipitation and drought. In addition, the Mann-Kendall trend could used to assess the significance of the monotonic trend in precipitation data time series, and it is one of the most effective methods for testing abrupt time series changes which can clarify the start time of the mutation. Therefore, the integrated approach (linear regression analysis, geostatistical analyst and Mann-Kendall trend detection test) was chosen to elucidate the spatio-temporal variation of precipitation and drought.

In this study, the spatio-temporal variation of precipitation and drought was analyzed using an integrated approach (linear regression analysis, moving average, geostatistical analyst and M-K). The aims of the present study is to: (1) Estimate the precipitation, and analyze the changing trends of precipitation seasonally and annually by using five years moving average and linear regression analysis; (2) Explain the spatio-temporal variation of precipitation and drought by using the geostatistical analyst; (3) Analyze the abrupt changes of annual precipitation by using M-K. Finally, the above analysis could elucidate the spatio-temporal variation of precipitation and drought, and provide theoretical guidance for drought resistance and agricultural production in SGAP of China.

**MATERIALS AND METHODS**

**Study area.** Southwest Guizhou Autonomous Prefecture (SGAP) of China is located in the southwest of China (104°35'E-106°32'18′E; 24°38'N-26°11'N). It has eight meteorological stations, including Anlong (AL), Ceheng (CH), Pu'an (PA), Qinglong (QL), Wangmo (WM), Xingren (XR), Xingyi (XY), and Zhenfeng (ZF). The state belongs to the Panjiang river basin of the Pearl River, which is a typical low-latitude and high-altitude mountain area, and most of the altitude is between 1000 and 2000 m. The SGAP is located in the sub-tropical climate zone with the annual mean temperature ranging between 13.8–19.4°C. The average annual sunshine hours is 1589.1 h.

**Data sources.** Fifty five years (1961-2015) daily precipitation data of eight meteorological stations (AL, CH, PA, QL, WM, XR, XY, and ZF) were obtained form Meteorologic Bureau of SGAP, and the partially missing data were downloaded from Climatic Data Center, National Meteorological Information Center, China Meteorological Administration (http://data.cma.cn). The four seasons are defined as spring (March to May), summer (June to August), autumn (September to November) and winter (December to February).

**Analyzing methodology.** Linear regression analysis and moving average. In this research, the 5-year moving average and least-squares fitting method to estimate the precipitation, the linear trend of the data sequence can be calculated following [23, 14], and 5-year moving average can be calculated following [18].

Geostatistical Analyst. In this study, we use the ArcGIS geostatistical analyst (kriging models) to interpolate and predict the precipitation in SGAP. In order to consider the elevation factor the cokriging interpolation and SPSS 17.0 were chosen to check the correlation. The results showed that the precipitation of the stations is significantly related to the elevation, so the precipitation data can be interpolated using the elevation data of the stations. The geostatistical analyst toolbox in the ArcGIS 10.2 soft-
ware was implemented in this research [19, 20].

Mann–Kendall (MK) trend detection test and abrupt change test. The nonparametric Mann–Kendall test [24, 25] has been commonly used to assess the significance of monotonic trend in climatological, meteorological and hydrological data time series [26, 27, 28, 29]. The MK test statistic \( S \) is calculated in the following equations:

\[
S = \sum_{i=1}^{n} \sum_{j=i+1}^{n} \text{sgn}(x_j - x_i)
\]

\( x_i \) is a time-series from \( i = 1, 2, 3 \ldots n-1 \), and \( x_j \) is another time-series from \( j = i + 1, \ldots n \), \( x_j \) is greater than \( x_i \) if the data set record length. Each point \( x_i \) is used as a reference point of \( x_j \), the results are recorded as \( \text{sgn}(\theta) \):

\[
\text{sgn}(\theta) = \begin{cases} 1, & \theta > 0 \\ 0, & \theta = 0 \\ -1, & \theta < 0 \end{cases}
\]

If the data set is identically and independently distributed, then the mean of \( S \) is zero and the variance of \( S \) is as follows:

\[
\text{Var}[S] = \frac{n(n-1)(2n+5) - \sum_i (t-1)(2t+5)}{18}
\]

(3)

where \( n \) is the length of the data set, \( t \) is the extent of any given time and represents the sum over all ties. Then, the statistic test is given as \( Z_c \). For a long time-series, statistical value \( S \) can be transformed into \( Z_c \), the equation is as follows:

\[
Z_c = \begin{cases} \frac{S-1}{\sqrt{\text{Var}(S)}}, & S > 0 \\ 0, & S = 0 \\ \frac{S+1}{\sqrt{\text{Var}(S)}}, & S < 0 \end{cases}
\]

(4)

When \( Z_c \) is \(-1.96 \leq Z_c \leq 1.96\), the null hypothesis \((H_0)\) is accepted, which indicates that there is no obvious trend. The trend is significant at the 95% confidence level if \(|Z| > 1.96 \) and at the 99% confidence level if \(|Z_c| > 2.58\). A positive \( Z_c \) indicates that the sequence has an increasing trend, while a negative \( Z_c \) reflects a declining trend [30].

The calculated standard \( Z \) value is compared with the standard normal distribution table with two-tailed confidence levels (\( \alpha=10\%, \alpha=5\%, \) and \( \alpha=1\% \)). If the calculated \( Z \) is greater than \(|Z| > |Z_{-\alpha}|\), the null hypothesis \((H_0)\) is invalid. Therefore, the trend is statistically significant. In this study, 90% and 95% two-tailed confidence levels were used for the M-K method.

Abrupt change denotes a fast transition from one state to another. It occurs when the climate system is forced to cross a threshold. For time series \( X_n \) ( \( n \) is the length of the data set), the order series \( (S_k) \) is given as follows [24]:

\[
S_k = \sum_{i=1}^{k} r_i, (k = 2, 3, 4, \ldots, n)
\]

(5)

\[
r_i = \begin{cases} +1, & x_i > x_j \\ 0, & x_i \leq x_j \end{cases}, \quad (j = 1, 2, 3, \ldots, i)
\]

(6)

where \( x_i \) and \( x_j \) are the sequential data values, and the statistic \((U_Fk)\) is defined as:

\[
U_Fk = \frac{S_k - E(S_k)}{\sqrt{\text{Var}(S_k)}}, (k = 1, 2, 3, \ldots, n)
\]

(7)

where \( U_Fk = 0 \), \( E(S_k) \) and \( \text{Var}(S_k) \) are the average value and variance of \( S_k \), which can be calculated by the following equations:

\[
E(S_k) = \frac{n(n+1)}{4}
\]

(8)

\[
\text{Var}(S_k) = \frac{n(n-1)(2n+5)}{72}
\]

(9)

then, the \( UB_k \) is calculated by repeating the above process in the order \( X_n, X_{n-1}, \ldots, X_i, X_j \) of the time series which makes \( UB_k = U_Fk \) \((UB_1 = 0, k = 2, n-1, \ldots, 3, 2, 1)\). In this research, the significance level is \( p = 0.05 \).

**RESULTS**

**Temporal variation of historical annual precipitation.** Fig. 1 (a-h) illustrates the annual time series of regionally averaged precipitation variation as well as the linear trend and the 5-year moving averages. From the aspect of interannual variation, the annual precipitation showed an increasing trend at Wangmo (WM) and Xingren (XR) stations, and a decreasing trend at Anlong (AL), Ceheng (CH), Pu'an (PA), Qinglong (QL), Xingyi (XY), and Zhenfeng (ZF) stations (Fig. 1). During 1961-2015, the average precipitation were 1184.06, 1345.67 and 1508.20 mm, with the linear trend of -2.419, -3.681 and -3.779 mm/year (Fig. 1c, d). The average annual precipitation in PA and QL stations, and a decreasing trend at Anlong (AL), Wangmo (WM) and Xingren (XR) stations, and a decreasing trend at Wangmo (WM) and Xingren (XR) stations, and a decreasing trend at Wangmo (WM) and Xingren (XR) stations. During 1961-2015, the average annual precipitation were 1088.47 mm and 980.71 mm, with the linear trend of 8.269 and 20.29 mm/year (Fig. 1e, f). The variations of seasonal and monthly mean
The average precipitation of summer in AL, CH, and PA showed a decreasing trend with average values of 631.44, 651.11, and 732.51 mm, while QL, WM, XR, XY, and ZF showed an increasing trend with 813.89, 573.33, 684.41, 805.32, and 703.63 mm. The decreasing rates of precipitation were -11.59 (AL), -0.40 (CH), and -4.91 (PA) mm/10a, while the increasing rates of precipitation were 0.40 (QL), 58.53 (WM), 10.89 (XR), 5.09 (XY) and 2.24 (ZF) mm/10a. Compared with the spring, the precipitation has increased a lot, which may be the reason that the annual average precipitation in SGAP is abundant, but the seasonal drought often occurs. It is found that the precipitation is relatively high in summer and is relatively evenly distributed compared to spring, with only a small amount of precipitation in the southeast (Fig. 3f, g, h).

FIGURE 1
Variations of annual precipitation as well as the linear trend and the 5-year moving averages.
In autumn, the precipitation in AL, CH, PA, QL, WM, XY, and ZF exhibit a decreasing trend with average values of 229.32, 251.01, 304.18, 314.16, 217.99, 308.54, and 260.42 mm, while XR showed an increasing trend of 251.33 mm. The decreasing rates of precipitation were -8.68 (AL), -4.89 (CH), -25.66 (PA), -27.89 (QL), -1.22 (WM), -19.13 (XY) and -16.46 (ZF) mm/10a, while the increasing precipitation rate was 4.32 (XR) mm/10a. The precipitation distribution in September and October shows a decreasing trend from northwest to southeast, while in November, the precipitation occurs in the northeast and southwest regions and it’s relatively less in the middle region (Fig. 3i, j, k).

The precipitation changes similarly on the spatial scale during winter with gradual decreasing from northwest to southeast (Fig. 3a, b, l). The precipitation decreasing rates were -2.50 (CH), -2.07 (PA), -0.90 (QL) and -1.65 (XR) mm/10a, while the precipitation increasing rates were 0.88 (AL), 1.69 (WM), 1.89 (XY) and 0.57 (ZF) mm/10a, respectively.

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\[ y = -0.7247x + 1788.8 \] (a) AL
\[ y = -1.7076x + 3683.2 \] (b) CH
\[ y = -0.5032x + 1238.3 \] (c) PA
\[ y = -0.9565x + 2196.6 \] (d) QL
\[ y = 0.8489x - 1410.5 \] (e) WM
\[ y = 1.2780x - 2313.7 \] (f) XR
\[ y = -0.9237x + 2080.5 \] (g) XY
\[ y = -0.3929x + 1069.7 \] (h) ZF
(ii) Summer

(a) AL  \( y = -0.8681x + 1955.2 \)

(b) CH  \( y = -0.4889x + 1223 \)

(c) PA  \( y = -2.5656x + 5404.6 \)

(d) QL  \( y = -2.7886x + 5857.9 \)

(e) WM  \( y = -0.1222x + 460.93 \)

(f) XR  \( y = 0.4318x - 607.11 \)

(g) XY  \( y = 0.5094x - 207.37 \)

(h) ZF  \( y = 0.224x + 258.34 \)
Variations of seasonal mean precipitation as well as the linear trends and the 5-year moving averages
(i. Spring, ii. Summer, iii. Autumn, and iv. Winter)
Fig. 4 shows the abrupt changes of annual precipitation in SGAP during 1961-2015. The UF(k) curves in each region exceed the critical value, which indicated that the precipitation has obvious changes in each region with a significant increasing or decreasing trend. Also, the intersections between UF(k) and UB(k) curves can be verified in each region, except in XR station, which indicated that the precipitation has seen a abrupt change in the other seven regions.

The precipitation in AL had a decreasing trend in 1961-1964, 1988-1994 and 2003-2015, and increasing trend during 1965-1988 and 1994-2003 (Fig. 1a, 4a). The overall fluctuations were relatively large, and the increasing or decreasing trend in 1963, 1967-1970, 1981-1983, 1994-2003 and 2011-2015 reached a significant level, exceeding the confidence level of 90% with linear trend of -2.419 mm/year. Meanwhile, there are two intersections of UF(k) and UB(k) curves in 1966 and 2007, and each intersection corresponding year is a turning point that represents precipitation changes. It can also be seen that the precipitation has been on a downward trend before 1965 until the abrupt intersection of UF (k) and UB (k) curves. It was followed by an increasing trend during 1988, while the precipitation turned to a decreasing trend in 2003. It shows that the downward trend has abruptly changed and the trend is more obvious until the UF(k) and UB(k) curves intersected in 2007. The precipitation in CH had an increasing trend in 1961-1988, 1995-1999 and 2001-2003, and decreasing trend in 1989-1994, 2000-2001 and 2003-2015 (Fig. 1b, 4b). The increasing or decreasing trend in 1963-1974, 1979, 1982-1984, 1997 and 2010-2015 reached a significant level, exceeding the confidence level of 90%. Meanwhile, there are two intersections of UF(k) and UB(k) curves in 1963 and 2010, which indicated that there were two obvious

FIGURE 3
Variations of month mean precipitation in SGAP during 1961-2015

The precipitation in PA and QL has a similar change trend with decreasing values during the initial analysis period. Subsequently it has fluctuations with an increasing or decreasing trends in the last measurements period (Fig. 4c, d). The overall trend decreased during 55-years, with a linear precipitation trend of -3.681 (PA) and -3.779 (QL) mm/year (Fig. 1c, d). The increasing or decreasing trends of PA and QL stations reached a significant level ($p<0.05$) only during the 2010-2015 period. For PA station, UF(k) and UB(k) curves had some intersections during the 1986-1988 period, which indicated the obvious abrupt changes occurrence. Similar behavior was determined during 55 years in QL station with a marked emphasis in 1967, 1981-1983, 1991, 1993 and 1997.

The precipitation in WM and XR showed a decreasing trend during the initial period with an increasing trend in the last measurements period (Fig. 4e, f). The overall trend increased during 55 years with linear precipitation trend of 8.269 (WM) and 20.29 (XR) mm/year (Fig. 1e, f). Increasing trend in 2007-2011 reaches a significant level in WM station, exceeding the 99% confidence level. Also, the XR station has an increasing trend during the 2006-2011 period with a significant level greater than 99% (Fig. 4e, f). During 55 years the WM station had four UF(k) and UB(k) curve intersections, which indicated a similar occurrence of obvious abrupt changes during the 1961-2015 period (1978, 1983, 1990 and 1996). However, the UF(k) and UB(k) curves do not have intersections during the 55 years in XR station, which indicates that abrupt changes have not occurred during the 1961-2015 period.

![Figure 4](image-url)  
**FIGURE 4** Abrupt change of annual precipitation by MK in SGAP during 1960-2015
The precipitation in XY station has a decreasing trend during the 1961-1966 and 2005-2015 periods and an increasing trend from 1966 to 2005. The overall decrease trend during 55 years with a linear trend of -1.758 mm/year (Fig. 1g, 4g). The increasing or decreasing trend during 1962-1964, 1971, 1978-1988, 1993-1998 and 2010-2015 reached a significant higher level than the 90% confidence level. During the evaluated period there were two UF(k) and UB(k) curve intersections in the 1967 and 2006 years. The corresponding year to each intersection is a turning point in precipitation change, which indicated that an obvious abrupt change occurred in 1967 with precipitation trend changes from decreasing to increasing, and also in 2006 when precipitation trend changes from increasing to decreasing. The precipitation in ZF station has a decreasing trend during 1961-1964, 1965-1967, 1971-1973, 1974-1976 and 2003-2015, while it has an increasing trend during 1964-1965, 1967-1971, 1973-1974 and 1976-2003. However, the overall decrease trend during the 55 years with a linear trend of -1.758 mm/year (Fig. 1h, 4h). The increasing or decreasing trend of ZF station during 1961-1964, 1978-1988 and 2011-2015 period reached a significant level \((p<0.05)\). The UF(k) and UB(k) curves have four intersections during 55 years, and these intersections indicate obvious abrupt changes during 1961 to 2015 period, as well in 1965, 1968, 2006 and 2009 years.

**DISCUSSION**

The tobacco leaf production has a crucial role in the Chinese agro-economic development with a particular interest in the SGAP as one strategic planting areas. SGAP is affected by drought, and its situation becomes more severe and frequent during the spring and summer seasons, which is the growth period of flue-cured tobacco. Until now, these events have affected the irrigation management and flue-cured tobacco yields severely. Therefore, the integrated approach has been used to expound the spatio-temporal variation of precipitation and drought.

In this study, an annual precipitation decreasing trend was found for SGAP during the 1961 to 2015 period. The result is consistent with the trend revealed by Li et al. [31] for SGAP over the last 30 years. However, the annual precipitation of WM and XR stations showed a significant increasing trend, different to the downward continuous trend in the previous study. This may result from the difference in statistical years. Moreover, the linear tendency rate found in this study ranges from -37.79 to -17.58 mm/10a, while other authors reported -71.06 mm/10a [31]. They also found that there existed multiple time-scale characteristics to annual precipitation in SGAP, and the periodic oscillation of the 30a timescale was obvious, and it showed a linear decrease in time. Wang et al. [32] showed that in nearly 30 years there were fewer rainstorm days in the middle SGAP than in the east and west, the number of heavy rain days had a downward trend.

There are multiple timescales of annual precipitation in SGAP, showing a linear decrease in time, and the spatial distribution is greater in the west and north of the area and lower in the east and south. The result is also consistent with the trend revealed by Li et al. [31]. Moreover, Li et al. [33] found that the reason why SGAP has suffered severe drought disasters, because it has been suffered the excessively hot and low-pressure weather process. Also, the abnormality of the atmospheric circulation has caused severely low precipitation, high temperatures, and abnormally high evaporation. Wang et al. [34] showed that the spatial distribution of short-time strong precipitation shows an “abundant in east and north, shortage in southwest” pattern. However, in this research, the spatial precipitation distribution is more extensive in the west, north, and southwest of the area and smaller in the southeast. In comparison with other authors, these differences could be due to the use of 55 years data because posterior studies only used ten years of data, and it was researched in the early summer. It can also be seen from Fig. 2(i) and Fig. 3(c,d,e) that there is relatively little precipitation in spring, which causes the spring drought. These results are also consistent with the trend revealed by Shi et al. [35]. Jiang et al. [36] pointed out that the precipitation (rainy season) during May-October accounted for 82%-85% of annual precipitation. However, the precipitation from November of last year to April of the next year (dry season) only accounted for 15%-18%, and there were rainless dry periods for six months, namely seasonal water shortages and the precipitation in dry season showed a decreasing trend and drought showed an increasing trend. The spring precipitation in SGAP is mainly caused by the stationary front, but the magnitude of precipitation is small. This behavior is because Siberian cold air is often blocked by the Qinghai-Tibet Plateau when it is southward, and the intensity is weakened. The low special terrain makes the temperature in the state about 3 °C higher than the same latitude area because of the low special terrain, which is also a cause of spring drought [37].

According to Matyasovszky [38], the abrupt change of climate denotes climate transition from one stable state to another, which occurs when the climate system is forced to cross a threshold, triggering a transition to a new state at a rate determined by the climate system. It is found that the UF(k) curves in each region exceed the critical value, the precipitation has obvious changes in each region and has a significant increasing or decreasing trend. Moreover, except XR station, the UF(k)
and UB(k) curves have intersections with abrupt precipitation change in the other regions. The result is consistent with Mo et al. [39], those authors founded that there is an obvious decrease in annual precipitation with a drop rate of 25.97 mm/10a in the region from 1958 to 2010. A descent of the precipitation in the rainy season played a dominant role in the annual precipitation reduction. We also found that the precipitation in SGAP is more common in the north and northeast as a whole, and is significantly lower in the central and south, and is also consistent with the trend revealed by Mo et al. [39]. These authors found that precipitation presented a clear regional spatial difference in the analyzed period, the precipitation reduced from northeast to southwest in the study area. In previous studies, Shi et al. [35] showed that drought and flood events appear a great transformation in the 1960s-1970s period, and spring and autumn tend to be drought, while summer and winter continue stable. Li et al. [31] have found that the annual precipitation in SGAP has many time-scale characteristics, the 30a time-scale periodic oscillation is obvious, the spatial distribution is abundant in the western and northern parts of the state, and shortage in the east and the spatial distribution in this study is abundant in the west and north of the area and less in the east and south.

CONCLUSIONS

The spatial and temporal distribution of precipitation and the drought were assessed by using the integrated approach. The main findings of this study are: (1) The annual precipitation in WM and XR stations showed an increasing trend, with the linear trend of 8.269 and 20.29 mm/year. While AL, CH, PA, QL, XY, and ZF stations have a decreasing trend, with the linear trend of -2.419, 1.848, -3.681, -3.779, -1.815 and -1.758 mm/year. From the variations of seasonal and monthly mean precipitation, spring precipitation in AL, CH, PA, QL, XY, and ZF stations showed a decreasing trend, while in WM and XR stations showed a increasing trend. At the north and northeast precipitations are more common, and the central and southwest precipitations are significantly lower. It could be concluded that the spring drought in SGAP had occurred frequently due to the precipitation decline year by year and the uneven spatial and temporal distribution. (2) The Mann-Kendall trend test indicated that the UF(k) curves in each region exceeded the critical value, and the precipitation had obvious changes in each region. The precipitation had a significant increasing or decreasing trend, and the UF(k) and UB(k) curves of each region had intersections with abrupt changes except XR station. The precipitation in AL, CH, PA, QL, XY, and ZF stations presented a decreasing trend overall. Finally, the above analysis has elucidated the spatio-temporal variation of precipitation and drought, which could provide theoretical guidance for drought resistance and agricultural production in the SGAP of China.

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ABSTRACT

Leaf senescence is an active and well-regulated degeneration process. Recent studies have shown that NAC transcription factors play an important role in the regulation of leaf senescence. ANAC019, ANAC055 and ANAC029 belong to same NAC sub-group (NAC-a) and they indicated as senescence-associated genes. The hormonal regulation of leaf senescence is a complex phenomenon and still not fully elucidated. ABA and me-JA are stress related plant hormones and accelerate leaf senescence. At the same time, these hormones not only stimulate leaf senescence but also make changes in gene expression. This study aimed to confirm to the effects of me-JA and ABA on the expression of ANAC019, ANAC055 and ANAC029 genes during leaf senescence in Arabidopsis thaliana. For this purpose, ABA and me-JA were applied to 34 day-old Arabidopsis leaves. After 6h of treatment, qPCR was performed to investigate relative expression of these genes on harvested leaves. The data showed that the expression level of ANAC019 was increased by ABA treatment but decreased after me-JA treatment. ABA treatment show increased expression of ANAC019, ANAC055 and ANAC029 genes while me-JA has induced effect on expression of ANAC055 gene. These results indicate that ABA is more effective than me-JA on expression of senescence-associated NACs, ANAC019, ANAC055 and ANAC029, in Arabidopsis.

KEYWORDS:
Leaf senescence, ANAC019, ANAC055, ANAC029, ABA, me-JA, gene expression

INTRODUCTION

Leaf senescence is the last phase of leaf development. It is not only controlled by developmental age but also affected by various internal and external environmental signals [1, 2]. During leaf senescence, physiological and biochemical changes are accompanied by changes in gene expression. Researchers reported the changes in the expression of thousands of senescence-related genes and more than 200 of transcription factor (TF) genes [2-5]. Among these TFs, NAC, WRKY and MYB family TFs are very important because they are principal players in regulating transcriptional changes during senescence [2, 6, 7].

NAC proteins are family of plant-specific TFs that have function in relation to plant development and they are involved in the regulation of stress responses [8-11]. The acronym of NAC is derived from three different proteins, namely NAM (for no apical meristem), ATAF1,2 and CUC2 (for cup shaped cotyledon) genes [12]. Molecular studies showed that a great number of genes from NAC family were involved in senescence processes. Stress-associated or senescence-associated NACs, including ANAC019, ANAC055, ANAC092 and ANAC029 genes were in the same cluster [7]. Researchers showed that expression of ANAC019 and ANAC055 was upregulated by age, drought, salt, ABA and JA [13, 14]. Similarly, expression of ANAC029 is upregulated by age, dark, ABA and drought [15, 16]. Promoter sequences of ANAC019 and ANAC055 are very similar and play an important role in senescence [7]. Also, ANAC029 is involved in cell expansion and senescence in Arabidopsis [17]. ANAC019, ANAC055 and ANAC029 have been reported to be positive regulator of leaf senescence [18].

Hormonal regulation of leaf senescence is really important to understand the leaf senescence process. Plant hormones are known to affect the time of leaf senescence, but they also affect plant growth and stress responses [19]. In fact, we know that all classical plant hormones play a role in the regulation of leaf senescence. For example, ethylene, jasmonic acid (JA), abscisic acid (ABA) and salicylic acid (SA) accelerate senescence, whereas, auxin, gibberellic acid (GA) and cytokinin delay it [19].

Abscisic acid (ABA), which is the central regulator of abiotic stress resistance in plants, is formed from three isoprene subunits [20, 21]. As well as its role in the regulation of abiotic stresses, ABA regulates a numerous of plant growth and developmental events including the induction of seed dormancy, seed germination, stomatal closure, regulation of shoot and root growth, fruit ripening,
abscission and leaf senescence [22, 23]. Previous studies related to ABA have been shown that it plays a role in the regulation of developmental leaf senescence. Foliar spraying with ABA promotes leaf senescence [24, 25, 26]. Leaf yellowing is an indicator of leaf senescence and ABA can induce it [27, 28]. ABA can also alter the expression of the specific senescence-associated genes when promoting senescence [21, 29]. Researchers reported that NAP (NAC-like, activated by apetala3/pistillata) was a positive regulator of leaf senescence in rice and Arabidopsis [17, 29-31]. Furthermore, ANAC029/AINAP, an ABA-inducible NAC family transcription factor, plays an important role in leaf senescence [17, 32]. Liang et al. [31] reported that knockdown of OsNAP (a NAC-like gene) could delay leaf senescence while overexpression of OsNAP accelerate it in rice [31]. In Arabidopsis, SNAC-A (including ANAC019 and ANAC055), a subfamily of stress responsive NAC transcription factors, play an important roles in ABA-induced leaf senescence [1]. The above-mentioned studies show us the importance of NAC-type transcription factors in the ABA-mediated leaf senescence.

Jasmonic acid (JA) and methyl jasmonate (me-JA), which is JA derivative, referred to as Jasmonates (JAs). JAs known as important signaling molecules that involved in several plant development processes, such as formation of the reproductive organs, root growth, tuber formation, fruit ripening, senescence, seed germination [33-35]. It is known that me-JA plays an important protective role in defense mechanisms [36]. In 1980, researchers reported me-JA as the senescence-promoting substance in wormwood [37]. So far, many studies have shown that JA induces senescence in various plant species [38-42]. During leaf senescence, JA biosynthesis-related genes were activated in Arabidopsis [39]. Likewise, JA application induced various senescence-associated genes (SAgs) [43, 44]. In Arabidopsis, ANAC019 and ANAC035 closely related NAC family proteins [45, 46]. Expression of ANAC035 and ANAC019 was induced by JA treatment after 2h and 5h, respectively [45]. Researchers showed that ANAC019 and ANAC035 expression was induced by me-JA in wild type Arabidopsis [46].

This study aimed to confirm the effects of me-JA and ABA on expression of ANAC019, ANAC035 and ANAC029 genes during leaf senescence in Arabidopsis thaliana. For this purpose, ABA and me-JA were applied to 34 day-old Arabidopsis leaves. After 6h of treatment, qPCR was performed to investigate relative expression of these genes in harvested leaves. The results of this experiment will assist in understanding the effects of ABA and me-JA on the expression of ANAC019, ANAC035 and ANAC029.

**MATERIALS AND METHODS**

**Plant Material.** Arabidopsis Col-0 seeds were stratified for two nights at 4 °C and then planted in an Arabidopsis soil mix (six parts Levington’s F2: one part dried silica sand: one-part vermiculite). Plants were grown in growth chamber in standardised conditions under a 16/8 h light/dark cycle at 22 °C and 70% relative humidity under 250 μmol.m⁻².s⁻¹ light.

**Hormones treatment.** Plants were sprayed with 100 μM ABA (dissolved in 0.1% ethanol) (Sigma), 100 μM me-JA (dissolved in 0.1% ethanol) (Sigma) and water (0.1% ethanol) as control on 34th day after sowing. After 6h of treatment, 4 rosette leaves were harvested with 5 biological replicates and freeze in liquid Nitrogen.

**qPCR.** Rosette leaves were harvested and frozen in liquid nitrogen. Total RNA was extracted from rosette leaves using TRIZOL reagent (Sigma Aldrich) according to manufacturer’s instructions. Total RNA was purified through an RNeasy column (QIAGEN) and treated with RNase-free DNase (Ambion TURBO DNase). cDNA synthesis was performed on 2 μg RNA as described previously [47]. Each reaction consisted of 7.5 μl Applied Biosystems SYBR Green PCR Mastermix, 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10 μM), 5 μl sterile water and 1 μl template cDNA. Tubulin primers used as an internal control. The primers are given Table 1. The real-time qPCR was carried out using the ABI 9700 real time detection system (Applied Biosystems). The reaction mixtures were incubated at 50°C for 2 min, then heated to 95 °C for 10 min followed by 50 cycles of 15 s at 95°C and 60 s at 60 °C (for ANAC019 primer 55 °C instead of 60 °C). Each reaction was optimised so that the PCR efficiency was between 90% and 110%. The relative expression was calculated from the threshold cycle (Ct value) and was normalised using beta tubulin expression as an internal control.

**Statistical analysis.** All data were presented as mean ± standard error. Each data was compared with the control data separately to understand whether they are significantly different from among other by Student’s t-test (*P < 0.05).

**RESULTS**

Leaf senescence is an important developmental process in the plant life cycle. It can occur developmentally or may be affected by internal and external factors. One of the most important internal factors is hormones and they cause significant changes in gene expression during leaf senescence.
It has been reported that expression of \textit{ANAC019}, \textit{ANAC055} and \textit{ANAC029} increases during stress and leaf senescence. In this study, the effects of me-JA and ABA on expression of \textit{ANAC019}, \textit{ANAC055} and \textit{ANAC029} during leaf senescence in \textit{Arabidopsis thaliana} were investigated. ABA and Me-JA were sprayed to 5-week old plants and rosette leaves were harvested after 6h of treatment. After 6h of treatment, qPCR was performed to investigate relative expression of these genes. On the 34th day, senescence begins at the leaf tips (Fig. 1).

\begin{table}
\centering
\caption{Primer list which are used.}
\begin{tabular}{lll}
\hline
\textbf{Forward} & \textbf{Reverse} \\
\hline
\textit{ANAC019} & At1g52890 TAAATGGATGATGAGGTTC & CGAAGTACCCTGTTGCTGA \\
\textit{ANAC029} & At1g69490 TCTGAGTGAAGCAGAGGTTC & ACAATGAGCCAGCGAACAC \\
\textit{ANAC055} & At3g1550 TGGTACACCGGAGCAAGAT & TCTACTTTGCTGCTCC \\
\textit{Beta-6-Tubulin} & At5g12250 TGGCAAGATGACACAAAG & AGACCTCAGGAGCTATG \\
\hline
\end{tabular}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{After 6h of treatment on 34th day (a) general view of plants, (b) image of harvested leaves.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{ABA treatment induced relative expression of \textit{ANAC019} but me-JA treatment decreased it. 5 week-old plants were treated with 100 \mu M ABA and 100 \mu M me-JA and rosette leaves were collected after 6h for RNA extraction and qPCR. Each value represents mean \pm SE. Student’s t-test *P<0.05 (compared with control).}
\end{figure}

The expression level of \textit{ANAC019} was increased by ABA treatment but decreased after me-JA treatment. It was examined how the expression of \textit{ANAC019} gene was affected by ABA and me-JA applications (Fig. 2). The data show that the expression of the ANAC019 gene was increased 5.6 fold after 6h of ABA treatment compared to control (P<0.05). After me-JA application, the expression of ANAC019 gene was decreased 2.6 fold compared to the control (P<0.05).
ABA and me-JA treatment induced relative expression of ANAC055.
5 week-old plants were treated with 100 µM ABA and 100 µM me-JA and rosette leaves were collected after 6h for RNA extraction and qPCR. Each value represents mean ±SE. Student’s t-test *P<0.05 (compared with control).

Both ABA and me-JA treatment shows increased expression of ANAC055 gene. In order to see the effect of ABA and me-JA application on the expression of ANAC055 gene, the results of PCR analysis are shown in Fig. 3. According to these results, not only ABA application increased relative expression of ANAC055 gene 16.1 fold compared to control (P<0.05), but also me-JA application increased it 1.8 fold compared to control (P<0.05).

ABA has induced effect on expression of ANAC029 gene. It was examined how the expression of ANAC029 gene was affected by ABA and me-JA applications (Fig. 4). The data showed that, expression of ANAC029 was significantly increased by ABA treatment compared to control (15 fold) (P<0.05). In contrast, me-JA treatment decreased 5.4 fold its expression compared to control (P<0.05).

DISCUSSION

Leaf senescence is a complex process controlled by internal and external signals such as age, darkness, plant hormones, environmental stresses and pathogen infection [14, 26]. The physiological and biochemical changes that occur during leaf senescence are followed by changes in the expression of thousands of SAGs [2]. Studies have shown that transcription factors play an important role in the control of SAG expression during leaf senescence. In particular, the NAC transcription factor family is one of the most important TFs that regulate leaf senescence [1]. Molecular studies showed that a great number of genes from NAC family were involved in senescence processes. Stress-associated or senescence-associated NACs, including ANAC019, ANAC055, ANAC092 and ANAC029 genes were in the same cluster [7]. ANAC019,
ANAC055 and ANAC029 have been reported to be positive regulator of leaf senescence [18].

The application of me-JA results in decreased expression of ANAC019 and ANAC029 genes while increasing expression of ANAC055 gene. When the results are examined, it is seen that the application of ABA is more effective than me-JA on expression of these genes in Arabidopsis (Figs. 2, 3, 4). Expression of all three NAC genes is induced by ABA. Pervious work showed that ANAC019 and ANAC055 play roles in ABA-induced senescence [1]. ANAC019 and ANAC055 that are closely related NAC-a subgroup proteins, have a clade of stress-associated NAC proteins, and their gene expression patterns have similar when induced by ABA and different abiotic stress factors [48].

Researchers showed that application of me-JA or JA induced expression of ANAC019 in Arabidopsis [45, 46]. These findings contradict the results that obtained in the present study. When the results are examined, it can be seen that the previous studies are the data obtained after the application to 2 or 3-weeks old plants. Our previous studies with IAA, SA and JA have shown that hormonal applications to Arabidopsis on different days have caused major changes in gene expression [49]. In the another study conducted with GUS and GFP reporter genes, expression of ANAC019 gene was determined by the presence of GFP in which the expression of this gene was increased in senescent leaves and the expression of this gene was in the nucleus of the epidermal cells and in the guard cells of the stomata during dark-induced senescence [50]. The data show that the application of me-JA on day 34th decreases the expression of the ANAC019 gene. When compared with other studies, it is thought that this may be closely related to the day of application. Researchers have shown that me-JA and JA applications increase expression of the ANAC055 gene [45, 46, 51]. The obtained data are in parallel with the previous results. It is known that the ANAC055 product is a putative JA regulatory protein [45]. Hickman et al. [13] showed that gene ontology analysis indicates that ANAC019 and ANAC055 may have opposite roles in the regulation of JA and salicylic acid (SA) signalling. ANAC055 may be required for normal JA signalling, while ANAC019 could enhance SA and repress JA signalling [13].

ANAC029 was firstly identified as a positive regulator of leaf senescence among the senescence-upregulated NAC TF genes [52]. It was found that its expression was upregulated by 3-fold in ANAC017-overexpressing plants and overexpression of ANAC017 leads to early leaf senescence in Arabidopsis [53]. ANAC019, ANAC055 and ANAC029 belong to same NAC subgroup (NAC-a) and they indicated as senescence-associated proteins [48]. Guo and Gan [17] reported that ANAC029 transcript was not detected in the young, green leaves but it was found in the old, senescing leaves. Also, their data showed that the yellow tip had stronger ANAC029 expression than the basal part of leaf [17]. The present data showed that expression of ANAC029 induced by ABA treatment at 34th day after 6h, while application of me-JA decreased it (Fig. 4). Ethylene, ABA, osmotic and salt stress induced gene expression level of ANAC029, while darkness, drought, oxidative stress, JA and SA did not have crucial effects on its gene expression level [17].

CONCLUSIONS

Overall, this study showed that ABA treatment induced expression of all three NAC TF genes, whereas application of me-JA increased only ANAC055 gene expression. These results indicate that ABA is more effective than me-JA on expression of senescence-associated NACs, ANAC019, ANAC055 and ANAC029, in Arabidopsis. When the obtained data are compared with previous studies, the effect on gene expression is confirmed to be related to time of application.

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Nihal Gören-Saglam designed and conducted the analyses and wrote the manuscript. The author read and approved the final manuscript.

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DEVELOPMENT OF A NOVEL SEWAGE MONITORING SYSTEM BASED ON LoRa MULTI-SENSOR FUSION USING FOR IMPROVING ENVIRONMENTAL PROTECTION EFFICIENCY

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ABSTRACT

Water pollution is one of the main factors affecting the natural ecological environment. Industrial, agricultural and domestic sewage lead to the pollution of surrounding groundwater and rivers, causing harm to human health. In order to monitor the discharge of sewage in real time and stably and improve the efficiency of environmental protection, a design scheme of multi-sensor technology sewage monitoring system is proposed. Sensors nodes of the system controlled by STM32L152, LoRa rf module sensors, temperature sensors, PH value, dissolved oxygen sensor, node data were transmitted LoRa communication technology to the gateway, the gateway was sent the data to the monitoring center, the PC displayed the data of the monitoring center, the system adopted multi-point sensing fusion calculation method to obtain the optimum data. The test results showed that the monitoring system is stable and reliable, which provides reference data for environmental protection and governance.

KEYWORDS:
Environmental efficiency, wireless sensor network, multipoint fusion, LoRa, node, the monitoring system

INTRODUCTION

Water pollution is an international concern. Water is a necessary resource for human survival. With the development of modern science and technology, water pollution is becoming more and more serious in urban and rural areas, especially in suburban rural areas. Accurate and rapid monitoring of sewage can help sewage treatment departments to speed up the determination of treatment schemes and improve the progress of sewage treatment, which promotes the efficiency of ecological environment protection. Chemical and physical methods are commonly used for sewage monitoring. Jiang, L., Wu, J. and Brito, D. [1-3] mentioned chemical and physical methods. McGrane, S. [4] mentioned the extensive monitoring of radiation catchments in the Upper Thames Basin in the United Kingdom using on-site water quality instrumentation. Lucero, N. and Leng et al. [5-6] used the model method to monitor the environment of sewage. Jaclyn, M., Abu-Zied, R., and Peschke, K. [7-9] uses biological method to analyze water quality pollution through the influence of sewage on specific organisms. Feng Libo, Ding Yuechang, Jiang Lan, Zong Feng, Yan Hong, Yang Jun and Ke Peipei [10-16] used remote sensing monitoring methods. Analyzing various detection methods, laboratory detection methods need to ensure the collection, transportation, preservation of water samples and laboratory environment. The detection conditions are strict, and the accuracy of data is high. However, the accuracy of the detection data will be affected if each link is not handled properly. On-site portable instrument testing requires people to bring instruments and reagents to the scene to collect which takes time. It is much more convenient than laboratory testing, but there is still a lot of human investment and time delay. The method of model analysis has some limitations. Some domestic researchers have studied the monitoring methods of remote sensor networks. The single-point sensor monitoring method cannot guarantee the accuracy of data very well, and is lower than the accuracy of laboratory detection methods. But it has the real-time and fast data acquisition. After comparing the monitoring methods, the multi-sensor sewage monitoring system is designed and developed to achieve data stability, real-time, fast, and improve the accuracy of single-point sensor monitoring system. It is of great significance to provide fast and stable data for sewage treatment and improve the efficiency of ecological environmental protection.
Based on the problems existing in the source of rural water pollution and the prevention and control of rural water pollution, a monitoring system for real-time monitoring of rural sewage treatment stations in Beijing suburbs using multiple wireless sensor technologies is proposed. By consulting Technical Specification for Surface Water and Sewage Monitoring and considering the situation of rural sewage, the monitoring parameters are selected as follows: temperature, pH and DO. Because the price of sensors abroad is generally high, the sensor of China is selected to collect temperature, pH and dissolved oxygen. The accuracy and real-time performance of the system are achieved by using wireless transmission technology and multi-sensor monitoring data fusion algorithm.

The whole system consists of data acquisition nodes. Each acquisition node is composed of ARM processor, LoRa radio frequency module, temperature sensor, pH sensor, dissolved oxygen sensor and power module. The data collected from the node is transmitted to the gateway through LoRa wireless communication technology.

The gateway transmits the data to the cloud monitoring center. Users log in to the account through PC or mobile phone to query the data. The overall structure of the system is shown in Figure 1.

**System design.** (1) **Hardware Design of Acquisition Node.** The acquisition node is composed of processor, LoRa RF module, sensor and power module. Among them, LoRa RF module needs to connect USART interface and wireless sensor network. The core of LoRa RF module is composed of low power ATM32L152 processor chip and low power SX1278 LoRa RF module. Temperature sensor, humidity sensor and pH value sensor are transformed into digital signal by data processing unit and transmitted to processor. The processor removes abnormal data and calculates the mean of effective value. The processor adaptively weights the data collected by multiple sensors and calculates the minimum error value. The processor can get the minimum error value by using this method and uploads the data to the gateway through the wireless module. A 5V lithium battery power supply is used to supply power to processors, sensors and wireless modules. The hardware block diagram of the acquisition node is shown in Figure 2.

1) **Sensor Unit.** The sensor unit selected domestic products. The measuring range of temperature sensor is $-55\sim 150^\circ C$, and the measuring accuracy is $\pm 0.2^\circ C$. The measurement range of pH sensor is $0\sim 14$, the accuracy is $\pm 0.02$ (pH4~9), and the resolution is 0.01. The measurement range of dissolved
oxygen sensor is 0–20 mg/L, the accuracy is less than ±0.1 mg/L (pH 4–9), and the resolution is 0.01 mg/L. Three kinds of sensors have their own A/D conversion module to convert analog signals into digital signals.

2) Wireless module. LoRa RF module uses SX1278 chip, which is connected with STM32L152 through USART interface. The serial port input terminal of the RXD chip SX1278 is connected with STM32L152 serial port output terminal. M0 and M1 are connected with data I/O ports PB5 and PB6 of STM32L152. Four modes of wireless module [17–18] are determined by setting the values of M0 and M1. AUX is used to indicate the mode of operation. It can change the mode of operation of wireless module by setting its state.

3) Power module. The working voltage of ATM32L152 processor chip is 1.8V~3.6V. Low-power operation mode current is 10.4uA. The working temperature range is -40~+85°C. The working voltage of SX1278 chip is 3.3V~5.2V. The SX1278 chip has 610 mA emission current, 20 mA receiving current, 5uA dormant current, 410 MHz to 441 MHz operating frequency band and operating temperature range of -40~+85°C. ATM32L152 processor chip cannot directly connect to 5V power supply. ADS830-3.3 chip is used to convert +5V voltage to 3.3V voltage. SX1278 wireless module is powered directly by 5V power supply. The working voltage of temperature sensor, PH sensor and dissolved oxygen sensor is 12V, and the +5V voltage is converted to +12V voltage by using PL7512 boost chip.

Gateway is the data exchange station of data acquisition node and monitoring center [17]. The gateway consists of an ATM32L152 processor chip and two low-power SX1278 LoRa RF modules. One of the LoRa RF modules is the receiving module of the acquisition node. The data of the TXD serial output port is transmitted to the ATM32L152 chip through the USART interface module. The ATM32L152 chip is transmitted to the LoRa RF module through the serial output port TXD. The LoRa RF module is uploaded to the gateway through wireless communication technology.

(2) System Software Design. 1) Software Design of Acquisition Node. The software of acquisition node is mainly composed of acquisition data processing program and LoRa wireless module sender program. Data processing of data acquisition node is designed by multi-sensor data fusion method. According to the mode of data fusion, it can be divided into distributed and centralized. Centralization is to integrate all sensor data into one operation, which increases the amount of computation and time. Distributed fusion reduces the pressure of system data processing and is a common method of multi-sensor data fusion [18]. According to the sequence of data fusion process and the classification of fusion level, it can be divided into three levels: data level fusion, feature level fusion and decision level fusion [19]. The processor reads the same sensor data according to the time setting requirement. After eliminating the abnormal data, it collects the set number of data and calculates the mean value to complete the data layer fusion. Then, the data of each sensor are fused at the feature level. The fused values are packaged into data frames and stored in the buffer, waiting for the LoRa module to send out. Nodes transmit data at intervals, and when nodes are idle, they enter a dormant state to reduce power consumption. The monitoring center can fuse the received data at the decision-making level, and judge and alarm the exceeded data.

Sometimes, there are some external or internal accidental factors affecting the acquisition value at the sensor acquisition point. In order to get the data close to the real value and satisfy the operation speed and real-time requirement of embedded microcontroller, we can collect data from the same sensor several times, then eliminate the abnormal data once, and then calculate the average value. That is to say, the Dixon criterion is adopted, which is suitable for the calculation of data in the range of 3-7 times of measurement. The criterion is simple to calculate, and the method of range ratio can be used to obtain more rigorous results [20]. The method is as follows:

Sort the collected n-times data from small to large into X1, X2, X3,… Xn, n is the number of acquisitions. Statistics γ_10 or γ'_10 that can be obtained by the following formula. When n = 3~7, the statistical formula is as follows:

\[
\gamma_{10} = -\frac{\chi^{(n)} - \chi^{(n-1)}}{\chi(n) - \chi(1)} \quad (1)
\]

or

\[
\gamma'_{10} = -\frac{\chi(2) - \chi(1)}{\chi(n) - \chi(1)} \quad (2)
\]

Set the data acquisition times of each sensor to 5 times, and check the critical value table of Dixon test, and then take significant level value \( \alpha = 0.01 \) and Statistical critical value \( D(\alpha, n) = D(0.01, 5) = 0.821 \).

If we can get \( \gamma_{10} > \gamma'_{10} \), then \( X_n \) is an outlier.

If we can get \( \gamma_{10} > \gamma'_{10}, \gamma''_{10} > D(\alpha, n) \), then \( X_1 \) is an outlier.

Data acquisition and processing program uses Dixon criterion to eliminate outliers and then compute the mean value. The average value is saved and the feature layer is fused. Feature level fusion mainly uses adaptive weighting calculation to improve the accuracy of data from sensors with different nodes. The optimal data after fusion, i.e. the estimated value, can be obtained from the adaptive weighted
correlation formula. The formula is as follows.

\[ \hat{x} = \sum_{p=1}^{n} W_p X_p \] (3)

Among them:

\[ \sum_{p=1}^{n} W_p = 1 \] (4)

When the total mean square error is the smallest, the corresponding weighting factor is:

\[ W_p = \frac{1}{\left(\sum_{i=1}^{n} 1/\sigma_i^2\right)^{1/p}}, p = 1, 2, 3, \ldots, n \] (5)

The corresponding minimum mean square error is:

\[ \sigma_{\min}^2 = \frac{1}{\sum_{p=1}^{n} \sigma_p^2} \] (6)

In the formula, \( X_p \) is the acquisition value of sensor, \( W_p \) is the weighting factor of sensor, \( P \) is the number of acquisition data, and \( \sigma^2 \) is the mean square deviation of each node.

2) Design of Data Transmitting Software for Acquisition Node.c. LoRa module has four working modes, general mode 0, wake-up mode 1, power saving mode 2 and sleep mode. The wireless module M0 and M1 are set up by MUC to decide the transmission mode. When the wireless module receives or transmits data, it can work in a set mode. After receiving or sending the data, it can be converted to a dormant mode, which reduces the energy consumption of the load. The working mode of the wireless module of the acquisition node is 0, and it is sent to the wireless receiving module of the concentrator at a fixed point. After power-on, the module initialization process is: AUX output keeps low level, hardware self-checking, and working mode is set as required; After initialization, AUX output high level, and works according to the set working mode. The flow chart of program design is shown in Figure 3.

Set the receiving module and sending module of gateway to work mode 1. Receiving data from different sensor nodes by polling node address. During each polling cycle, the data of each node is read and the next polling is waiting after the transmission. The receiving module of the gateway receives data and stores it in the buffer. The data is transmitted to the cloud server according to the present time, and the data is transmitted to the client through the internet. The data can be monitored in real time by the host computer. The working mode of gateway wireless transceiver module and node wireless module is set to receive data and upload according to time, which reduces the number of LoRa module sending and energy consumption.

RESULTS

Packet Loss Rate Test of Data Transmission. Packet Loss Rate of LoRa Transmission Data and Gateway Receiving Data in the System is analyzed by Sending Analog Data Analysis. Data were sent at intervals of 1 minute, 2 minute and 3 minute for 7 days. The test results are shown in Table 1.

Through the test, data is sent every 1 minute and 7,000 data are sent every 7 days. The received data is 6875, and the packet loss rate is 1.79%. Packet loss rate is 1.43% and 1.15% when sending data in 2 minutes interval and 3 minutes interval. Data loss rate decreases with the increase of sending interval. If the distance between the node module and the gateway increases, or there are other environmental impacts in the transmission, the packet loss rate will increase. Therefore, the LoRa module should be selected as far as possible in the transmission distance and few obstacle avoidance environments. The system can collect data every two hours within 2000 meters. Therefore, the packet loss rate is very small, which can ensure the stable transmission of the system data.

System Acquisition Data Testing. The outlet of a sewage treatment station in the suburbs of Beijing was selected for system test and the data were recorded and analyzed. Five sensors were installed on the diagonal line of the drainage outlet to collect the temperature, PH value and dissolved oxygen value of five points, and compared with the test value of the operation station of the treatment station. The
PH value of the operation station is measured by acidity meter, and the result of PH value is analyzed by glass electrode method. Dissolved oxygen is analyzed by iodometry and water quality thermometer is used for temperature measurement. Considering the influence of other values of temperature, the temperature is generally chosen to be 25°C. When the temperature is about 25°C, the data are collected to obtain the standard detection conditions as far as possible. Select three-week monitoring data, and then select one day of monitoring data from each week to compare the temperature, PH value and dissolved oxygen value of the system and operation station as shown in Table 2, Table 3 and Table 4.

By comparing Tables 2, 3 and 4, it is found that the errors of the three monitoring values are smaller than those of other temperatures in 24°C—26°C. The errors between temperature and PH values monitored by the system and those measured by the operation station are in the range of -1%—+1%, which meets the requirements of automatic monitoring equipment. The errors between the dissolved oxygen data measured by the system and the test data of the operation station are within the range of -2%—+2%. The system works steadily. Because of the different precision of the test instrument and the time deviation of the collected data, the error of the test data is affected to a certain extent.

### TABLE 1
LoRa Module and Gateway Data Transmission Loss Rate Test Results

<table>
<thead>
<tr>
<th>Time</th>
<th>Run time/day</th>
<th>Send interval/min</th>
<th>Send data/piece</th>
<th>Accept data/piece</th>
<th>Acceptance rate/%</th>
<th>Loss rate/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td>7</td>
<td>1</td>
<td>7000</td>
<td>6875</td>
<td>98.21</td>
<td>1.79</td>
</tr>
<tr>
<td>10:00</td>
<td>7</td>
<td>2</td>
<td>7000</td>
<td>6900</td>
<td>98.57</td>
<td>1.43</td>
</tr>
<tr>
<td>12:00</td>
<td>7</td>
<td>3</td>
<td>7000</td>
<td>6920</td>
<td>98.85</td>
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<td>21</td>
<td></td>
<td>21000</td>
<td>20695</td>
<td>98.54</td>
<td>1.46</td>
</tr>
</tbody>
</table>

### TABLE 2
Comparisons between system measurements and operation station Measurements (Week 1)

<table>
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<tr>
<th>Time</th>
<th>8:00</th>
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<th>12:00</th>
<th>14:00</th>
<th>16:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systematic data Temperature/°C</td>
<td>21.5</td>
<td>21.7</td>
<td>22.3</td>
<td>22.9</td>
<td>23.8</td>
</tr>
<tr>
<td>Operating station data temperature/°C</td>
<td>21.0</td>
<td>21.4</td>
<td>22.9</td>
<td>23.0</td>
<td>24.1</td>
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<tr>
<td>Experimental error/%</td>
<td>+0.5</td>
<td>+0.3</td>
<td>-0.6</td>
<td>-0.1</td>
<td>-0.3</td>
</tr>
<tr>
<td>Systematic data PH value</td>
<td>7.41</td>
<td>7.63</td>
<td>7.05</td>
<td>7.03</td>
<td>7.98</td>
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<tr>
<td>Operating station data PH value</td>
<td>7.73</td>
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<td>7.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental error/%</td>
<td>-0.31</td>
<td>-0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systematic data dissolved oxygen (mg/l)</td>
<td>7.6</td>
<td>9.3</td>
<td>7.9</td>
<td>9.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Operating station data dissolved oxygen (mg/l)</td>
<td>7.9</td>
<td></td>
<td>8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental error/%</td>
<td>+1.4</td>
<td></td>
<td>+0.6</td>
<td></td>
<td></td>
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### TABLE 3
Comparisons between system measurements and operation station measurements (Week 2)

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<tr>
<td>Systematic data Temperature/°C</td>
<td>22.5</td>
<td>23.1</td>
<td>24.0</td>
<td>25.1</td>
<td>26.0</td>
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<tr>
<td>Operating station data temperature/°C</td>
<td>22.1</td>
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<td>23.5</td>
<td>24.8</td>
<td>26.5</td>
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<tr>
<td>Experimental error/%</td>
<td>+0.4</td>
<td>-0.5</td>
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<td>-0.5</td>
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<td>7.70</td>
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</tr>
<tr>
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<td>-0.31</td>
<td>-0.15</td>
<td></td>
<td></td>
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<tr>
<td>Systematic data dissolved oxygen (mg/l)</td>
<td>7.9</td>
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<td>7.9</td>
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<td>9.3</td>
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<tr>
<td>Operating station data dissolved oxygen (mg/l)</td>
<td>8.7</td>
<td></td>
<td>8.6</td>
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</tr>
<tr>
<td>Experimental error/%</td>
<td>-1.1</td>
<td></td>
<td>+0.3</td>
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CONCLUSIONS

In order to improve the efficiency of ecological environment protection, China supports the installation of automatic monitoring equipment system to provide real-time data and provide timely and effective reference data for pollution source control. In this paper, a multi-sensor sewage monitoring system is designed to improve the efficiency of environmental protection. The hardware of sensor node is designed. The method and flow chart of LoRa module transceiver software and data fusion are given. The system realizes the real-time monitoring of sewage treatment. It can be used for self-monitoring and self-management of pollution source units, and also can provide reference data for monitoring and management departments. The management department can choose other parameters according to the actual situation for further detection. This not only improves the efficiency of environmental management, but also provides reference for other areas of pollution source monitoring. After testing, the system works stably and meets the monitoring requirements. It has certain reference value. In practical operation, the influence of sensor performance and acquisition conditions on accuracy should be considered.

ACKNOWLEDGEMENTS

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REFERENCES


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<tr>
<td>Temperature/°C</td>
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<tr>
<td>Experimental error/%</td>
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<td>+0.7</td>
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EFFECT OF VERMICOMPOST APPLICATION ON FERTILITY OF CALCAREOUS SOIL AT SUMMER SQUASH (CUCURBITA PEPO) CULTIVATION

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ABSTRACT

The study investigated the effect vermicompost compared with the effect of chicken manure on the number of soil bacteria (heterotrophic aerobic mesophilic) and enzyme activities (urease, alkaline phosphatase, β-glycosidase, dehydrogenase) in the same field cultivated with summer squash for two consecutive years. The study was established as a factorial design according to randomized block design and in the experiment, organic fertilizers which were planned into 6 factors as 2 different fertilizers (vermicompost-VC and chicken manure-CM) and 3 different mineral fertilization doses (control-0, 20 t ha⁻¹ and 40 t ha⁻¹) were applied to 24 plots with 4 replications. The details of the test subjects are as follows: a) VC 0, b) VC 20, c) VC 40, d) CM 0, e) CM 20; and f) CM 40.

According to the results obtained, it was determined that the soil was significantly affected by organic fertilization both in terms of enzyme activities (except urease and dehydrogenase) and the presence of bacteria. It was found that the measured parameters were higher in the first year (except urease) compared to the second year and the differences between the applications in the first year were statistically significant. In this context, it was determined that alkaline phosphatase and β-glycosidase activities dominated by VC 20 and number of bacteria VC 40 for the first year. In addition, the differences between the sampling periods (excluding β-glycosidase in the second sampling period) of the measured parameters over the years were significant. As a result, in the light of the obtained data, it was concluded that the vermicompost is an important organic fertilizer that can be used to maintain fertility of calcareous soil at summer squash cultivation.

KEYWORDS:
High lime, manure, number of bacteria, organic fertilizer, soil enzymes.

INTRODUCTION

Among the cucurbit species in the world vegetable production, especially in summer fields and low-tunnels produced in the cooking squash occupies an important place. While the fruit properties of squashes vary according to the varieties, the color of the fruit is usually dark, medium and light green, and some varieties are yellow-orange in color. While gourd, crete, water and vine gourd are among the summer squashes, light green (gum) and dark green (crete) squashes are produced [1]. Chemical fertilizers are generally used in the fertilization of summer squash which has an important place in the world squash production and therefore has high economic importance. Due to the fact that squash loves organic matter-rich soils and the physical and chemical properties of vermicompost, which is an organic fertilizer, vermicompost is one of the organic materials that can be used in summer squash cultivation [2].

Organic fertilizers used in agricultural production are not only beneficial for the plant to which they are applied, they can provide a better environment for the next plant. They increase the water and nutrient retention capacity and cation exchange capacity of the soil. Again, chemical fertilizers are less important than organic fertilizers because of nitrogen loss by washing is also important in terms of environmental protection [3]. At this point, an organic fertilizer called vermicompost, which is a supporter of sustainable agriculture and food security and whose recognition is increasing day by day, emerges worldwide. Vermicompost can produce significant effects on soil fertility and plant growth through the activity of beneficial microorganisms produced by worms [4]. Many researchers have used vermicompost directly or in combination with chemical fertilizers in their studies [5, 6, 7, 8, 9, 10].

Soils rich in calcium carbonate (containing more than 10%) are generally alkaline reaction soils. And these soils are called calcareous soils. Such soils constitute an important part of the soil in the world and their pH range is between 7-8.5. Especially in soils with a pH above 7.5, chlorosis is common because the plant cannot absorb microelements such as zinc, iron and manganese [11, 12]. Generally, the amount of organic matter in these soils is very small.
It is necessary to increase the organic matter in order to lower the pH and increase the usefulness of the nutrients. The most suitable method for this is to add organic fertilizer to the soil. In addition, adjusting the pH of the fertilizer by using nitric acid is an effective method to reduce the negative impact of calcareous soil in fertilization where irrigation and fertilization are performed together [13].

In order to determine the fertility status of soils based on microbial presence and activity, methods of counting and/or isolating specific groups of microorganisms according to different carbon and energy sources can be used in addition to determining the general microbial presence of the soil. With these methods, important clues about the fertility status of soils can be obtained. In addition, soil enzyme activity analysis is used as an effective indicator in determining soil fertility [14]. Research has shown that there is a reliable relationship between enzyme activities and soil fertility [15, 16]. Therefore, the disintegration of organic materials in the soil, humus formation and nutrient cycles that guide the soil microorganisms that are one of the most important determinants of soil fertility [17]. In this study, the effects of vermicompost application on fertility of calcareous soil at summer squash cultivation were tried to be compared by another organic fertilizer, chicken manure.

**MATERIALS AND METHODS**

**Experiment area, factor and design.** This study was carried out in a farm field located in Akçay town (36° 36’05.14’’K, 29° 44’26.27’’D) at 1150 m of surface in Elmali district of Antalya in Turkey. The climatic characteristics of the experiment area are given in Table 1.

In the experiment, commercially sold vermicompost and chicken manure were used as the source of organic fertilizers. The vermicompost used had a pH of 7.71, an EC of 2900 μS cm⁻¹, organic matter of 47.75% and a C: N ratio of 14: 1; chicken manure had a pH of 6.98, an EC of 3270 μS cm⁻¹, organic matter of 49.81% and a C:N ratio of 13:1 (Table 2). However, some physical and chemical properties of the test soil are given in the table below (Table 3).

The study was established as a factorial design according to randomized block design and in the experiment, fertilizers which were planned into 6 factor as 2 different fertilizers (vermicompost-VC and chicken manure-CM) and 3 different fertilization doses (control-0, 20 t ha⁻¹-20 and 40 t ha⁻¹-40) were applied to 24 plots with 4 replications. The details of the test subjects are as follows: a) VC 0, b) VC 20, c) VC 40, d) CM 0, e) CM 20; and f) CM 40.

**TABLE 1**

<table>
<thead>
<tr>
<th>Months</th>
<th>Precipitation (mm)</th>
<th>Maximum temperature (°C)</th>
<th>Minimum temperature (°C)</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>68.4</td>
<td>25.6</td>
<td>11.9</td>
<td>49.37</td>
</tr>
<tr>
<td>June</td>
<td>57.3</td>
<td>28.1</td>
<td>14.3</td>
<td>48.43</td>
</tr>
<tr>
<td>July</td>
<td>68.4</td>
<td>32.6</td>
<td>14.9</td>
<td>49.37</td>
</tr>
<tr>
<td>August</td>
<td>52.4</td>
<td>38.7</td>
<td>15.6</td>
<td>45.76</td>
</tr>
<tr>
<td>September</td>
<td>96.3</td>
<td>26.2</td>
<td>8.3</td>
<td>62.52</td>
</tr>
<tr>
<td>October</td>
<td>100.4</td>
<td>20.2</td>
<td>5.8</td>
<td>71.28</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Fe (%)</th>
<th>Zn (%)</th>
<th>Mn (%)</th>
<th>Cu (%)</th>
<th>mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermicompost</td>
<td>1.98</td>
<td>2.08</td>
<td>1.3</td>
<td>1.89</td>
<td>0.82</td>
<td>1561</td>
<td>112</td>
<td>459</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Chicken manure</td>
<td>2.19</td>
<td>2.31</td>
<td>4.55</td>
<td>2.81</td>
<td>1.24</td>
<td>1278</td>
<td>175</td>
<td>283</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Texture</th>
<th>Clay</th>
<th>Total N (%)</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.92</td>
<td>Available P (P₂O₅ kg ha⁻¹)</td>
<td>170.23</td>
</tr>
<tr>
<td>EC (dS m⁻¹)</td>
<td>0.652</td>
<td>Changeable K (K₂O kg ha⁻¹)</td>
<td>1860</td>
</tr>
<tr>
<td>Lime (%)</td>
<td>22.8</td>
<td>Changeable Ca (CaO kg ha⁻¹)</td>
<td>20810</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>2.2</td>
<td>Changeable Mg (MgO kg ha⁻¹)</td>
<td>1510</td>
</tr>
</tbody>
</table>
Vermicompost and chicken manure were mixed into plots and allowed to incubate for approximately 2 weeks. On the other hand, Vural et al. [1] reported that the amount of pure nutrients to be given to the soil for squash cultivation is 200 kg ha\(^{-1}\) N, 200 kg ha\(^{-1}\) P\(_2\)O\(_5\) and 200 kg ha\(^{-1}\) K\(_2\)O, respectively. Accordingly, 15.15.15 compound fertilizer was applied to the plots considering the nutrient content of the test soil. Thus, the basic development of the plants in the control plots has been ensured in a way that does not overshadow the effect of vermicompost applications which are the main subject of the experiment.

In the experiment, summer squash (\textit{Cucurbita pepo}) was used as plant material and squash seeds were grown as seedlings before planting in the field. For this purpose, seeds were planted in 2:1 peat:perlite medium and necessary maintenance was done until the seedling stage (3-4 weeks). Squash seedlings which are ready for planting have been planted in 5 m\(^2\) plots with 20 plants (60 x 40cm) in each plot. In the experiment carried out for approximately 6 months (May-November) in one season (1\(^{st}\) and 2\(^{nd}\) year) with seedling and growth period, the seedlings were planted in the soil and irrigation and other cultural processes has been done with care.

**Sample collection and analysis methods.** Within the scope of the study, a soil sample was taken before the experiment was set up and the chemical analyzes of the methods mentioned below were performed. On the other hand, biological analyzes were performed on soil samples, which were in 2 (I), 4 (II) and 6 (III) months of the plant development during the experiment. In order to represent the experiment area at the highest level, soil samples were obtained from all plots at 0-30 cm depth from the locations where the drippers closest to the plant root were located.

Chemical analyzes of soil samples taken before the experiment was established were as follows: texture [18], lime [19], pH and EC [20] (1/2.5 soil water mixture), organic matter [21], total nitrogen (modified Kjeldahl) [22], available phosphorus [23], exchangeable potassium, calcium and magnesium [22]. Soil samples brought to the laboratory for chemical analysis were dried in air and passed through a 2 mm sieve and made ready for analysis.

Biological analyses of soil samples obtained during the experiment are as follows: urease [24], alkaline phosphatase [25], β-glycosidase [26], dehydrogenase [27] enzyme activities and total heterotrophic aerobic mesophilic bacterial count [28]. In addition, soil samples were taken from the soil moisture for biological analysis and were brought to the laboratory immediately and stored at +4°C until analysis.

**Statistical analysis.** The findings obtained from the study were grouped according to LSD test (p<0.05) by SPSS 17.0 package program by using repeated measure analysis (RM) according to the fertilizer and dose interaction effects and time-dependent changes of the subjects and Duncan multiple comparison test [29].

**RESULTS AND DISCUSSION**

**Urease activity.** The effect of organic fertilization on urease activity of the soil at different doses in the experiment area for two consecutive years is shown in Table 4. It is observed that urease activity of the soil is almost 2 times lower in the first year compared to the second year. However, it is seen that urease activity is in a continuous fluctuation trend starting from the first sampling period in both years. On the other hand, the change in urease activity of the soil was not found to be statistically significant in terms of neither fertilizers and doses nor the interaction of these two. However, it was determined that the changes in enzyme activity during sampling periods were statistically significant (p<0.05).

In general, the factors affecting the activity of a soil enzyme are the amount of substrate, as well as the amount of the soil organic matter, temperature, pH and humidity. In addition, the stimulation of microorganisms secreting these enzymes by any external factor (eg organic fertilization) directly affects enzyme activity. Urease is an enzyme secreted by microorganisms outside the cell and is an important enzyme that passes urea nitrogen bound to soil organic matter to the soil solution [31]. It is considered that the amount of organic matter (2.2%) and total N (0.10%) content of the experiment soil is within the limits of sufficiency does not create a noticeable change in urease activity. As a matter of fact, it has been reported that the increase in urease activity by adding organic fertilizer to soil is mainly related with organic matter and nitrogen content of fertilizer and nitrogen in fertilizer acts as a substrate for urease enzyme [32].

**Alkaline phosphatase activity.** It is seen that the effect of applications on the alkaline phosphatase activity of the soil varies from year to year (Table 4). In the first year, enzyme activity was quite high, but was significantly lower from the second year onwards. In addition, a significant jump was observed in the enzyme activity in the second sampling period in the first year. On the other hand, the effect of interactions of fertilizer and dose applications on alkaline phosphatase activity of the soil was found to be statistically significant in both years (p<0.01). In addition, only in the first year organic fertilization was significantly increased enzyme activity (p<0.05, respectively), and it was determined to be VC 20 application. However, the changes in enzyme activity during the sampling periods were found to be statistically significant (p<0.05).
Phosphatases, which are called as acid and alkali due to soil reaction, are enzymes that enable phosphate which is bound to soil organic matter to pass into soil solution. In this context, the phosphorus cycle; It is known as a miraculous event that gives life to high plants, microorganisms and soil animals through a series of enzymatic reactions that occur only in the soil environment. The amount of phosphatases activity in soil solution, while the amount of substrate organic matter content, pH and temperature are in a positive relationship with the soil’s phosphorus content (inorganic phosphorus) shows a negative relationship [26].

In this study, it is difficult to predict that alkaline phosphatase activity of soil is positively affected by organic fertilization. Because the experiment soil mineral phosphorus (170.23 P2O5 kg ha⁻¹) is good in terms of essentially the potential to reduce enzyme activity. However, especially in the first year, the presence of slightly alkaline reaction (pH 7.92) in the soil with a high lime content of the test soil (22.8%), may have made the existing phosphorus fixed. Furthermore, the test plant may have increased alkaline phosphatase activity in inversely proportional to the reduced inorganic phosphorus, since it is a high phosphorus-exploiting plant. However, although the activity of phosphatases varies with soil reaction, organic fertilization increases and the activity of inorganic fertilization is generally inhibited [34, 35, 36, 37, 38]. On the other hand, in relation to the sharp decrease in enzyme activity in the second year, the decrease in the amount of organic matter, especially due to the accumulation of inorganic phosphorus in the soil and the lack of organic fertilization, is directly related.

### β-Glycosidase activity

In this study, one of the measured parameters was β-glycosidase activity. As it can be seen in Table 4, when the changes in enzyme activity are examined in years, it is observed that it is high in the first year and low in the second year. However, in the first year, it was found that the enzyme activity was generally close to each other in all sampling periods, and in the second year, activity

| TABLE 4 |
| The effects of vermicompost and chicken manure applications on soil biological properties |

<table>
<thead>
<tr>
<th>Cultivation season (year)</th>
<th>Contents (g kg⁻¹ dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC</td>
<td>CM</td>
</tr>
<tr>
<td>1st year</td>
<td>2nd year</td>
</tr>
<tr>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>1st</td>
<td>2nd</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (D)</th>
<th>Fertilizer (F)</th>
<th>Time</th>
<th>DxF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>3.647*</td>
<td>3.647*</td>
</tr>
<tr>
<td>NS</td>
<td>3.575*</td>
<td>4.945*</td>
<td>4.945*</td>
</tr>
<tr>
<td>NS</td>
<td>3.278*</td>
<td>3.775*</td>
<td>3.775*</td>
</tr>
<tr>
<td>NS</td>
<td>3.278*</td>
<td>4.514*</td>
<td>4.514*</td>
</tr>
<tr>
<td>NS</td>
<td>8.352*</td>
<td>3.227*</td>
<td>3.227*</td>
</tr>
<tr>
<td>NS</td>
<td>8.352*</td>
<td>4.729*</td>
<td>4.729*</td>
</tr>
<tr>
<td>NS</td>
<td>9.257*</td>
<td>9.257*</td>
<td>9.257*</td>
</tr>
<tr>
<td>NS</td>
<td>4.729*</td>
<td>8.798*</td>
<td>8.798*</td>
</tr>
<tr>
<td>NS</td>
<td>3.532*</td>
<td>3.238*</td>
<td>3.238*</td>
</tr>
</tbody>
</table>

1 The difference between values not shown with the same letter is significant
2 ***; Important at 1% level
3 *; Important at 5% level
4 NS: not significant
increased especially in the third sampling period. It was determined that fertilizer and dose interaction significantly affected soil β-glycosidase activity in both years (p<0.01). In addition, it was determined that vermicompost applications significantly increased enzyme activity (maximum increase in VC 20 application; p<0.05) for the first year and the effects of applications were insignificant in the second year. On the other hand, only the change in β-glycosidase activity between sampling periods in the second year was found to be statistically significant (p<0.01).

β-glycosidase is an enzyme responsible for the decompose of cellulose, a complex carbohydrate compound in the soil. This enzyme plays a very important role in converting cellulose to glucose, providing energy for high plants, microorganisms and soil animals [39]. As with other extracellular enzymes, the activity of this enzyme is directly and/or indirectly affected by many factors such as soil organic matter quantity and quality, types and amounts of organic and inorganic fertilizers given to soil, soil pH, temperature and humidity [40, 41]. In this study, the results obtained in terms of β-glycosidase activity showed similarities with alkaline phosphatase activity. In fact, it is noteworthy that enzyme activity was positively affected by organic fertigation only in the first year and there was no difference in enzyme activity between the applications in the second year. Although the amount of organic matter (2.2%) of the test soil is within the adequacy limit, it may still reveal a high measurement of the enzyme activity for the first year, possibly stimulating the presence of cellulose-degrading microorganisms in the soil with organic fertilization. However, despite the application of organic fertilizer to the soil, the decrease in the diversity of microorganisms due to factors such as nutrient accumulation, precipitation and plant use in the soil may have caused a decrease in enzyme activity at 2nd year. In fact, studies on β-glycosidase activity, microorganism diversity and soil organic matter coincide with the findings obtained in this study [42, 43, 44, 45].

**Dehydrogenase activity.** Time-related changes in soil dehydrogenase activity with organic fertigation are shown in Table 4. Dehydrogenase activity showed fluctuations during the two-year experiment period. Therefore, it showed a significant increase in the second sampling period in the first year and then decreased. In the second year, the enzyme activity has been in a general decreasing trend since the first sampling period. On the other hand, according to the data obtained from the statistical analysis, it was found that the interaction effects of fertilizer and dose applications alone and together did not reveal any significant changes on the dehydrogenase activity of the soil. However, it was concluded that the change in enzyme activity between sampling periods in both years was statistically significant (p<0.05).

The microbiological activity of the soil can be determined by dehydrogenase activity, an intracellular enzyme showing the total amount of oxidative activity of the soil microflora [46, 47]. In addition, by measuring the activity of this enzyme, it is possible to examine the changes caused by environmental pollution (soil, water, etc.) and to reveal the soil fertility status [48, 49, 50, 51]. In this study, it is interesting to note that dehydrogenase activity does not change significantly with fertigation applications. It can be expected that this result would be considered normal when considering the fact that of heavy metals with increasing concentrations in soil solution (Fe, Zn, Mn, Cu) and NO₃⁻ and NO₂⁻ accumulation may also inhibit the enzyme. In the literature, results supporting this approach have been reported [52, 53, 54]. Also, soil-organic material deposition decreasing year by year with the use of plant-microorganisms. On the other hand, the importance of time-dependent changes in enzyme activity suggests that the presence and diversity of microorganisms, which is a reflection of enzyme activity, may be influenced by certain soil properties (particularly temperature and humidity).

**Bacteria Count.** One of the parameters measured in this study is the total number of bacteria which are important indicators of soil fertility. It is seen that the bacteria in the soil tend to increase significantly in the following periods compared to the first sampling period in both years with fertigation applications (Table 4). On the other hand, it was found that the number of soil bacteria was lower in the second year as in enzyme activities (except urease activity). According to the statistical analysis, it was determined that fertilizer and dose interaction had positive and significant effects on the number of bacteria in the soil in both years (p<0.05). In addition, it was found that only the first year applications significantly increased the number of bacteria (maximum increase in VC 40 application; p<0.01, p<0.05, respectively). The changes in the number of bacteria during the sampling periods were found to be statistically significant (p<0.01).

Soil microorganisms play an active role in many chemical and physical changes occurring in the soil. In particular, they are essential elements of soil fertility because they are involved in the cycle of essential nutrients such as nitrogen, phosphorus and carbon, which are essential for plant development [55, 56]. Microorganisms need carbon and energy resources to survive in the soil. The main nutrient and energy source of heterotrophic microorganisms in soil is organic matter. In this study, it is not surprising that the number of bacteria in soil increased significantly with organic fertilization applications for the first year. The majority of soil bacterial communities are heterotrophic; it is thought that the data obtained in this study should be considered as
normal, based on the fact that they develop better in soils with neutral and slightly alkaline reaction, sufficient organic matter and rich in inorganic nutrients [57]. In the second year, there is no difference between the decreases and applications determined; soil temperature varies from the first year, not organic fertilization and some nutrients (phosphorus, zinc, iron, etc.) due to high lime may have been effective. As a matter of fact, the results suggest that organic fertilization in calcareous soils can increase the microbial presence and their activities in the soil [58, 59, 60].

CONCLUSION

In this study, the effects of organic fertilization on soil bacterial presence and enzyme activities of squash grown soil were investigated separately and together with the interaction effects of the applications. According to the results, it was determined that the soil was significantly affected by organic fertilization in terms of both enzyme activities (except urease and dehydrogenase) and bacteria numbers. The remarkable point here is that the mentioned parameters are higher in the first year (except urease) compared to the second year and the differences between the applications in the first year are statistically significant. In terms of statistical significance, it was determined that alkaline phosphatase and β-glycosidase activities were dominated by VC 20 and number of bacteria VC 40 for the first year. Another result is that the differences between the sampling periods (excluding β-glycosidase second sampling period) of all measured parameters are statistically significant over the years.

Under the use of intensive inputs, modern plant cultivation leads to an increase in soil and water pollution, chemical accumulation that may threaten the plants and human health, and decrease in soil fertility, which is one of the most important agricultural production problems. The aim of this study is to determine how vermicompost fertilization affects soil fertility and especially the change of microbial presence and enzyme activity. In the light of the obtained data, it was concluded that the vermicompost is an important organic fertilizer that can be used to maintain fertility of calcareous soil at summer squash cultivation. In addition, studies covering different plants, different climatic zones and soil types will be able to reach large masses in terms of the effects of vermicompost on both soil properties and plant growth.

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THE INFLUENCE OF LOADING RATES ON KAISER EFFECT OF ROCKS WITH DIFFERENT LITHOLOGIES FOR IN-SITU STRESS DETERMINATION DURING OIL AND GAS EXPLORATION

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ABSTRACT

The Kaiser Effect experiment is an efficient method to obtain the in-situ stress in the laboratory, which is of guiding meaning for drilling and fracturing in the petroleum industry. However, different loading rates have an unknown influence on this effect. Therefore, it is inevitable to investigate in its influence of loading rates on Kaiser Effect to obtain accurate in-situ stress. With the combination of MTS816 Rock Testing System and SAMOSTM Acoustic Emission Detection System, the curves demonstrating the relationships between the stresses corresponding to Kaiser Effect of sandstone, gritstone as well as mudstone and acoustic hits under different confining pressures determined by simulated well depths were obtained, which were subsequently utilized to calculate and further correct the in-situ stresses. The results showed that the curves could be subdivided into four stages: micro-fissure compaction stage, acuteness zone, descent zone, and quiet area. As for sandstone, gritstone, and mudstone, the stress of the Kaiser Points increased dramatically with the rise of the loading rate, while regarding brittle rocks such as limestone, loading rates had a mild influence on Kaiser Effect. After corrected by loading rates, the test values got closer to the real values and the relative error declined from 9.028% to 1.954% averagely. In conclusion, when calculating in-situ stress with Kaiser Effect, a reasonable loading rate is necessary. This is because at low loading rates, the large cracks and the microcracks inside the core will be displaced and expanded, resulting in an obvious acoustic emission signal, and thus a smaller stress corresponding to Kaiser Point while at high loading rate, only large cracks will be displaced and expanded, and hence the stress is relatively large.

KEYWORDS:
Kaiser effect, in-situ stress, lithology, loading rate, acoustic emission, oil and gas exploration

INTRODUCTION

In drilling and development of oil and gas fields, the effect of in-situ stress will cause reservoir compaction or expansion, resulting in changes in porosity and permeability. In-situ stress is very important to the design of the injection-production development program for its arrangement of the well pattern [1]. It also plays an important role in hydraulic fracturing design, extension and propagation of hydraulic fractures, and production during mining, water injection-induced earthquakes, formation slip, and creep, and extensive casing damage, etc. In the drilling process of oil and gas wells, in-situ stress is also an important factor for wellbore stability, fracture pressure gradient, the preventive inclination of vertical wells or drilling directional inclined wells, trajectory control of horizontal wells, and casing deformation in the rheological formation and so on. In short, with the mature development and comprehensive application of geology, geophysical prospecting, logging, drilling, oil production, water injection, fracturing, and other technologies, there will be more abundant research methods for the in-situ stress, its research will also be worth a deeper look. More and more people will realize the significance and broad prospects of in-situ stress research for enhancing oil and gas recovery ratio, improving the overall efficiency of oil and gas exploration and development, and boosting the level of oil and gas exploration and development [2-3].

In the oilfield of in-situ stress measurement, there is mainly the measurement of the hole with the stress which contains hydraulic fracturing stress measurements, wellbore caving stress azimuth measurements, casing stress relief, etc. and core analysis methods. The more accurate results can be given by those methods which can describe the characteristics of the stress field accurately, but deep in-situ stress measurement is expensive. Most of the cores of theirs analysis methods come from boreholes and can be measured indoors, such as acoustic emission Kaiser effect method, differential strain analysis method, wave velocity anisotropy method
and so on [2]. Core analysis method does not require a large number of field equipment and people, but through indoor equipment measurement and analysis to obtain in-situ stress. Therefore, researchers have done a lot of research on the measurement of in-situ stress in the laboratory, especially the acoustic emission Kaiser effect method that can not only determine the size of in-situ stress but also assist the analysis of its orientation [4].

In the in-situ stress measurement of by acoustic emission, the Kaiser effect method has been widely applied. For different lithology in the oil field, different artificial loading rates have great influence on the determination of in-situ stress by acoustic emission Kaiser effect, which is not conducive to obtain in-situ stress accurately, thus affecting the site construction design or decision-making. All in all, it’s necessary for our research.

The Kaiser effect of rocks refers to the memory ability of anisotropic solids such as stress and strain [5]. For example, deformation memory effect, strain hardening (deformation rate analysis stress measurement technology), ultrasonic memory effect, charged memory effect and so on. In 1963, Goodman first discovered the Kaiser effect of rocks [6], he considered that rocks had memories of the maximum stress or strain experienced. According to the property of the Kaiser effect, T. Kanagawa and H. Nakasa first used it to measure the crustal stress in 1978 [7]. They measured the results through the acoustic emission test of rocks in different stratum [8-9]. With the intensive study of in-situ stress measured by Kaiser effect, the effect of water saturation and time effect on Kaiser effect, which conclude that the Kaiser effect of some lithologies gradually disappears as time goes by [10-11]. After a large amount of research on the Kaiser effect of rocks, domestic and overseas scholars explored that some other factors influenced the results of the Kaiser effect measurement such as time [12], temperature, lithology [13], confining pressure [14], and the variation of the main stress axis in the multistage loading [15]. In 2016, Valery Toksarov, et al. [16] discovered Kaiser effect expresses itself in different lithology rocks in indoor experiments. In 1997, Y. Li and D. R. Schmite [17] discovered the effect of the coring process on the Kaiser effect. A. Lavrov discovered that the loading rate of brittle rock in multistage loading process influences Kaiser Effect in 2002 [15]. But in practical application, the measurement of crustal stress in the oil field is mainly aimed at rocks with various complex mechanical properties in the formation [18-23]. So far, whether different loading rates will influence the measurement of in-situ stress is still an urgent problem to be solved.

In the experiment of measuring in-situ stress by Kaiser effect in the formation rocks of Jidong Oilfield, we found that loading rate has a significant effect on the in-situ stress measured by Kaiser effect as for different lithology rocks (such as sandstone, mudstone, limestone, etc.). Different lithology with different mechanical properties and existing forms of cracks. If the same rate is used to load, some lithology may not get the true in-situ stress value, which will affect its oilfield application. This paper makes a quantitative study on the effect of loading rate on the Kaiser effect. Besides, the real value of measurement of in-situ stress by Kaiser effect is obtained by evaluating the effect of different loading rates on the Kaiser effect experiments with different lithology. Considering the influence of well depth for Kaiser Effect, we simulate the stratum condition by confining pressure.

**MATERIALS AND METHODS**

**Experimental instruments.** The experimental instruments are made up of the MTS816 rock testing system and the SAMOSTM acoustic emission detection system of the United States (as shown in Figure 1).

---

**FIGURE 1**

The flow chart of in-situ stress measured by Kaiser effect
The MTS816 (Mechanical Testing & Simulation) rock testing system is a full digital computer automatic control system, which can record load, stress, displacement and strain values exactly, and synchronously draw load-displacement, stress-strain curves. The SAMOSTM acoustic emission detection system is the third generation of the fully digital system, which developed by PAC (physical acoustics corporation) in America. The core is to process the PCI-8 (Peripheral Component Interconnect) acoustic emission function card of the PCI bus in parallel. The materials are adopted cylindrical specimens by taking from the cores of different layers in Jidong Oilfield. In order to avoid the influence of anisotropy, the core is taken from the same large one in the vertical direction. The ratio of diameter to axis length is 1: 2, the diameter is generally 25 millimeters, and its parallelism and verticality are all in accordance with the laboratory requirement. A thin section and a directional block are arranged between the two ends and the pads of the specimen, so as to reduce the friction of the faces and ensure the loads uniformly distributed on the axial of the specimen. The pressure at both ends of the specimen and the contact with the acoustic emission sensor are all polished to eliminate the interference signals in the initial loading and make the acoustic emission sensors more effective to receive the AE signals from the internal cracks in the specimen [24-25]. In order to ensure the coupling effect of the sensor and the specimen, the couplant is applied to the two parts of the contact, and then the sensor is fixed on the side of the specimen by the rubber tube, and the oscilloscope is used to display the waveform signal.

Experimental methods. The confining pressure is calculated according to the stratum in the rock samples, and then the stratum is simulated in the laboratory. As for different cores with different lithology in vertical direction, the MTS electro-hydraulic servo control loading system loads with different loading rates of different axial. In the axial loading process, the confining pressure is also increased at the same time. When the confining pressure raises to a predetermined value, then keep it constant with continuing increasing axial loads. The acoustic emission probe is used to detect acoustic emission signals generated during loading of rock samples, and the induction frequency is 40~100 kHz. The acoustic emission signal is sent to the SAMOSTM acoustic emission instrument for recording and processing after magnified, the number and the energy of acoustic emission, and the change curve of the change rate with the load can be given by the instrument.

RESULTS AND DISCUSSION

The relationship between Kaiser Effect of different types of rocks with loading rates. The experimental results of acoustic emission during the whole process were obtained by loading different rates at 0.01, 0.02, 0.04, and 0.05 MPa per second (As shown in Figures 2~4). According to the curve of the cumulative hits of acoustic emission with time, we can get the stress values corresponding to the Kaiser effect of each experiment (As shown in Table 1). According to the Kaiser Effect experiment to obtain the size of in-situ stress, which the formula is as follows:

\[
\sigma_v = \sigma_{\perp} + \alpha P_p \tag{1}
\]

\[
\sigma_h = \frac{\sigma_{90} + \sigma_{0^\circ} + \sigma_{120}}{2} + \alpha P_p \tag{2}
\]

\[
\sigma_b = \frac{\sigma_{90} - \sigma_{0^\circ} - \sigma_{120}}{2} + \alpha P_p \tag{3}
\]

\[
tg 2 \theta = \frac{\sigma_{90} - \sigma_{0^\circ} - \sigma_{45^\circ}}{\sigma_{0^\circ} + \sigma_{90^\circ}} \tag{4}
\]

Where \( \sigma_v \) is the overlying strata stress, \( \sigma_{\perp} \) is the maximum horizontal principal stress, \( \sigma_b \) is the minimum horizontal principal stress, \( P_p \) is the formation pore pressure, \( \alpha \) is the effective stress coefficient, \( \sigma_{\perp} \) is the Kaiser point stress of vertical core, \( \sigma_0 \) is the the Kaiser point stress of horizontal core, \( \sigma_{45^\circ} \) is the Kaiser point stress of 45° horizontal core, \( \sigma_{90^\circ} \) is the Kaiser point stress of 90° horizontal core. \( \theta \) is the angle between 0° the horizontal core and maximum horizontal principal stress. On the basis of Formula (1), \( \sigma_v \) can be determined according to the Kaiser Effect experiment, which compared with logging data to determine overburden stress. The logging data to determine \( \sigma_v \) can be expressed as follows:

\[
\sigma_v = \int \rho g dH \tag{5}
\]

Where \( \rho \) is the formation density from the logging, \( g \) is the gravitational acceleration, \( H \) is the well depth.

As for sandstone, gritstone, and mudstone, the stress of the Kaiser Points increased dramatically with the rise of the loading rate, while regarding brittle rocks such as limestone, loading rates had a mild influence on Kaiser Effect. After corrected by loading rates, the test values got closer to the real values and the relative error declined from 11.85% to 2.61%.
From the Figures 2~4, we can see that the AE can basically be divided into 4 stages under different loading rates [26]: (1) The first stage, the microfracture compaction stage, reflects that the fracture compression closure has existed in the rock specimen after loading. The second is the acuteness area, it enters upon the elastic deformation stage with the loads increasing, and there is neither plastic deformation nor crack propagation.

The original fracture in the specimens was continuously pressed, and acoustic emission activities were few or even absent. Entering the stage of stable expansion and dilatation of cracks with loading continuing, fractures are controlled by applied stress in a stable state. The emergence of acoustic emission is mostly a sudden style with an increasing tendency. The main reason is that a large number of microcracks begin nucleating, nucleation, then confluence, and the stable propagation of microcracks at last. The last stage is the calmness area, it enters the stage of unsteady propagation to the failures with loading continuing. The fracture propagation in the direction of oblique or parallel loading is fast, then gets into the unstable development stage, however, the acoustic emission activity increases sharply. The sliding surface was formed by fracture propagation and connection, as a result, the rock specimen was broken completely. The mechanical nature of the Kaiser effect reflects the reactivity and extension of a specific micro crack formed by the stress action of rocks under the load of the original stress. Microcracks emitted stronger acoustic signals when it was generated and expanded under external loads. According to these signals, we can judge whether microcracks are generated or expanded [27].

It can be seen from Table 1 that the stress values corresponding to the same cores are different under different loading rates. For different loading rates, the measured Elastic modulus values in mudstone vary greatly. In this paper, the real values of in-situ stress obtained from the comprehensive evaluation of the density log calculation and the hydraulic fracturing measurement are compared with the measured ones, and we can also get the stress deviations at the same time.

### TABLE 1

<table>
<thead>
<tr>
<th>The number of wells</th>
<th>The depth (m)</th>
<th>Lithologic</th>
<th>The true value of stress $^*$ (MPa)</th>
<th>Loading rate (MPa/s)</th>
<th>Corresponding stress (MPa)</th>
<th>Elastic modulus (MPa)</th>
<th>Deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G60-23</td>
<td>3806</td>
<td>glutenite</td>
<td>82.35</td>
<td>0.02</td>
<td>72.59</td>
<td>14402</td>
<td>11.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>80.2</td>
<td>14497</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>84.91</td>
<td>15012</td>
<td>-3.11</td>
</tr>
<tr>
<td>Np1-4</td>
<td>3792</td>
<td>Limestone</td>
<td>80.52</td>
<td>0.04</td>
<td>80.91</td>
<td>12560</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>80.99</td>
<td>12596</td>
<td>0.58</td>
</tr>
<tr>
<td>I90-4</td>
<td>2962</td>
<td>Medium sandstone</td>
<td>63.02</td>
<td>0.02</td>
<td>60.92</td>
<td>35264</td>
<td>-3.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(containing mud)</td>
<td></td>
<td>0.04</td>
<td>68.48</td>
<td>42314</td>
<td>8.66</td>
</tr>
<tr>
<td>G32-30</td>
<td>3515</td>
<td>Fine sand-mudstone</td>
<td>75.21</td>
<td>0.04</td>
<td>70.12</td>
<td>17563</td>
<td>-6.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>76.25</td>
<td>19658</td>
<td>1.38</td>
</tr>
<tr>
<td>G60-23</td>
<td>3883</td>
<td>Sand-mudstone</td>
<td>84.12</td>
<td>0.04</td>
<td>78.02</td>
<td>14436</td>
<td>-7.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>86.93</td>
<td>15329</td>
<td>3.34</td>
</tr>
<tr>
<td>M29-1</td>
<td>2479</td>
<td>Gritstone</td>
<td>49.96</td>
<td>0.01</td>
<td>49.56</td>
<td>8956</td>
<td>-0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>55.26</td>
<td>9562</td>
<td>10.61</td>
</tr>
</tbody>
</table>

Note: The true value of stress was gotten from the comparison of hydraulic fracturing and logging data.

### FIGURE 2

The relationship between the stresses corresponding to Acoustic emission Hits of the glutenite under different loading rates
The acoustic emission cumulative curves of grit stone at 2 different loading rates. The Kaiser Effect is a measure of the elastic modulus and is observed when the stress-strain curve becomes linear. Figure 3 shows the Kaiser Effect for different loading rates, with the slope of the curve corresponding to loading rates of 0.02 MPa per second, 0.05 MPa per second, and 0.04 MPa per second. The Kaiser Effect increases with the loading rate, indicating that loading rate has great influence on the Kaiser effect of glutenite rocks.

Figure 2 is an acoustic emission cumulative curve of gravel rock at 3 different loading rates. According to Table 1, the corresponding stress value of the Kaiser point increases with the loading rate. The slope of the curve corresponding to 0.02 MPa per second and 0.04 MPa per second change greatly, which indicates that loading rate has great influence on the Kaiser effect of glutenite rocks.

Figure 3 is an acoustic emission cumulative curve of grit stone at 2 different loading rates. The specimen is taken from the formation core of more than 2000 meters in the Jidong Oilfield. Due to the low strength of the gritstone, a small loading rate was carried out in experiments. Under different loading rates, the slope of the curve changes greatly, and the corresponding in-situ stress value increases with the loading rate. With the loading rate increasing, rocks occurred destruction in a short time.

The acoustic emission cumulative curves of limestone at 2 different loading rates are presented in Figure 4. For the limestone, the slope of the 2 curves is different, but the corresponding stress values are basically identical, which shows that the loading rate has little influence on the Kaiser effect of brittle rocks.

As for sand and mudstone, the Kaiser values obtained by different loading rates are quite different. This is because the larger and smaller cracks will be dislocated and closed when the loading rate is slower, resulting in smaller Kaiser Effect values. When loading rate is faster, only large cracks will extend to cause dislocation and closure, which produce a larger stress value of the Kaiser effect. The above views are quantitatively analyzed by using the measurement of the Elastic modulus. The Elastic modulus of the core is measured at different loading rates by taking the same core as above experiment, and the data are shown in Table 1. With the loading rate increasing, the Elastic modulus of rock increases as well, besides, and the Elastic modulus of the core
The correlation between calculation results of Kaiser effect and hydrofracturing

<table>
<thead>
<tr>
<th>The number of wells</th>
<th>The depth of wells (m)</th>
<th>The maximum horizontal in-situ stress (g/cm³)</th>
<th>The minimum horizontal in-situ stress (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kaiser effect method</td>
<td>Hydraulic fracturing method</td>
</tr>
<tr>
<td>G19–10</td>
<td>2 457</td>
<td>1.901</td>
<td>1.967</td>
</tr>
<tr>
<td>L19X1</td>
<td>3 214</td>
<td>2.120</td>
<td>2.165</td>
</tr>
<tr>
<td>MRX1</td>
<td>2 963</td>
<td>2.190</td>
<td>2.200</td>
</tr>
<tr>
<td>NP1</td>
<td>2 654</td>
<td>1.910</td>
<td>1.900</td>
</tr>
</tbody>
</table>

Note: The unit of stress equivalent mud density: g/cm³.

through data comparison, we can see that the in-situ stress obtained by hydraulic fracturing method is basically consistent with that of Kaiser effect experiment, which demonstrates that different loading rates will affect the measured values in the Kaiser effect measurement under loading confining pressure, so the reasonable rate must be evaluated accurately to get the true value.

CONCLUSIONS

1. Different loading rates have little effect on the Kaiser effect of brittle rocks such as limestone. For rocks such as sandstone and mudstone, the stress value of Kaiser effect points increases with loading rate. The appropriate loading rate should be selected for the Kaiser effect experiments. Because of the special mechanical properties of the plastic rocks, such as salt rock, clay, etc., the effect of their Kaiser effect is more obvious. Thus, the effect of loading rate of the plastic rocks on the Kaiser effect should be further studied.

2. When loading rate is smaller, the smaller cracks will also occur dislocation and expansion, and the stress value corresponding to the Kaiser effect point is smaller. However, when the loading rate is larger, only the larger cracks will be dislocated and expanded, the stress value is relatively large, and the increasing range is 5% to 15%.

3. The loading rate also has a certain effect on the Elastic modulus and other rock mechanics parameters. Therefore, it is suggested that concrete analysis should be carried out to get more accurate test results in other experiments.

4. In the practical application, for the effect of loading rate on the Kaiser effect, because the loading rate of the laboratory is far higher than the loading rate of the historical activities in the actual formation, the stress values obtained by the Kaiser effect in the laboratory are different from the actual conditions, and the loading rate has an important influence on the Kaiser effect.

with lower compressive strength is smaller, which is basically consistent with the effect of loading rate on the stress value.

**Verification with hydraulic fracturing method.** From the above experiments, we can see that the stress value measured by the Kaiser effect of limestone is not related to different loading rates. But as for glutenite rock, sand-mudstone and fine sand-mudstone, as the rock itself is more compact and when the depth is over 2500 m, the rate of 0.04 MPa per second is relatively appropriate by comparing the data in Table 1, that is to say, the stress value obtained by loading at a larger rate is more accurate. However, for these loose rocks, such as grit stone and medium sandstone, their strength is relatively small. In the process of loading, if the speed is too fast, the deviation of the Kaiser effect point value is relatively large (about 12% deviation), so a smaller rate should be selected for loading.

The experimental conclusion is applied to the in-situ stress measurement of many wells in Jidong Oilfield. The stress measurement of four wells is taken as an example. According to the convention of the petroleum industry, the main in-situ stress is generally expressed by equivalent mud density, and the unit is Grams per cubic centimeter. The known parameters and measurement results are shown in Table 2.

In order to verify the accuracy of the results, we compared them with the results of micro hydraulic fracturing oilfield experiments which are shown in Table 2. The in-situ stress obtained from hydraulic fracturing can be expressed as follows:

\[ S_t = P_f - P_r \]  
\[ \sigma_h = P_s \]  
\[ \sigma_{ff} = 3\sigma_h - P_f - \alpha P_p + S_r \]

Where \( S_t \) is the Tensile Strength, \( P_f \) is the formation fracture pressure, \( P_r \) is the reopening pressure, \( P_s \) is the instantaneous shut-in pressure.
ACKNOWLEDGEMENTS

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EVALUATION OF THE IN VITRO AND IN VIVO EFFECTS OF THE ETHANOLIC LYOPHILIZED EXTRACT OBTAINED FROM PHOLIOTA AURIVELLA MUSHROOM ON CCl₄-INDUCED TOXICITY IN RATS

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ABSTRACT

The aim of this study was to evaluate Pholiota aurivella extract’s chemical and mineral composition, total antioxidant capacity, histopathological changes in liver and kidney, as well as its possible protective/harmful role against carbon tetrachloride (CCl₄)-induced oxidative stress in rats. After the toxicity test, twenty four rats were divided into four experimental groups: Control, CCl₄, CCl₄+P.aurivella (100 mg/kg, extract) and CCl₄+P.aurivella (500 mg/kg, extract) groups. Some biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) in serum, malondialdehyde (MDA) and antioxidant defense system (ADS) constituents such as reduced glutathione (GSH) levels, glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities in the liver, brain and kidney tissues, histopathological changes in liver and kidney of rats were observed. P.aurivella extract was rich in phenolic (p-coumaric and protocatechuic acids) and fatty acid (linoleic, oleic and palmitic acids) compounds and high level of Arsenic. In vivo studies revealed that P.aurivella extract suppressed the levels of ADS enzymes and increased the MDA content and AST, ALT and LDH in treated groups. According to histopathological investigation, liver and kidney revealed severe histopathological changes both in CCl₄ group and P.aurivella- treated groups. These results have shown that oral administration of P.aurivella extract caused a significant toxic effects in rats. Therefore, in the context of the data obtained from this study the P.aurivella could not be regarded as a safe food source.

KEYWORDS: Pholiota aurivella, Carbon tetrachloride, Bioactive compounds, Oxidative stress, Histopathological changes, Rats.

INTRODUCTION

Nowadays, mushrooms are used in many areas such as food, medicine, agricultural pests and parasites, alternative treatments for cancer, cosmetics, skin care and recycling of natural biomaterials. Antioxidant components such as polysaccharides, tocopherols, phenolics, carotenoids, ergosterol and ascorbic acid present in fruit bodies of the mushrooms enable to neutralize free radicals and protect cells against oxidative damage, aging and various degenerative diseases [1]. In addition, mushrooms are considered as functional foods containing bioactive compounds which exhibit beneficial effects on human health [2-4]. It was propounded that some mushrooms have toxic effects contrary to their protective effects. Some wild edible mushrooms consuming as an important food sources by people might cause significant health problems due to their heavy metal deposition properties such as arsenic a highly toxic chemical [5]. For instance, it was reported that Pleurotus ostreatus, Volvariella volvacea and Flammulina velutipes caused serious complications such as cardiovascular, neurodegenerative and reproductive disorders and various tissue damages caused by the long-term experimental applications or due to their various toxic substances [6-8]. Amatoxin found Galerina sulphureus caused cell death in liver and kidneys by inhibiting RNA polymerase II activity [9]. Additionally, it was reported that Agrocybe cylindracea caused severe hepatotoxicity in mice fed with 25 and 250 mg / kg / day extract for 6 days [10].

Pholiota aurivella (Batsch) P. Kumm. has spicy smell and milky taste which is grown on the
bodies of *Fagus, Fraxinus, Salix* and *Alnus* trees, in the late summer and autumn seasons. There is no certain judgement regards to edibility of this species) [11]. It is a cosmopolite mushroom distributed in various countries such as China, Japan, Far East Russia, Europe, North America, Morocco [12] and Turkey [11].

There is no or limited study in scientific literature regards to chemical composition and potential health benefits and toxicity effects of *P. aurivella*. However, in our previous study, it was reported that *P. aurivella* mushroom caused decrease in rat erythrocyte antioxidant defense system, increased erythrocyte hemolysis and fluctuations on hematological parameters [13]. Therefore, we aimed to determine the phytochemical composition and in *vitro* and in *vivo* antioxidant capacity of *P. aurivella* lyophilized extract in this study. Additionally, adverse and toxicity effects of the extract on histopathological changes in liver and kidney, biochemical indices and antioxidant defence system in brain, kidney and liver tissues of CCl4-induced rats were evaluated.

**MATERIALS AND METHODS**

**Mushroom material.** Fruit bodies of *Pholiota aurivella* (Batsch) P. Kumm. were harvested by Cemil Sadullahoglu and Prof. Dr. Yusuf Uzun in Gürpınar district, Van City, in the Eastern Anatolia Region of Turkey, (GPS coordinates 38° 19' 53.4" N; 43° 23' 86.7" E) on September-October, 2016. The identity of samples was confirmed by Prof. Dr. Kenan DEMIREL at the Department of Biology, Science Faculty, Van Yuzuncu Yıl University, Turkey and a voucher specimen was deposited at the university’s fungarium (Fungarium code: YYYVAN, FUNGARIUM-7488). The fruiting bodies of fresh mushroom material were cleaned properly and dried at room temperature in the dark until dry. Subsequently, the samples were ground and stored at -20°C until analysed.

**Preparation of lyophilized extract.** The lyophilized extract was prepared according to method [14]. Briefly, the ground mushroom material was mixed with a 10-fold volume of aqueous ethanol (80 % ethanol, 20 % H2O, v/v), shaken for 2 h at room temperature (25°C) and centrifuged for 20 min (15320 g) at 4°C (Sorvall RC-5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial No. 02U8152, USA) with the supernatant collected. The extraction was repeated one more time. The supernatants from the consecutive extractions were combined. Solvent was evaporated under reduced pressure at 40°C. The derived fraction was dissolved in purified water and freeze-dried under a vacuum at -51°C to obtain a fine lyophilized powder used for analysis.

**Chemicals.** Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich, Inc. (St Louis, MO, USA) and were of analytical or HPLC grade. Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Standardized Reishi-Shiitake-Maitake extract was purchased from Solgar (Istanbul, Turkey). Kits for antioxidant enzymes analysis were supplied by Randox Laboratories L.T.D. (Antrim, UK).

**In vitro antioxidant studies. Folin-ciocalteu (total phenolic content) assay.** Folin-Ciocalteu reducing capacity (Total phenolic content) of the extract was determined as described previously [14] and the results were expressed as mg gallic acid equivalents per gram of dry weight of the lyophilized extract (mg GAE/g DW), based on Gallic acid standard curve and against a blank control. Standardized Reishi-Shiitake-Maitake extract was used as positive control. The analyses were conducted in triplicate.

**Ferric reducing antioxidant power (FRAP) assay.** Total reducing capacity was determined using the FRAP assay as described previously [14] and the reducing capacity of the extract was expressed as μM of iron (Fe^{2+}) per gram of dry weight of lyophilized extract (μM Fe^{2+}/g DW) based on an iron sulphate standard (FeSO4) curve against a blank control. Standardized Reishi-Shiitake-Maitake extract was used as positive control. The analyses were conducted in triplicate.

**Oxygen radical absorbance capacity (ORAC) assay.** Oxygen radical scavenging capacity was determined using the ORAC assay according to [14] and antioxidant capacity of the sample was expressed as μM of trolox equivalent per gram of dry weight of lyophilized extract (μM T Eq./g DW) based on a trolox standard curve. Standardized Reishi-Shiitake-Maitake extract was used as positive control. The analyses were conducted in triplicate.

**Chemical Composition. Identification of phenolic compounds by liquid chromatography – diode array – mass spectrometry (LC-DAD-MS/MS).** LC-DAD-MS/MS analysis was conducted as described previously with minor modifications [14] on a Quantum triple stage quadrupole (TSQ) mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an auto sampler. An aliquot (3 μL) of each ethanolic extract was chromatographed on a 150 x 2.1 mm i.d., 5 μm Luna Synergy Hydro column (Phenomenex) which was heated to 30°C. Analytes were separated using 0.5% formic acid in purified water (A) and 0.5% formic acid in acetonitrile (B) under a flow rate of
200 μL/min. The gradient employed was 0% B for 2 min, then 40% B for 6 min, following 60% of B for 8 min and 100% B for 4 min. Then the gradient was held for 4 min at the rate of 100% of B. The photodiode array detector was used to acquire data from 190-520 nm. Ions for mass spectrometry were generated using an electrospray source in either the positive or negative mode (depending on analyte) under conditions set following optimization using chlorogenic acid (negative) or quercetin-3-glucoside (positive). MS experiments in the full scan (parent and product-specific) and the selected reaction monitoring (SRM) mode were conducted. The composition of phenolic compounds was characterized based on their UV spectrum, retention time, co-chromatography with commercial standards, when available, and MS fragmentation patterns.

Quantification of phenolic compounds by high performance liquid chromatography-diode array detector (HPLC-DAD). Quantification of phenolic compounds by the HPLC was conducted as described previously with minor modifications [14] on HPLC system, which consisted of two LC-10ADVP pumps, SPD-M10ADVP diode array detector, CTO-1-ADVP column oven, DGU-12A degasser, SIL-10ADVP auto injector, and SCL-10A system controller (Shimadzu Corporation, Kyoto, Japan) equipped with an Atlantis column (dC18, 4.6 mm i.d x 100 mm length, 5 μm particle size, Waters Associates, Chippendale, NSW, Australia). Analytical HPLC was run at 30 °C and monitored at 280 nm, 320 nm, 370 and 520 nm. Injection volume was 10 μL. The following solvents in purified water with a flow rate of 1.0 mL/min were used: A, 0.5% trifluoroacetic acid (TFA) and B, 0.5% TFA + 95% acetonitrile. The elution profile was a linear gradient elution for B of 0% to 20% for 3 min, to 40% for 5 min, to 60% for 5 min, to 80 for 5 min and to 100% for 5 min. The gradient elution was held at the rate of 100% B for 5 min. The identity of compounds was confirmed by co-chromatography and comparison of their spectral characteristics with those of authentic standards.

Volatile Compounds. Head space solid phase micro extraction (HS-SPME) procedure. Lyophilized P.aurivella extract (2 g) were weighed into a 40 mL vial and treated by divinylbenzene / carbosil / polydimethylsiloxane fiber with 50/30 mm film thickness. The fiber was preconditioned in the injection port of the gas chromatography (GC) as indicated by the producer before the analysis. The samples were placed in Mininert® push-button valves and kept at 35 °C with continuous internal stirring and equilibrated for 30 min. Subsequently, the solid phase micro extraction (SPME) fiber was exposed for 40 min to the headspace at 35°C. SPME fiber was introduced into the GC injector after sampling and was left for 3 min to allow the analysis of thermal desorption. The headspace volume, heating temperature and time of the extraction were optimized according to [15].

Gas chromatography–mass spectrometry (GC-MS) analysis. Volatile compounds present in P.aurivella extract were analysed by gas chromatography mass spectrometry (GC/MS) using a head space solid phase micro extraction and identified by the fragment ions and relative retention indices of their peaks with those of the MS library standards. Volatile compounds prepared by HS-SPME procedure were analysed on Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) equipped with a flame ionization detector (FID), at the injector temperature of 260°C in injection mode. The lyophilized extract was chromatographed on a 60 x 0.25 mm i.d., 0.2 μm HP 88 column (Agilent, USA). Oven temperature was programmed as follows: 80°C at the start, followed by an increase to 190°C at a rate of 5°C /min, held at 190°C for 3 min, followed by an increase at a rate of 5°C /min to 260°C and held for 3 min. The subsequent temperature increase was at a rate of 2°C /min to 275°C and held for 12.5 min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature was 250°C; the ionization mode, electron impact (EI); acquisition ion range, 40 to 200 m/z; scan rate, 1 us-1. The compounds were identified using the National Institute of Standards and Technology (NIST) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices, calculated as described by Van Den Dool and Kratz [16]. Results were expressed as FID response area in relative percentages.

Mineral Composition Analysis. Mineral composition of the P.aurivella extract was evaluated by using AAS, ICP-MS VE ICP-OES. The analysis solution was prepared by dissolving the extract in HNO3. Subsequently the solution was subjected to microwave assisted extraction procedure. The identity of mineral compounds was confirmed comparison of their characteristics with those of authentic standards.

In vivo studies. Animals. Twenty-four Wistar albino male rats 2-6 months old, weighing 200-350 g, were provided by the Experimental Animal Research Centre, Van Yuzuncu Yil University. The animals were housed at 25 ± 1°C in a daily light/dark (~12/12) cycle. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethic Committee of the Van Yuzuncu Yil University (Protocol number: 27552122-
Acute toxicity test. The method of (OECD guidelines, test 423: acute oral toxicity method; 2002) were employed in this study. Three rats were dosed with 20, 50, 100, 250, 500, 1000 and 2000 mg/kg of the P. aurivella extract by orally via gastric gavage for 0.5, 2, 4, 8, 24, 48 and 72 hours respectively. The animals were fed food and water ad libitum. No signs of toxicity and mortality were observed over a period of 72 h. 100 mg/kg and 500 mg/kg doses were chosen after toxicity test and no sign of the morbidity and mortality.

Experimental design. The rats were randomly divided into four groups: Control, CCl₄, CCl₄+P. aurivella (100 mg/kg, extract) and CCl₄+P. aurivella (500 mg/kg, extract). Food and tap water were supplied for all animals as ad libitum during experimental period.

Control Group (n=5): The rats received tap water and fed with standard pellet diet as ad libitum.

CCl₄ Group (n=5): The rats received 0.5 mL CCl₄/kg rat weight intraperitoneally injection twice per week [17].

CCl₄+P. aurivella Group (n=6): The rats received 0.5 mL CCl₄/kg rat weight intraperitoneally injection twice per week and P. aurivella lyophilized extract (100 mg/kg, per day) was treated to rats via orally during 28 days.

CCl₄+P. aurivella Group (n=6): The rats received 0.5 mL CCl₄/kg rat weight intraperitoneally injection twice per week and P. aurivella lyophilized extract (500 mg/kg, per day) was treated to rats via orally during 28 days.

Antioxidant defence system enzyme analysis. The rats were anesthetized by injection of ketamine (5 mg/100 g body weight) intraperitoneally. Liver, brain and kidney tissues were dissected and stored at -78°C. The tissue were homogenization was carried out using stainless steel probe homogenezier (SONOPULS HD 2200, Bandelin, Berlin, Germany) as described previously by Dogan et al. [17]. Reduced glutathione (GSH) levels, malondialdehyde (MDA) content, glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were measured in liver, brain and kidney supernatants as described previously by Dogan et al. [17].

Measurement of biochemical parameters. Serum biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea, uric acid (UA), creatinine (CREA), total protein (TP), sodium (Na), potassium (K), chloride (Cl), cholesterol (CHOL), triglyceride (TG) and HDL-cholesterol (HDL-C) were measured by an auto analyser (ARCHITECT 16200, Abbott Park, IL 60064, USA) using the Abbott biochemistry kits (USA).

Histopathological examination. At the end of the trial period animals were sacrificed under anesthesia and then necropsy was performed. For microscopic investigation liver and kidney tissues were fixed in 10% neutral buffered formalin for 72 h and then were washed in running fresh water for 12 h. They were treated with alcohol series and a series of xylene during the routine tissue control period. They were embedded in paraffin, sectioned at 4 μm and then stained with hematoxylin/eosin (HE) and the relevant areas were photographed with light microscope.

Statistical analysis. All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the Minitab 13 for Windows package program. One-way analysis of variance (ANOVA) statistical test was used to compare the differences between means of the experimental groups in 95% confidence interval.

RESULTS

In vitro antioxidant capacity and chemical compositions of P. aurivella. Hydrophilic extract obtained from fruiting bodies of Pholiota aurivella had a high extraction yield (19.7%) (Table 1). Total phenolic content measured by Folin-Ciocalteu method of the extract had a 3 fold that of the positive control (Reishi-Shiitake-Maitake extract). Similarly the extract exhibited 3 fold higher capacity of scavenging peroxyl radicals (ORAC) than the positive control. With regards to total reducing capacity, the extract showed a high antioxidant power than that of the positive control (Table 1).

Bioactive compounds (phenolics, volatiles and minerals) in the extract were presented in (Table 1). Spectral characteristics of HPLC peaks revealed that phenolic acids were the dominating group of phenolic compounds. The dominating compounds present in the extract were detected at 326 nm (Figure 1), which indicated the presence of hydroxycinnamic acids, and stilbens. No any compounds were detected at 370 and 520 nm. Mass spectrometric data confirmed these results (Table 1). p-Coumaric acid was the major phenolic compound of the extract and made up over 66 % of total phenolics. Other phenolic compound tentatively identified based on m/z transition data was pro catastechic acid (Table 1).

GC-MS analysis revealed that fatty acids were the main component of the volatile composition of the extract. Linoleic acid (56.7% contribution) was the dominant fatty acid detected in the extract,
followed by oleic and palmitic acids (23.9 and 16.4% contributions, respectively) (Figure 2, Table 1).

Mg, K, Na and Si were the dominant components in the extract. The amounts of of heavy metal components (Pb, Mn, Cr, Cd and Co) in the extract were detected in low levels. However, a high level of As (62 μg/g), a highly toxic element, was determined in the extract (Table 1).

**FIGURE 1**
HPLC profile of *P. aurivella* mushroom extract

**FIGURE 2**
GC-MS profile of *P. aurivella* mushroom extract
TABLE 1
Chemical composition and in vitro antioxidant capacity of P. aurivella

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Protocatechuic acid (mg/g extract)</th>
<th>p-Coumaric acid (mg/g extract)</th>
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</thead>
<tbody>
<tr>
<td>MS/MS ([M+1]/[M-1])</td>
<td>&lt;153</td>
<td>&gt;163</td>
</tr>
<tr>
<td>Fragment ions (m/z (+/-))</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>Concentration</td>
<td>2.2±0.0</td>
<td>15.5±0.8</td>
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Volatile composition

<table>
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<tr>
<th>Retention Time (min.)</th>
<th>Compound name</th>
<th>Level (µg/g extract)</th>
<th>Relative concentration (%)</th>
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</thead>
<tbody>
<tr>
<td>36.5</td>
<td>Palmitic acid</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>40.8</td>
<td>Oleic acid</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>42.1</td>
<td>Linoleic acid</td>
<td>56.7</td>
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<table>
<thead>
<tr>
<th>Mineral</th>
<th>Level (µg/g extract)</th>
<th>Mineral compound</th>
<th>Level (µg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>T</td>
<td>Mn</td>
<td>3.9±0.0</td>
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<tr>
<td>As</td>
<td>62.1±0.1</td>
<td>Mo</td>
<td>0.12±0.0</td>
</tr>
<tr>
<td>B</td>
<td>207.4±3.6</td>
<td>Pb</td>
<td>0.19±0.0</td>
</tr>
<tr>
<td>Cd</td>
<td>0.34±0.0</td>
<td>Sc</td>
<td>2.32±0.1</td>
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<tr>
<td>Co</td>
<td>0.03±0.0</td>
<td>Si</td>
<td>444.1±10.8</td>
</tr>
<tr>
<td>Cr</td>
<td>1.37±0.0</td>
<td>Zn</td>
<td>18.4±0.6</td>
</tr>
<tr>
<td>Cu</td>
<td>16.7±1.0</td>
<td>Na</td>
<td>578.9±10.4</td>
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<tr>
<td>K</td>
<td>737.1±26.4</td>
<td>Fe</td>
<td>21.6±1.2</td>
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<tr>
<td>Mg</td>
<td>1113.3±8.5</td>
<td>Ca</td>
<td>194.4±2.6</td>
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</table>

Means with different letters in the same raw were significantly different at the level (p<0.05). 

n=3. mg Gallic acid/g extract, μmol Fe2+/g extract, μmol Trolox Eq./g extract.  *Reishi-Shiitake-Maitake extract; FCR, folin-ciocalteu reagent; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity. Ag, silver; As, Arsenic; B, boron; Ca, calcium; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Mo, molybdenum; Na, sodium; Pb, lead; Se, selenium; Si, silicon; Na, sodium; Zn, zinc.

TABLE 2
Effects of P. aurivella extract on biochemical parameters CCL-induced in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCL</th>
<th>CCL + P. aurivella (100 mg/kg)</th>
<th>CCL + P. aurivella (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>117.40±8.65</td>
<td>154.75±23.30</td>
<td>129.67±15.23</td>
<td>133.80±12.62</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>37.40±7.7</td>
<td>77.80±13.16</td>
<td>72.33±14.76</td>
<td>69.80±14.31</td>
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<tr>
<td>LDH (U/L)</td>
<td>954.20±64.02</td>
<td>1878.75±111.58</td>
<td>1197.33±229.15</td>
<td>1270.40±255.67</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>57.20±6.7</td>
<td>47.25±4.57</td>
<td>48.00±9.94</td>
<td>45.20±3.64</td>
</tr>
<tr>
<td>UA (mg/dL)</td>
<td>1.70±0.37</td>
<td>1.40±0.14</td>
<td>1.82±0.53</td>
<td>1.50±0.40</td>
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<tr>
<td>CREA (mg/dL)</td>
<td>0.49±0.02</td>
<td>0.46±0.04</td>
<td>0.53±0.03</td>
<td>0.48±0.04</td>
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<tr>
<td>TP (g/dL)</td>
<td>5.60±0.23</td>
<td>6.20±0.08</td>
<td>6.00±0.21</td>
<td>6.16±0.09</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>141.60±1.14</td>
<td>140.75±2.22</td>
<td>140.00±1.10</td>
<td>140.80±0.84</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>5.42±0.40</td>
<td>4.98±0.65</td>
<td>5.32±0.60</td>
<td>5.46±0.54</td>
</tr>
<tr>
<td>CI (mEq/L)</td>
<td>100.80±3.35</td>
<td>102.50±3.11</td>
<td>104.17±1.72</td>
<td>103.80±0.84</td>
</tr>
<tr>
<td>CHOL (mg/dL)</td>
<td>32.60±6.23</td>
<td>29.25±4.40</td>
<td>19.33±5.50</td>
<td>19.00±3.54</td>
</tr>
<tr>
<td>TRIG (mg/dL)</td>
<td>65.20±14.38</td>
<td>41.75±10.81</td>
<td>26.50±7.70</td>
<td>16.80±3.49</td>
</tr>
<tr>
<td>HDL_C (mg/dL)</td>
<td>51.23±6.14</td>
<td>46.73±6.82</td>
<td>38.32±7.20</td>
<td>40.54±4.58</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD. One-way ANOVA followed by Tukey test, when appropriate (n= 6 or 5 animals for each four groups).

a Different from the Control group.

b Different from the CCL group.

c CCL+P. aurivella (100 mg/kg, extract) different from the CCL+P. aurivella (500 mg/kg, extract) group.

The effects of P. aurivella extract on biochemical parameters. As presented in Table 2, AST, ALT and TP levels in serum were significantly increased in CCL, CCL+P. aurivella (100 mg/kg) and CCL+P. aurivella (500 mg/kg) groups according to Control group. While markedly an increase in serum LDH level was measured in CCL, CCL+P. aurivella (100 mg/kg) and CCL+P. aurivella (500 mg/kg) groups compared to Control, it was significantly decreased in CCL+P. aurivella (100 mg/kg) and CCL+P. aurivella (500 mg/kg) groups according to control.
CCL_{4} group. CREA level was substantially higher in CCL_{4}+P.aurivella (100 mg/kg) group than Control and CCL_{4} groups. CHOL level was importantly reduced in CCL_{4}+P.aurivella (100 mg/kg) and CCL_{4}+P.aurivella (500 mg/kg) groups according to Control and CCL_{4} groups. HDL-C level was significantly lessened in CCL_{4}+P.aurivella (100 mg/kg) and CCL_{4}+P.aurivella (500 mg/kg) groups according to Control group. TRIG one of the important lipid profile parameters was measured markedly lower in CCL_{4}, CCL_{4}+P.aurivella (100 mg/kg) and CCL_{4}+P.aurivella (500 mg/kg) groups than Control group. Additionally, it was decreased in CCL_{4}+P.aurivella (100 mg/kg) and CCL_{4}+P.aurivella (500 mg/kg) groups according to only CCL_{4} group. Moreover, significant reduction in TRIG was observed depending on dose increase in mushroom extract.

The effects of *P.aurivella* extract on lipid peroxidation and antioxidant enzymes in brain, kidney and liver. As shown in Table 3, MDA content was significantly increased in CCL_{4} group according to Control group in brain, kidney and liver. Lipid peroxidation was markedly decreased in CCL_{4}+P.aurivella (100 mg/kg) and CCL_{4}+P.aurivella (500 mg/kg) groups compared to CCL_{4} group in brain. Additionally, MDA content in brain was measured importantly higher in CCL_{4}+P.aurivella (500 mg/kg) group than Control and CCL_{4}+P.aurivella (100 mg/kg) groups. Lipid peroxidation in kidney of CCL_{4}+P.aurivella (500 mg/kg) group was significantly higher than Control group and similar to CCL_{4} group level. MDA content in liver was significantly elevated in CCL_{4}+P.aurivella (500 mg/kg) group according to other groups. Also, significant increase in MDA content in liver of CCL_{4}+P.aurivella (100 mg/kg) group was measured according to Control group. The effects of *P.aurivella* extract treatment on oxidative stress parameters such as GSH, GST, GR, CAT, GPx and SOD in CCL_{4} induced toxicity in the brain, liver and kidney tissues of rats were presented in Table 3. The GSH levels in the CCL_{4}+P.aurivella extract treated groups were significantly decreased (p < 0.05) in the kidney tissue according to Control group. Additionally, kidney tissue of CCL_{4}+P.aurivella (100 mg/kg) group had significant decrease in GSH level compared to CCL_{4} group. Similarly, the GSH levels in the CCL_{4} and CCL_{4}+P.aurivella extract treated groups were significantly decreased in the liver tissue compared to the Control group. Also, there was a significant increase in GSH level in the CCL_{4}+P.aurivella (500 mg/kg) group in liver according to CCL_{4} group.

### Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parameters</th>
<th>Control</th>
<th>CCL_{4}</th>
<th>CCL_{4}+P.aurivella (100 mg/kg)</th>
<th>CCL_{4}+P.aurivella (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>MDA nmol/g</td>
<td>102.2+±9.14</td>
<td>202.5+±23.51</td>
<td>78.3+±19.78</td>
<td>137.6+±15.21</td>
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<tr>
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<td>GSH mg/g</td>
<td>38.19+±9.88</td>
<td>34.49+±2.35</td>
<td>34.21+±4.86</td>
<td>33.93+±2.66</td>
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<tr>
<td></td>
<td>GST U/g</td>
<td>8.3+±2.21</td>
<td>6.78+±1.52</td>
<td>5.62+±1.39</td>
<td>5.26+±1.07</td>
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<tr>
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<td>GR U/g</td>
<td>1.03+±0.31</td>
<td>0.92+±0.10</td>
<td>1.16+±0.17</td>
<td>1.20+±0.15</td>
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<td>CAT U/g</td>
<td>42.17+±9.80</td>
<td>8.88+±0.62</td>
<td>6.18+±0.80</td>
<td>6.25+±1.26</td>
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<td>GPx U/g</td>
<td>67.3+±17.61</td>
<td>48.8+±10.91</td>
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<td>54.08±11.71</td>
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<td>SOD U/g</td>
<td>2174.85±38.75</td>
<td>2198.08±17.36</td>
<td>2198.57±21.35</td>
<td>2168.95±34.52</td>
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<tr>
<td>Kidney</td>
<td>MDA nmol/g</td>
<td>146.62±36.43</td>
<td>285.77±66.29</td>
<td>211.52±61.46</td>
<td>280.04±49.17</td>
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<td>GSH mg/g</td>
<td>17.60±1.14</td>
<td>16.08±1.91</td>
<td>12.52±1.98</td>
<td>13.55±2.64</td>
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<td>GST U/g</td>
<td>7.80±1.89</td>
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<td>GR U/g</td>
<td>0.33±0.10</td>
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<td>CAT U/g</td>
<td>255.33±48.87</td>
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<td>GPx U/g</td>
<td>675.57±174.70</td>
<td>538.76±142.52</td>
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<td>SOD U/g</td>
<td>2201.39±29.94</td>
<td>2216.26±11.58</td>
<td>2207.90±62.26</td>
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<tr>
<td>Liver</td>
<td>MDA nmol/g</td>
<td>133.15±33.23</td>
<td>210.32±52.36</td>
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<td>GSH mg/g</td>
<td>19.02±1.56</td>
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<td>GST U/g</td>
<td>27.58±6.59</td>
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<td>GR U/g</td>
<td>0.31±0.09</td>
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<td>CAT U/g</td>
<td>508.03±118.55</td>
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<td>GPx U/g</td>
<td>596.00±91.78</td>
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<td>423.13±95.24</td>
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<td>SOD U/g</td>
<td>2123.39±83.09</td>
<td>2177.09±31.52</td>
<td>2172.02±77.90</td>
<td>2048.92±87.59</td>
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Data were expressed as mean ± SD. One-way ANOVA followed by Tukey test. when appropriate (n= 6 or 5 animals for each four groups).

* Different from the Control group.

* Different from the CCL_{4} group.

* CCL_{4}+P. aurivella (100 mg/kg, extract) different from the CCL_{4}+P. aurivella (500 mg/kg. extract) group.
**FIGURE 3**
The normal histological architecture of liver tissue of the control group (A). Macrovesicular and microvesicular fatty degenerations, necrosis and hydropic degeneration in hepatocytes, sinusoidal congestion in 
CCI-treated group (B). Severe the same lesions in CCl4+P.aurivella groups (C and D). HxE, Bar: 100 μ.

**FIGURE 4**
The normal histological architecture of kidney tissue of the control group (A). Decrease in the mezengial 
cells hydropic degeneration of tubular epithelium, vacuolisation in the podocyte, coagulation necrosis, 
dilatation of Bowman space, dilatation of tubular lumen of kidney tissue of the CCl4 group (B). Severe the 
same lesions in CCl4+P.aurivella groups (C and D). HxE, Bar: 100 μ.
The GST enzyme activity in brain of CCl₄+P.aurivella extract treated groups was significantly decreased compared to the Control group. The GST enzyme activity in kidney and liver tissues of CCl₄ group was significantly decreased compared to Control and CCl₄+P.aurivella extract treated groups, except CCl₄+P.aurivella (500 mg/kg) group in liver.

With regards to GR enzyme activity in brain, there were relatively increases in CCl₄+P.aurivella extract treated groups compared to the CCl₄ group. There was significantly decrease in GR activity of CCl₄ group and CCl₄+P.aurivella extract treated groups compared to Control group in liver tissue. Its activity was significantly decreased in kidney of CCl₄+P.aurivella (500 mg/kg) group compared Control group.

A sharp decrease (p < 0.01) in the CAT enzyme activity was measured in brain tissue of CCl₄ group and CCl₄+P.aurivella extract treated groups compared to the Control group. Additionally, the CAT enzyme activity in brain of CCl₄+P.aurivella extract treated groups was significantly decreased compared to the CCl₄ group. There was significantly decrease in the CAT activity of CCl₄+P.aurivella extract treated groups compared to Control group in kidney tissue. The GPx enzyme activity in brain and liver tissues of CCl₄+P.aurivella extract treated groups was significantly decreased compared to Control, except CCl₄+P.aurivella (500 mg/kg) group in brain.

The effects of P.aurivella extract on histopathological changes in liver and kidney. Histopathological findings in the liver and kidney tissue samples from all groups are presented in (Figure 3 A-D) and (Figure 4 A-D), respectively. Control group liver had normal color and consistency, macroscopical change was not detected. All CCl₄ and CCl₄+P.aurivella treated group livers had yellowish colored and crumbly-fatty consistency noticed at grossly. The livers of rats from the control group had a normal histological architecture of the sinusoids, remark cord, and hepatic portal and central vein (Figure 3 A). Liver histopathology revealed some significant histopathological changes in CCl₄-induced group. The principal lesions were focal disseminated midzonal and centriflobular macrovesicular and microvesicular fatty degenerations, necrosis and hydropic degeneration in hepatocytes, sinusoidal congestion and inflammatory cell infiltration (Figure 3 B). These changes were more severe in groups CCl₄+P.aurivella (100 and 500 mg/kg extract) than in group CCl₄. In addition, the most severe lesions were seen in the CCl₄+P.aurivella (500 mg/kg) group depending on the dose of P.aurivella supplementation. Due to all these alterations, the normal histological appearance of the hepatic parenchyma was distorted (Figure 3 C, D). The histological structure of the liver was not preserved via P.aurivella administration. Moreover, P.aurivella significantly increased fatty degeneration and the other histopathological changes.

The kidneys of the control groups showed normal histological architecture (Figure 4 A). In the CCl₄ group, severe lesions was observed. Focal glomerular necrosis, hypocellularity in glomeruli and glomerular shrinkage was detected in this group. In additional, some of the proximal tubules and Bowman space were dilated and some tubuli epithelial cells and podocyte showed hydropic degeneration with picnotic nuclei and vacuolation. Also rare intraluminal casts were in some tubules. Inflammatory cell infiltration was also detected in the CCl₄ group. The affected glomeruli had a thickened basal membrane (Figure 4 B) P.aurivella supplementation resulted in worse these lesions. The most severe lesions were detected in the CCl₄+P.aurivella (500 mg/kg) group (Figure 4 C, D).

DISCUSSION

Recently, bioactive substances such as phenolic compounds, vitamin B, mineral compounds (selenium, copper, potassium, silisium), sulfur amino acids (ergothioneine), fiber, vitamin D2, chitin and beta-glucan present in wild edible mushrooms have been a popular research area due to their high antioxidant potential [18-21]. Antioxidant-rich mushrooms inhibited the formation of various free radical sources such as hydroxyl radical (OH), superoxide anion (O2·), hydroxyl ion (OH·), nitric oxide (NO) and hydrogen peroxide (H₂O₂) and reduced the risk of possible disease formation [1,22]. However, the toxic effects of some wild edible mushrooms have been also reported [23]. Liver failure, bradycardia, seizures, chest pain, gastroenteritis, intestinal fibrosis, renal failure, erythromelalgia and rhadomyolysis were previously reported in a mushroom poisoning study [24]. The authors suggested that addition to toxic mushrooms, some edible mushrooms might contain poisonous compounds and trigger poisoning severity depending on the dose [25]. Therefore analytic, bioactivity and toxicity studies on wild edible or inedible mushrooms are vital to reveal their in vivo and in vitro properties.

In this study, we firstly determined in vitro antioxidant capacity and phytochemical content of P.aurivella ethanol-based lyophilized hydrophilic extract. Our findings showed that the extract contained high level of total phenolics and exhibited pronounced antioxidant capacities in vitro, which was higher than total phenolics and in vitro antioxidant activities of several mushroom samples reported previously [25-27]. Phenolic acids (coumaric and protocatechuic acid) were detected as key phe-
nolic components of the extract. Islam et al. [26] evaluated 43 commonly consumed mushroom species and detected high quantities of phenolic acids including gallic, gentisic and p-coumaric acids in the extracts. Moreover, various researchers reported coumaric and protocatechuic acids as main phenolic compounds of several mushroom species [1, 25, 27-29].

Hydroxycinnamic acid derivatives are accepted as potential natural phenolic compounds in the management of lipid metabolism and obesity due to their inhibitory properties of macrophage infiltration, preventing adipocyte differentiation and lowering lipid profile [30]. Yoon et al. [31] detected modulating properties of coumaric acid on glucose and lipid metabolism via AMP-activated protein kinase in L6 skeletal muscle cells. Hsu and Yen [32] investigated the effects of multiple phenolic compounds and concluded coumaric acid as one of the most effective inhibitor of adipogenesis in 3T3-L1 adipocytes. In another study conducted by Hsu et al. [33] ameliorative effect of coumaric acid on lipid metabolism was reported. Effective antioxidant capacities of coumaric acid and protocatechuic acid both in vitro and in vivo were determined in several experimental studies [34, 35]. No any flavonoid compounds were determined in the extract which is in agreement to [36] who reported that mushrooms do not contain flavonoids due to the non-presence of key enzymes (chalcone isomerase or chalcone synthase) involved in the flavonoid biosynthetic pathway.

Fatty acids identified in the extract were composed of linoleic, oleic and palmitic acids which are common fatty acids found in mushroom species [25]. Linoleic acid which has various biological effects is one of the essential requirement compound for mammalian health and development. Oxidized metabolites of linoleic acid were reported for their strong activation properties of antioxidant response elements which may initiate responses that able to lessen damage caused by oxidative stress [37]. Chemical compounds detected in the extract including phenolic and fatty acid compounds are biologically active non-toxic compounds and major contributors of antioxidant activity both in vitro and in vivo and regulative properties on serum parameters.

In the light of high in vitro antioxidant activity results and identification of biologically active compounds in the extract, we aimed to evaluate serum biochemical parameters, lipid peroxidation and antioxidant defense system enzymes in order to determine possible bioactivity and toxicity effects of *P. aurivella* extract against oxidative stress induced by CCl₄ in vivo rat model.

Our findings showed that serum AST, ALT and LDH enzyme levels were increased in rats treated with two different doses of *P. aurivella* extract according to Control group. The AST, ALT and LDH activities in the serum are commonly used as biochemical indicators for tissue damage, particularly in liver. Leakage of these indicators from hepatic cells into blood because of damage on liver is resulted with increases in these enzyme activities. It was reported that lectin isolated from the *A. cylindracea*, known as an edible mushroom, caused severe hepatotoxicity by dose-dependent increase in the AST and ALT levels [10]. Similarly, in our study, the increase of ALT, AST and LDH activities after treatment with *P. aurivella* extract did not stabilize the plasma membrane and regulate liver damage caused by CCl₄. CREA, an indicator of kidney function produced from creatine, is a molecule of major importance for energy production in muscles. Increase in the level of CREA is usually linked to renal dysfunctions or renal diseases [38]. Although the significant increase in CREA was measured in serum of CCl₄+*P. aurivella* (100 mg / kg, extract) group compared to Control and CCl₄ groups, this increase was at normal limit range (0.2 – 0.8 mg/dL) for rats. TP content in serum of CCl₄ group and *P. aurivella* extract treated groups was significantly higher than Control group. Tissue damage might have caused this elevated protein content in serum. As similar to our results, increases in TP levels in the toxic groups were previously reported [39,40].

Significant decreases in some lipid parameters CHOL and HDL_C were measured in group *P. aurivella* treated groups compared to Control and CCl₄ groups. Additionally, TRIG level was markedly decreased in CCl₄ group and *P. aurivella* treated groups according to Control. Moreover, *P. aurivella* caused drastically dose-dependent decrease in TRIG level. In the current study, the suppression of the molecules responsible for the lipid synthesis pathway such as 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) key enzyme for cholesterol synthesis and sterol-regulatory element-binding protein (SREBP)-2 transcription factor regulating cholesterol gene expression or the active substances presence in *P. aurivella* might have caused decline in lipid parameters.

According to our lipid peroxidation results, *P. aurivella* had no protective role against hepatotoxicity, nephrotoxicity and brain damage induced by CCl₄. On the contrary, the increase in the dose-dependent MDA was measured in liver, brain and kidney. MDA, a major toxic product of the lipid peroxidation, is produced as a result of ROS attacking to lipids in cell membranes, proteins and nucleic acids. It was reported that lipid peroxidation was decreased in treated groups by feeding rats with sweetgum oil and carob pods supplemented food while CCl₄ caused increase in lipid peroxidation in liver, brain and kidney tissues [41]. Some *Pleurotus* extracts reduced lipid peroxidation in the heart, liver, brain and kidneys of the CCl₄ induced rats [42, 43]. However, *P. aurivella* extract used in this
study promoted lipid peroxidation and in this aspect, this mushroom might have toxic properties.

In this study, we evaluated in vitro and in vivo antioxidant capacities of *P. aurivella* extract. Fluctuations on the antioxidant defense system enzyme levels were observed in the CCl₄ and *P. aurivella* treated groups compared to Control group. However, *P. aurivella* extract caused decrease of GSH and CAT levels in kidney, decrease of GR and GPX activities in liver and particularly sharp decrease of CAT activity in brain. Antioxidants play vital roles for protection of harmful effects of free radicals produced by endogenous or exogenous stresses in organisms. GSH, GST, GR, CAT, GPX and SOD enzymes are the main components of antioxidant defense system in organisms. GSH, GST, GR and GPX which have thiol structure are important antioxidant enzymes which suppress directly or indirectly formation of free radicals in the metabolism of xenobiotics [44-46]. Additionally, the superoxide radicals (O₂⁻) are converted to the hydrogen peroxides (H₂O₂) by SOD, then the CAT enzyme converts these hydrogen peroxides to H₂O and O₂ [47]. Levels of these enzymes are indicators of oxidative stress. Consumption of antioxidant rich sources might help to protect against oxidative stress and its complications. Although there are many studies suggesting that mushrooms have antioxidant potential, a few studies have reported for investigation of their in vivo effects on experimental animals [1, 4, 21]. On the other hand in various mushrooms were reported some toxin substances such as amatoxin, orellanine, coprine, muscarine, muscinol, psilocin, psilocybin, choline, lampterol fasiculol, agaritín, gyromitrin and ibotenic acid and these toxin can cause adverse effects such as liver failure, brady- cardia, chest pain, seizures, gastroenteritis, intestinal fibrosis, renal failure, erythromelalgia and rhabdomyolysis [24]. According to our findings, *P. aurivella* extract generally had negative effects on lipid peroxidation and antioxidant defense system enzymes instead of contribution of their activities. Since phenolic compounds and fatty acids detected in this study are not toxic substances, we also evaluated mineral compounds particularly heavy metals of the extract. Heavy metals present in the extract such as Pb, Mn, Cr, Cd and Co were above the legal health limits. However, the extract contained high level of As (62 µg/g) which represent a serious risk to health. Arsenic is a widespread metalloid in nature and one of the elements present in concentrations that raise chemical and toxicological concerns. The normal levels of arsenic in wild mushrooms are usually less than 1 µg/g dry weight and recommended not to be higher than 3 µg/g dry weight in mushroom samples [48]. The content of As in *P. aurivella* extract was 20 fold of normal level, which might cause toxic potential of the extract according to our in vivo experimental findings.

Toxic effects of *P. aurivella* extract used for against CCl₄ induced damage came into prominence according to our results. Addition to accumulation of toxic substances particularly As, consumption of the mushroom without boiling or some lectin-like proteins in the mushroom samples might be possible reasons for these toxic effects of *P. aurivella* extract.

Local people generally consume wild edible mushrooms as treated with high temperatures such as boiling and grilling in order to break down possible toxic substances. In this study, *P. aurivella* extract was directly lyophilized without boiling, following feeding of rats with this extract via orally. Regarding this, possible toxic substances were not broken down because of using mushroom extract which was not treated with high temperature. It was reported that CCl₄ caused increase in formaldehyde concentration in urine of CCl₄-induced rats [49, 50]. Some reactive aldehydes such as formaldehyde, acetaldehyde and crotonaldehyde are produced as a result of generated free radicals by CCl₄ metabolism [51]. The high concentration of formaldehyde is toxic for organisms. It was reported that the high concentration of formaldehyde formed as a result of lignin degradation was detected in *P. aurivella* and *Sphingobium* sp. SYK-6 bacterium culture, while other eleven fungi sources could convert formaldehyde and methanol into formic acid [52].

According to our histopathological findings, *P. aurivella* mushroom extract didn’t have tissue protective role against CCl₄-induced tissues damage. Moreover, rats fed with *P. aurivella* extract showed severe and common degenerative and necrotic changes and disrupted architecture in the liver lobules. The histological changes in the liver and kidney injury induced by CCl₄ are know as apoptosis, necrosis, steatosis and mononuclear cell infiltration in both lobular area and portal septa [53, 54].

When all the data are evaluated, it has been concluded that some wild mushrooms that are rich in antioxidants and preservative contents do not always have positive effects on their in vivo studies, on the contrary they can cause very serious toxic effects due to the accumulation of heavy metals from environmental pollution.

**CONCLUSION**

The data obtained from this study suggest *P. aurivella* lyophilized extract had no protective role against CCl₄ damage and on the contrary it was contributed to negative effects of CCl₄ in rats. We detected that hydrophilic ethanol-based lyophilized extract of *P. aurivella* was not able to ameliorate CCl₄ induced hepatotoxicity, nephrotoxicity and lipid peroxidation in the kidney, brain and liver. The extract was found as a rich source of phenolics
and fatty acids which exerting antioxidant activities. However, the content of As in P. aurivella extract was 20 fold of normal level, which might caused toxic potential of the extract according to our in vivo experimental findings. When histopathological findings are evaluated together with biochemical parameters, it was concluded that the mushroom extract used for preservative purposes against CCl₄ induced oxidative stress in rats increased the dose dependent damage in the kidney and liver tissues. Therefore, people should careful random consuming mushrooms as a food source because of their heavy metal accumulation abilities.

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BIOLGICAL CONTROL OF STEM ROT, 
SCLEROTINIA SCLEROTIORUM BY INDIGENOUS 
BACILLUS SUBTILIS ISOLATES

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ABSTRACT

Sclerotinia stem rot is a plant disease caused by the pathogenic fungus Sclerotinia sclerotiorum, and considered as one of the most serious and devastating diseases that affect many crop plants in Jordan. Biological control has recently become a successful alternative to the chemical treatment. In this study, five bacterial isolates (U1-U5) were obtained from the external surface of non-germinated Sclerotia collected from soil samples of infected cucurbit plants in the Jordan Valley. Bacterial isolates were evaluated for their potential antifungal activity against two S. sclerotiorum isolates; S23 and E15. Results showed significant inhibition of both sclerotal and mycelial formations, with highest antifungal activity observed for U2, U3 and U5 isolates. These three isolates were identified as Bacillus subtilis and subjected to an in vivo evaluation of antifungal activity on squash seeds under greenhouse conditions. Results revealed a significant improvement in seedling growth parameters and high survival percentages, with normal overall appearance.

KEYWORDS:
Sclerotinia sclerotiorum, Sclerotia, Bacillus subtilis, Antifungal, Squash, Biocontrol

INTRODUCTION

Sclerotinia sclerotiorum, generally known as stem rot or white mold, is one of the most devastating and aggressive soil-borne phytopathogens with a universal ecological distribution [1]. This pathogen attacks wide range of plant hosts and families, including several economically important crops that accordingly suffered huge losses [2]. In Jordan, S. sclerotiorum has been reported to infect many plant species, especially in the Jordan valley [7,8].

S. sclerotiorum is difficult to control due to several reasons include the wide host range, the formation of persistent Sclerotia structures, and the limitation of the cultural practices as a control method [3,4]. Using fungicides has many disadvantages including botanic phytotoxicity, negative impact on the environment, development of fungicide resistant strains, lack of selectivity against different organisms, and the vulnerability of fungicides to degradation by microorganisms when used in soil applications [5,6]. All that reasons mentioned highlighted the need for finding alternative methods such as Biological control which safer and so it is worth attempting [9,10,11].

Several bacterial antagonists were reported to play role as biological control agents [12,13]. Bacillus spp. and B. subtilis has many advantages over other microorganisms such as the ease and fastness in the reproduction, the capability of forming spores which are resistant to unfavorable conditions and the production of many useful and effective antagonistic compounds [14,15,55]. B. subtilis reported to be effective in controlling several fungal plant diseases including S. sclerotiorum [16,17,18,19,55].

MATERIALS AND METHODS

Isolation of Sclerotinia sclerotiorum. Two isolates of S. sclerotiorum (S23 and E15) from our collection of plant disease laboratory, plant production department, Jordan University of Science and Technology were used in this study. Isolates were described by Osso et al. [8] and isolated as Sclerotia from soil samples of infected plots in the Jordan valley. Stock Sclerotia were treated with sodium hypochlorite 3% for 5 minutes, and subsequently washed three times with sterile distilled water (SDW) [20]. The treated Sclerotia were cultured on potato dextrose agar (PDA) then incubated at 20±2°C to reproduce new Sclerotia and mycelia and stored in refrigerator at 4°C until use, mycelial growth was renewed weekly to keep it active.

Bacterial Isolates, Culture and Identification. Five bacterial isolates (labelled as U1- U5) were isolated from the external surfaces of non-germinated Sclerotia. These Sclerotia were obtained
from cucurbit plant fields (debris and soil) infected by *S. sclerotiorum* in Jordan Valley. The isolates were maintained in glycerol at (-20°C) for long-term storage or stored at 4 °C on nutrient agar media (NA) for short-term storage.

In this study, a loop full of each bacterial isolate was cultivated in 50 ml sterilized nutrient broth (NB). Cultures were placed on a rotary shaker at 180 rounds per minute (RPM) and 30°C until the culture turbidity has an optical density = (0.8). The bacterial growth was monitored by measuring the absorbance (turbidity) at different periods with the aid of spectrophotometer at 600 NM [21]. Bacterial concentrations were adjusted to 1.2×10^8 colony forming cell (CFU) using plating and dilution technique [22], then maintained and stored in refrigerator until use in this study.

Bacterial isolates were identified firstly to the genus level by Gram staining, shape, endospore formation and whether it were aerobic or anaerobic using the manual of Bergey et al. [23]. In detail: smear was prepared for each bacterial isolates, using Gram stain kit (Aromax, Jordan), smear was stained with crystal violet for one minute, and rinsed with water. The smear was then stained with iodine for one minute, followed by decolorization with ethanol and rinsing with water. The smear was then stained with safranin for one minute, rinsed with water, and observed under the microscope for shape of cells, the colors, whether pink-red or purple-blue in gram staining [24]. For endospore-forming staining: smear was stained with malachite green (Aromax, Jordan) and remained under steam for 5 minutes, followed by rinsing with water. The smear was then stained with safranin for one minute, rinsed with water, and observed under microscope to check whether the spores were green and the vegetative cells were red [25]. Finally, bacteria were observed whether it was aerobic or anaerobic by inoculating bacteria in a test tube contain liquefied media, then solidified it with ice cold water and incubate at 25°C [22]. For species identification, bacteria were identified to the species level with VITEK® BCL Test Kit (BioMerieux Direct, USA) [26].

**In vitro Antifungal Activity of the Bacterial Isolates against** *S. sclerotiorum*. The antifungal activity of the bacterial isolates (U1-U5) against *S. sclerotiorum* mycelial growth and Sclerotia formation was performed by the dual culture technique [27]. This experiment was performed by using two kinds of culture media, PDA and NA. Bacterial culture was inoculated as a line near the Petri dish periphery by a micropipette then incubated at 30°C for 24 hours. Mycelial plug (8 mm) was cut from the margins of an active fungal growth, and then transferred to the centre of these plates. Plates with fungal plug and with a line of only broth medium serve as a control. Plates were incubated at 25°C and dark until the full growth in the control plates was reached [28]. Mycelial growth inhibition was measured by calculating parallel lines extending from the edge of the growth to the end of the plate. Growth inhibition percentage over the control was calculated by using the Equation:

\[ \text{Inhibition\%} = \frac{Y^c - T}{Y} \times 100(A) \]

Whereas Y: the average growth distance (cm) of control and T: the average growth distance (cm) of treatment. Also, the number of Sclerotia formed in each plate was recorded after the end of two weeks to investigate the Sclerotia formation inhibition. Sclerotia formation inhibition was calculated as mentioned by Vincent [29] using the Equation:

\[ \text{Inhibition\%} = \frac{F - E}{E} \times 100(B) \]

Whereas F: the average number of Sclerotia formed in control plate and E: the average number of Sclerotia formed in treatment plate. Each treatment was performed in five replicates. Finally, the bacterial isolates that showed the highest inhibitory activity (isolates U2, U3 and U5) were chosen for the next investigation.

**The in vivo Investigation of the Antifungal Activity under Greenhouse Conditions.** Suspensions of bacterial cells of U2, U3 and U5 were prepared as follows: A 50 ml of prepared bacterial culture was centrifuged (6000 RPM for 15 minutes), then washed with SDW, and resuspended in 50 ml of SDW to obtain the same origin volume without bacterial metabolites and media. The fungal inoculum was prepared as described by Wang *et al.* [30] with a slight modification. Mycelial plugs (8 mm) were inoculated in sterilized potato dextrose broth (PDB) and put under orbital shaker at 80-100 RPM and 20±2°C for 7 days. The produced mycelial mat was collected and resuspended in SDW. Mycelia were homogenized by the aid of a sterile blender to make homogenized fungal inoculum, and kept under 4°C until use.

Squash seeds (‘Anita’, Petseed (PS) Sharikat Al-Asmida, Jordan) were disinfected with 3% sodium hypochlorite for 3 minutes, then rinsed with SDW twice and allowed to dry under laminar flow. Sterilized seeds were Inoculated with bacteria suspension as described by Abdelazeez *et al.* [31]. The sterilized seeds were steeped in the bacterial suspensions mixed with 10% Arabic gum for one hour and dried in sterile Petri dishes under laminar, seeds steeped only in 10% Arabic gum solution were included as control treatments. In addition. Also, seeds pre-treated with Thiram fungicide were used as positive controls. One third filled pots with sterile peat moss were inoculated with mycelial suspension at the ratio of 10 ml/pot. After an incubation period of 3 days at (20±2°C) in the dark, four seeds were located equidistantly on the pots, then pots were refilled with sterilized peat moss and placed under greenhouse conditions with regular
irrigation. The number of survived seedlings was recorded during the experiment to calculate the survival percentage by applying the equation:

\[
\text{Survival} \% = \frac{s}{r} \times 10^{(C)}
\]

Whereas R: the total squash seed number and S: the survived squash seed number. Seeds that germinated and emerged up considered as survived [32]. Five weeks later, the survived plants were uprooted, and the morphological characters (e.g. Root and plant length (cm), fresh weight (g)) were recorded. Pots with seeds treated by only gum Arabic and untreated peat moss served as an absolute control. Pots inoculated only with the pathogen served as pathogen control. Pots with seeds treated by Arabic gum and antagonistic bacteria, but peat not treated serve as a positive control while pots with peat treated with E15 or S23 and seeds treated by only Arabic gum serve as a pathogen (negative) controls. The experiment was arranged in a randomized complete block design (RCBD) under greenhouse conditions (temperature 25±2 °C, air condition and relative humidity RH= 85%). The experiment was performed twice; each treatment includes five replicates (pots) with four seeds per replicate (twenty seeds per treatment in total).

**Experimental Design and Statistical Analysis.** In this study, the experimental design was a randomized complete design (RCD) for the in vitro study while the in vivo under-greenhouse experiment was arranged in a randomized complete block design (RCBD). The data were analyzed by standard analysis of variance (ANOVA) one way and two way. The mean values among treatments were compared to each other by Fisher’s least significant difference (LSD) at \( p \leq 0.05 \) level of significance using Microsoft Office 2010 software.

**RESULTS**

**In vitro Antifungal Activity of the Bacterial Isolates against S. sclerotiorum.** Five bacterial isolates were investigated for their antifungal activity against S. sclerotiorum S23 and E15 by using the dual culture technique. All bacterial isolates showed significant inhibition of mycelial growth and sclerotia formation over the control (Figure 1). Ranges of inhibition varied according to the fungal and bacterial isolates, and media. Results are shown in Table (1) and Table (2). The mycelial growth inhibition in PDA appeared more pronounced than in NA against S23 cultures, while the opposite was observed and more significantly against E15 (Table 2). Generally, it can be also observed from Table (1) that bacterial isolates U2, U3 and U5 showed the highest inhibitory. As such; these isolates were chosen to carry out further experiments.

**Bacteria Identification.** Bacteria isolates (U1-U5) were originally identified to the genus level by Gram staining, shape, spore formation and Oxygen requirement; these isolates were found to be aerobic Gram-positive endospore-forming rods, and so were identified as Bacilli. Moreover, U2, U3 and U5 were identified to their species level using BCL Test Kit VTK2 (BioMerieux Direct, USA) as *Bacillus subtilis*.

**TABLE I**

*In vitro* mycelial growth and sclerotia formation inhibition of *S. sclerotiorum* S23 and E15 by five antagonistic bacterial isolates (U1-U5) using two types of media, PDA and NA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mycelial growth inhibition (%)</th>
<th>Sclerotia formation inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA media</td>
<td>NA media</td>
</tr>
<tr>
<td>S23 with U1</td>
<td>51±2.4 (bc)</td>
<td>47±1.5 (c)</td>
</tr>
<tr>
<td>S23 with U2</td>
<td>53±2.0 (b)</td>
<td>51±2.3 (a)</td>
</tr>
<tr>
<td>S23 with U3</td>
<td>61±3.3 (a)</td>
<td>51±1.9 (a)</td>
</tr>
<tr>
<td>S23 with U4</td>
<td>47±1.2 (c)</td>
<td>44±2.4 (c)</td>
</tr>
<tr>
<td>S23 with U5</td>
<td>60±2.8 (a)</td>
<td>50±1.7 (b)</td>
</tr>
<tr>
<td>S23 (control)</td>
<td>0±0.0 (d)</td>
<td>0±0.0 (d)</td>
</tr>
<tr>
<td>E15 with U1</td>
<td>47±0.7 (b)</td>
<td>86±1.1 (a)</td>
</tr>
<tr>
<td>E15 with U2</td>
<td>49±0.8 (a)</td>
<td>87±0.9 (a)</td>
</tr>
<tr>
<td>E15 with U3</td>
<td>49±1.0 (a)</td>
<td>88±2.3 (a)</td>
</tr>
<tr>
<td>E15 with U4</td>
<td>46±0.4 (b)</td>
<td>82±1.5 (a)</td>
</tr>
<tr>
<td>E15 with U5</td>
<td>49±1.9 (a)</td>
<td>88±2.8 (a)</td>
</tr>
<tr>
<td>E15 (control)</td>
<td>0±0.0 (d)</td>
<td>0±0.0 (d)</td>
</tr>
</tbody>
</table>

* Values represent inhibition % over 7 days incubation periods ± standard deviation, and calculated by applying Equation A.
** Values represent the inhibition % over 14 day incubation period ± standard deviation, and calculated by applying Equation B. Means were calculated as an average of five replicates. Means followed by the same letter within a column are significantly not different according to Fisher’s least significant difference (LSD) test at \( p < 0.05 \). (U1-U5) are isolates of antagonistic bacteria, E15 and S23 are *S. sclerotiorum* isolates. (-) Indicates that no Sclerotia formed within the period of incubation.
TABLE 2

*In vitro* mycelial growth and sclerotia formation inhibition of *S. sclerotiorum* S23 and E15 by five antagonistic bacterial isolates (U1- U5) with the role of two types of media (PDA and NA)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mycelial growth inhibition (%)*</th>
<th>Sclerotia formation inhibition (%***)</th>
<th>PDA media</th>
<th>NA media</th>
<th>PDA media</th>
<th>NA media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Against S23</td>
<td>54±6.4(a)</td>
<td>48±3.4(b)</td>
<td>65±7.3(b)</td>
<td>73±6.2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Against E15</td>
<td>48±1.6(b)</td>
<td>86±0.5(a)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values represent the mean of inhibition % of all the bacterial isolates± standard deviation over 7 day incubation period, and calculated by applying Equation A. ** Values represent the mean of inhibition % of all the bacterial isolates± standard deviation over a 14 day incubation periods, and calculated by applying Equation B. Means were calculated as an overall average of (U1-U5) replicates to measure the effect between groups against S23 and E15. Means followed by the same letter within a row are significantly not different according to Fisher’s least significant difference (LSD) test at P<0.05. E15 and S23 are *S. sclerotiorum* isolates. (-) Indicate that no Sclerotia formed within the period of incubation.

![Figure 1](image-url)  
**FIGURE 1**

Effect of antagonistic bacterial isolates (U1-U5) against *S. sclerotiorum* S23 on PDA media by dual culture technique
In vivo observations of fungal resistance of squash seed bacterized with *B. subtilis* against *S. sclerotiorum* S23 and E15 under greenhouse conditions: (A) shoot view in pots of seeds treated with S23 alone (top) and E15 alone (down), (B) treated with S23 and U3 (top) and E15 and U3 (down), (C) roots of seeds treated with S23 and U3 (top) and E15 and U3 (down), (D) treated with S23 alone (top) and E15 alone (down).

**TABLE 3**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Survived plants percentage (%) *</th>
<th>Growth parameters: **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh Shoot Weight (g)</td>
</tr>
<tr>
<td>S23+Thiram</td>
<td>65±1.0 (AB)</td>
<td>10.66±1.4(A)</td>
</tr>
<tr>
<td>S23+U2</td>
<td>40±1.4(B)</td>
<td>09.43±2.0(A)</td>
</tr>
<tr>
<td>S23+U3</td>
<td>55±1.2(B)</td>
<td>10.21±1.6(A)</td>
</tr>
<tr>
<td>S23+U5</td>
<td>55±1.3(B)</td>
<td>09.07±1.9(A)</td>
</tr>
<tr>
<td>S23(alone)</td>
<td>10±0.5(C)</td>
<td>04.75±1.1(B)</td>
</tr>
<tr>
<td>E15+Thiram</td>
<td>80±1.0(AB)</td>
<td>10.61±1.3(A)</td>
</tr>
<tr>
<td>E15+U2</td>
<td>65±3.9(AB)</td>
<td>10.02±1.8(A)</td>
</tr>
<tr>
<td>E15+U3</td>
<td>70±0.7(AB)</td>
<td>10.27±1.7(A)</td>
</tr>
<tr>
<td>E15+U5</td>
<td>65±1.2(B)</td>
<td>10.63±2.1(A)</td>
</tr>
<tr>
<td>E15(alone)</td>
<td>10±0.5(C)</td>
<td>04.90±2.2(B)</td>
</tr>
<tr>
<td>U2 (alone)</td>
<td>10±0.0(AB)</td>
<td>11.01±2.1(A)</td>
</tr>
<tr>
<td>U3 (alone)</td>
<td>10±0.0(AB)</td>
<td>11.60±2.0(A)</td>
</tr>
<tr>
<td>U5 (alone)</td>
<td>95±4.0(AB)</td>
<td>11.03±2.2(A)</td>
</tr>
<tr>
<td>C</td>
<td>100±0.0(AB)</td>
<td>11.31±1.9(A)</td>
</tr>
</tbody>
</table>

* Values were calculated by applying Equation E (section 3.7). ** Values represent the mean values ± standard deviation measured for survived plants. C is the absolute control. The mean was calculated as an average of five replicates. Means followed by the same letter within a column are significantly not different according to Fisher’s least significant difference (LSD) test at *P* < 0.05. U2, U3 and U5 are isolates of antagonistic bacteria *Bacillus subtilis*, E15 and S23 are *S. sclerotiorum* isolates.
The in vivo Investigation of the Antifungal Activity under Greenhouse Conditions. The efficacy of U2, U3 and U5 isolates as a biological control on squash seeds against S. sclerotiorum isolates (S23 and E15) was investigated as a seed bacterization under greenhouse conditions. The results shown in Table (3) indicate that the measured parameters of plant treated with pathogen and antagonists were slightly lower than those treated by pathogen and Thiram as a fungicide, but significantly higher than those treated with only fungal isolates (pathogen control). In addition, the plant treated with bacterial isolates only showed the highest values of the measured parameters among the treated plants, and were same as those in absolute control. Figure (2) shows plants that were grown after their treatment either with fungal isolates alone (Panels A and D), or in combination with the bacterial isolates (Panels B and C). The former one (A) showed pots of the pathogen control any germinated seeds or rarely germinated but with diminished growth (B), while the latter showed a more obvious growth in contrast.

DISCUSSION

Biological control of S. sclerotiorum has been demonstrated in numerous studies. This disease has been reported to infect cultivations in Jordan valley [8,33]. S. sclerotiorum S23 and E15 are fungal isolates characterized by the high germination percentage and aggressiveness [8]. The present study makes an evaluation for five indigenous isolates against S23 and E15. Bacterial isolates were isolated from the external surface of S. sclerotiorum sclerotia associated with infected plants and soil of growing fields in the Jordan valley. The best biological control thought to be that one originated from the natural-occurring organisms since these organisms are adapted to the environmental conditions such as temperature, pH and humidity [34].

Inhibition of mycelia and Sclerotia represent an important way for preventing and limiting disease spread. The in vitro activity of the bacterial isolates (U1-U5) against mycelial growth and Sclerotia formation of S. sclerotiorum isolates (S23 and E15) showed interesting results, on both mycelial growth and Sclerotia formation. The results appeared in Table (1) indicate that all bacterial isolates inhibited the mycelial growth and sclerotia formation to a certain extent, with highest inhibitory activity obtained for U2, U3 and U5. Furthermore, as all bacterial isolates caused an inhibition zone when combined with the fungi, this may suggest that the antibiotic play a role as a mechanism for antifungal activity [35]. Antibiosis is important comparing to other mechanism because of its rapid diffusible nature [36]. These results were agreed with those obtained by Walia et al. [37] who found that the inhibitory rate of Bacillus subtilis CKT1 reached 75% against S. sclerotiorum in vitro. Also, these results were similar to those obtained by Zhang et al. [38] who reported that B. subtilis caused the inhibition zone up to (2) cm against S. sclerotiorum.

Media is a key factor affecting quantitative and qualitative production of bacteria biomass and its metabolites, so it is worth mentioning that according to the culture media used in this experiment (PDA or NA) some variations were observed in the results (Table 2). This may indicate that the media affects the bacterial or fungal growth, as has been presumed by Gilbert and Lindeman[39], and by Savchuk [40], in which the latter has supposed that media composition can affect the bacterial or fungal growth.

U2, U3 and U5 bacterial isolates were consequently chosen to undergo further investigations, including species identification and the in vivo investigation of the biological control under greenhouse conditions. U2, U3 and U5 were identified by their species level and found to be B. subtilis. Fortunately, B. subtilis is a human non-pathogenic bacterium and known as a powerful biocontrol agent because of its high spore production, and the fact that the bacterial spores are commonly resistant to heat, desiccation, and U.V light (15). Moreover, B. subtilis is an agent that has antifungal activity against many plant fungal pathogens by multiple mechanisms such as antibiotic, enzymatic activity (such as chitinase), and antifungal volatiles (such as Acetoin) [41,42,43,44].

In this study, the potential of B. subtilisU2, U3 and U5m biological control against the pathogen S. sclerotiorum was evaluated in vivo under greenhouse conditions by seed bacterization. The in vivo experimental part is important, it has been reviewed and not surprised that In vitro assays may be limited by many reasons. In addition, biological control potential may not have the same expression or even may do not correlate In vivo conditions [45,46]. Generally, Seed treatment consider as effective against many problems include seed decay, seedling diseases and soil pathogen [47,48]. The strategy of seed bacterization has been identified for protecting seed from being infected by Sclerotinia[49], also Bacillus spp. has become a safe biocontrol agent, especially as seed protectants [50].Results showed successful seedling growth parameters of a squash plant treated with bacterial isolates, while the untreated seeds mostly none germinated or showed trivial growth (Table 3 and Figure 2). Comparable results have been reported in the literature with similar experiments.Errakhi et al.[51] demonstrated the capability of antagonist to protect sugar beet by preventing the root rot disease caused by Sclerotinia rolfsii incidence up to 63%. In another study, Hernández-Suárez et al. [16] found that B. subtilis strains protect tomato seeds
by reducing disease incidence and severity caused by *Rizoctonia solani* and *F. oxysporum*. Elias et al. [52] found that the antagonist suppresses *S. sclerotiorum* the causal agent of lettuce drop disease under greenhouse conditions this suppression represented by survived seedling and dry mass over the pathogen control. The high percentages of the survived plants and plant growth parameters over the pathogen control may indicate that these *B. subtilis* isolates were effective as a seed treatment in the suppression of the soil borne pathogen *S. sclerotiorum* under greenhouse conditions in both pre- and post-emergence stages. The positive control seedlings (squash seeds treated by *B. subtilis* alone) were observed to survive and grow like the absolute control (Table 3). This indicates that these bacteria may not cause harmful effect on the survived plant percentage or growth parameters. This has been similarly reported by El-Tarabily et al.[53] who found that treating lettuce plants with *Streptomyces spp.* alone was not harmful.

The results of the in vivo investigation were related with those obtained in vitro for U2, U3 and U5 by inhibiting the disease infection and development (Table 1, Table 3). Trojo-Estrada et al. [54] reported a similar relationship between in vitro and in vivo investigation. Results in this experiment look promising as no disease symptoms were detected in the survived plants in all bacterial isolates treatments, this may indicate that these isolates were powerful as a biological control agent.

**CONCLUSION**

*In vitro* results were examined for their applicability by in vivo investigation on squash plants under greenhouse condition. The disease was suppressed as the survived plants numbers and their characteristics were obviously higher than those in the pathogen controls. The achieved results indicate that *B. subtilis* could be a successful alternative to the chemical fungicides as a solution for the indicated agricultural problem.

**ACKNOWLEDGEMENTS**

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INVESTIGATION OF TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT POTENTIALS OF SOME VETCH GENOTYPES (VICIA SP.) GROWN IN TURKEY

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ABSTRACT

The aim of this research was to investigate the total phenolic contents and antioxidant potentials of some vetch genotypes obtained from seed methanol extracts which are cultivated in different areas of Turkey. In this study, five genotypes from three vetch species (common vetch (Vicia sativa L.), Hungarian vetch (Vicia pannonica Crantz.) and Narbon vetch (Vicia narbonensis L.)) were used as materials. The total phenolic content (TPC) of the samples was determined by using Folin-Ciocalteu’s reagents and the antioxidant power determined by using DPPH* scavenging activity and ferric-reducing antioxidant activity (FRAP) assays. According to the research results, the TPC values of extracts were determined between 14.91- 31.63 mg GAE g⁻¹. The Narbon vetch genotypes had the lowest TPC and antioxidant capacity, while Hungarian vetch genotypes had higher total phenolic content and more DPPH* scavenging activity. The interaction of species × genotypes was found significant at level of P< 0.05. The differences between the total phenolic content and antioxidant activity potentials of the species indicate that the species with high phenolic content and antioxidant activity can be evaluate in terms of feed technology studies for animal nutrition, animal welfare, and meat quality.

KEYWORDS:
Common vetch, Hungarian vetch, Narbon vetch, total phenolic constitutes, FRAP.

INTRODUCTION

The Vicia L., which has nearly 190 species in the world, has approximately 66 species, 27 subspecies and 29 varieties have been identified in Turkey [1, 2]. In Turkey, this genus is one of the most common cultivated forage crops [3]. Common vetch (Vicia sativa L.) mainly cultivated as a winter crop in the Mediterranean coastal region of Turkey and some cultivated varieties can be intensively used for high-quality animal feeding [4]. Hungarian vetch (Vicia pannonica Crantz) is a cool season forage legume species, able to survive freezing without any damage, as well as water-efficient and drought-tolerant, and can be grown in every region in our country [5]. Narbon vetch (Vicia narbonensis L.), which is an annual legume species and tolerant to cold and drought stresses, has been cultivated for many years in Turkey, and it is readily found among native flora of Turkey [4, 6].

In recent years, product safety and animal welfare are some of the most discussed topics in modern animal feeding and recent studies have a significant impact on the roles of polyphenols and antioxidants to improve meat quality and animal welfare [7, 8]. Polyphenol compounds are produced as secondary metabolites from plants and they have several positive effects on humans and animals. In the intestinal tract, these compounds help to proliferate of useful bacteria against pathogenic bacteria [9]. Polyphenol constituents possess important roles to develop a better health status of the animals [10]. They can also have a positive influence on the gut of animals [11]. Dietary supplementation with a rich source of phenolic compounds significantly decreased some pathogenic bacteria in animals [12]. Although the antioxidants can be used in commercial feeds [13], there is a consideration for research the new sources of antioxidant materials from plants in animal feeding to improve the meat quality [8].

The antioxidant potential of common vetch has also been explored by Amarowicz et al [14]. A recently reported study revealed that common vetch extracts inhibited lipid peroxidation, comparable to the soybean extracts [15]. However, any previous literature on the comparison of total phenolic content and antioxidant activities in seeds of V. sativa L., V. pannonica Crantz and V. narbonensis L. species has not been found. The scope of this research was the determination of the total phenolic constituents and antioxidant potential of seeds from fifteen vetch genotypes belonging to V. pannonica Crantz., V. narbonensis L. and V. sativa L. species which represent the diversity of the Vicia species in Turkey.
MATERIALS AND METHODS

Material. The seed materials were provided from Field Crops Department of Namık Kemal University. Common vetch (V. sativa L) genotypes Selçuk (CVs), Orakfe (CVOE), Doruk (CVD), Urkmez (CVU) and Alper (CVA); Hungarian vetch (V. pannonica Crantz) genotypes 56.3 (Hv56.3), Altmova (HvA), 47.2 (Hv47.2), Ege Beyazı (HvEB) and Narbon vetch (V. narbonensis L.) genotypes Sarıfe (HvSE), 236 (Nv236), Bozdağ (NvB), Özgen (NvO), Karakaya (NvK) and Dikili (NvD) were used in the study (Figure 1). The seeds were sampled from harvested plants at the dry matured stage in July.

Extraction of samples. Dried seed samples were milled with a laboratory mill and extraction were done by using methanol 80 % (v/v) as the solvent. Sample: extraction ratio was 1:10 (v/w), and suspensions were placed in a shaking water bath heated to 70 °C for 15 min. The extractions were repeated three times and solvents were evaporated under vacuum using a rotary evaporator. Samples were lyophilized with a Labconco freeze dryer (FT 33; Armfield, UK) for analyses.

Total phenolic content (TPC). TPC of vetch samples were determined by using with Folin-Ciocalteu’s reagent (725 nm; Hitachi U-2000 spectrophotometer, Model 121-0002), results were expressed as mg gallic acid equivalents (GAE) per g of extract [16].

The DPPH radical scavenging activity. This activity was determined according to method described by Brand-Williams et al [17]. EC50 values were deducted from the plots for determination of the extract concentration of needed to scavenging 50 % of the initial DPPH.

The ferric-reducing antioxidant power (FRAP). FRAP assayed as previously described by Benzie & Strain [18] and FRAP values of samples were calculated and expressed as µmol Fe2+ equivalent (FE) per g extract by using the calibration curve of Fe2+.

Statistical analyses were done at three analytical repetitions. The significance levels of means were evaluated by the least significant difference (LSD) test. Differences were considered to be significant at P < 0.05 level. Data were analyzed by using the MSTAT-C statistical computer package to evaluate the differences. The relationships between analyzed characters of vetch genotypes were calculated as the Pearson correlation coefficient.

RESULTS AND DISCUSSION

Extraction yields were determined from the mass of prior extraction and the mass of extract obtained after removal of solvent. The extraction yield of vetch seed in methanol extract varied from 6.15 % (Nv236) to 9.70 % (HvSE). As seen from Table 1, the lowest extract yield was obtained from V. narbonensis. TPC of vetch genotypes ranged between 15.46 mg GA equivalents (g-1 extract) and 31.63 mg GA equivalents (g-1 extract) in methanol extracts (Table 1). According to mean values of phenolic content, V. pannonica showed the highest TPC value and decreased in the following order; V. pannonica > V. sativa > V. narbonensis. Pastor & Cavada [19] determined the V. sativa was attracted with highest polyphenol constitutes of all 28 Vicia genotypes and V. narbonensis showed the lowest polyphenol concentration. These results were similar to our findings. The demonstrated TPC values of V. sativa were also slightly higher than the results (23.0 µg GA mg-1) obtained by Kyung Jun et al. [20].

The DPPH radical scavenging activity results of vetch genotypes are given in Table 1 and Figure 2, according to EC50 values and their % inhibition activities. The lower EC50 value of DPPH radical scavenging activity gave the higher antioxidant activity of a sample. The scavenging activity of each vetch extract was tested according to the concentration range of 0.2-0.8 mg assay-1. The inhibitory activity of samples depends on concentration as seen in Figure 2. The highest increase in antiradical...
activity towards DPPH with increasing extract content was noted in HvSE genotype. At the highest assayed concentration the percent DPPH inhibitory activity of HvSE, NvO, Hv47.2, Hv56.3 were 65.3 %, 55.5 %, 49.0 %, and 46.9 %, respectively. The lowest inhibition effect against DPPH was found in NvD and in NvK genotypes. The activity of α-tocopherol was 69.8 % at 0.4 mg assay⁻¹, and 89.9 % at 0.6 mg assay⁻¹ when compared to standards compounds (Figure 2).

**FIGURE 2**

DPPH scavenging activity of vetch genotypes at different concentrations.

Data are expressed as the mean ± standard deviation (n = 3); bars having different letters differ significantly (P<0.05). Mean values: *V. sativa*: 36.64, *V. pannonica*: 49.54, *V. narbonensis*: 26.38. (LSD 5 % = 0.559)

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Extraction yield (%)</th>
<th>TPC (^{ab}) (mg GAE g⁻¹)</th>
<th>DPPH (^c) (EC (^\text{50})) (mg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. sativa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selcuk (CvS)</td>
<td>8.22</td>
<td>25.47 ± 0.33 see</td>
<td>0.32 ± 0.03 e</td>
</tr>
<tr>
<td>Orakefi (CvOE)</td>
<td>8.97</td>
<td>28.47 ± 1.65c</td>
<td>0.30 ± 0.04e</td>
</tr>
<tr>
<td>Doruk (CvD)</td>
<td>8.06</td>
<td>25.14 ± 1.42a</td>
<td>0.54 ± 0.02 e</td>
</tr>
<tr>
<td>Urmeyz (CvU)</td>
<td>7.71</td>
<td>28.17 ± 0.07d</td>
<td>0.38 ± 0.03f</td>
</tr>
<tr>
<td>Alper (CvA)</td>
<td>8.41</td>
<td>24.39 ± 0.53e</td>
<td>0.47 ± 0.05e</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>8.27</strong></td>
<td><strong>26.33</strong></td>
<td><strong>0.41</strong></td>
</tr>
<tr>
<td>56.3 (Hv56.3)</td>
<td>7.53</td>
<td>31.63 ± 1.52a</td>
<td>0.25 ± 0.09</td>
</tr>
<tr>
<td>Altinova (HvA)</td>
<td>9.32</td>
<td>30.56 ± 0.52b</td>
<td>0.37 ± 0.02f</td>
</tr>
<tr>
<td>47.2 (Hv47.2)</td>
<td>9.12</td>
<td>29.06 ± 1.32c</td>
<td>0.24 ± 0.02f</td>
</tr>
<tr>
<td>Saraf (HvSE)</td>
<td>9.70</td>
<td>25.52 ± 0.66d</td>
<td>0.17 ± 0.01f</td>
</tr>
<tr>
<td>Ege Beyazi (HvEB)</td>
<td>8.02</td>
<td>26.86 ± 0.59c</td>
<td>0.34 ± 0.04f</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>8.74</strong></td>
<td><strong>28.72</strong></td>
<td><strong>0.27</strong></td>
</tr>
<tr>
<td><em>V. pannonica</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>236 (Nv236)</td>
<td>6.15</td>
<td>17.99 ± 0.90c</td>
<td>0.70 ± 0.05c</td>
</tr>
<tr>
<td>Bozdog (NvB)</td>
<td>7.94</td>
<td>14.91 ± 0.33a</td>
<td>0.87 ± 0.11b</td>
</tr>
<tr>
<td>Ozgen (NvO)</td>
<td>6.84</td>
<td>20.55 ± 0.13b</td>
<td>0.26 ± 0.02b</td>
</tr>
<tr>
<td>Karakaya (NvK)</td>
<td>8.15</td>
<td>19.29 ± 0.33c</td>
<td>0.98 ± 0.07b</td>
</tr>
<tr>
<td>Dikili (NvD)</td>
<td>8.05</td>
<td>15.46 ± 0.46d</td>
<td>3.09 ± 0.20e</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>7.42</strong></td>
<td><strong>17.64</strong></td>
<td><strong>1.18</strong></td>
</tr>
<tr>
<td>LSD 5 %</td>
<td>0.365</td>
<td>0.040</td>
<td></td>
</tr>
</tbody>
</table>

* Data are statement as the mean ± standard deviation (n=3).

Values in the same column having different letters differ significantly (P<0.05).

\(^c\) \(\text{EC}_{50}\) value of DPPH for α-tocopherol determined as; 0.010 ± 0.02 mg mL⁻¹.
FIGURE 3
Ferric-reducing antioxidant power (FRAP) of vetch genotypes.
Data are expressed as mean ± standard deviation (n = 3). Bars having different letters differ significantly (P<0.05). Mean values: V. sativa: 0.46\(^6\), V. pannonica: 0.41\(^8\), V. narbonensis: 0.31\(^8\). (LSD 5% = 0.27.32)

TABLE 2
Correlation coefficients values (r\(^2\))

<table>
<thead>
<tr>
<th></th>
<th>TPC</th>
<th>FRAP</th>
<th>DPPH(^*)(EC(_{50}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>1</td>
<td>0.612**</td>
<td>-0.517**</td>
</tr>
<tr>
<td>FRAP</td>
<td>1</td>
<td>1</td>
<td>-0.767**</td>
</tr>
<tr>
<td>DPPH(^*)(EC(_{50}))</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** shows significant at P< 0.05

According to the EC\(_{50}\) values of DPPH\(^*\) scavenge activity, while the lowest EC\(_{50}\) value was found in HvSE (0.17 mg mL\(^{-1}\)), the highest value was determined in NvD extract (3.09 mg mL\(^{-1}\)) (Table 1). Following HvSE, the highest DPPH\(^*\) radical scavenging activities were determined in Hv47.2 (0.24 mg mL\(^{-1}\)), Hv56.3 (0.25 mg mL\(^{-1}\)), and NvO (0.26 mg mL\(^{-1}\)). The EC\(_{50}\) value was determined as 0.010 for standard compounds \(\alpha\)-tocopherol. The EC\(_{50}\) value of HvSE seems to be nearly one-seventeen of the activity of \(\alpha\)-tocopherol. According to statistical mean values, it could be seen significant differences among genotypes (P < 0.05) and DPPH\(^*\) scavenge activity in the following order; V. pannonica > V. sativa > V. narbonensis.

Antioxidant compounds can act as reductants, and Fe\(^{3+}\) reducing potential of extract to Fe\(^{2+}\) could be determined by FRAP assay. While the highest activity was determined in HvSE (0.53 mmol Fe\(^{2+}\) g\(^{-1}\)), the lowest TEAC activity was found in the extract in NvD (0.24 mmol Fe\(^{2+}\) g\(^{-1}\)) and in NvB (0.25 mmol Fe\(^{2+}\) g\(^{-1}\)). The results of the FRAP assay of the fifteen genotypes of vetch reveal that the common vetch genotypes had higher activity than Hungarian and Narbon vetch. When compared with standard compounds, HvSE extract exhibited 7.85 fold lower activities than \(\alpha\)-tocopherol (Figure 3). The evaluation of the strength of FRAP activity of vetch genotypes showed that the mean value of V. sativa had the highest value and it was followed by V. pannonica and V. narbonensis, respectively (Figure 3).

According to Pearson's correlation analyses, TPC was strongly correlated with determined antioxidant activity assays. However, the highest significant correlation was found with FRAP (r = 0.612**, P < 0.05) (Table 2). The relationship between TPC and EC\(_{50}\) values of DPPH\(^*\) scavenge activity was also significant (r = 0.517**, P < 0.05). On the other hand, FRAP showed significant relationship with DPPH\(^*\) (r = 0.767**, P < 0.05). Similar relationships were determined by Orak et al. [21] among TPC and FRAP in bean, and similar to FRAP and EC\(_{50}\) of DPPH\(^*\) scavenge activity in olive leaves [22].
CONCLUSIONS

This study discloses the total phenolic constituents and antioxidant potentials of methanolic extracts obtained from fifteen vetch genotypes from common vetch, Hungarian vetch and Narbon vetch. The relationships among the investigated characters were also determined by Pearson's correlation coefficients. Based on the analysis, genotypes can show similarity or differences in respect to analyzed characters. Some genotypes differentiated and showed high activity in their cluster. As a result, while Narbon vetch genotypes showed lower TPC and antioxidant capacity than other genotypes, Ozgen (NvO) was differentiated in this cluster with high FRAP and DPPH scavenging activity. Genotypes from Hungarian vetch seemed to have higher total phenolic constituents and more DPPH scavenging activity. Sarıefe (HySE) has attracted attention with the highest FRAP and DPPH scavenging activity, when compared similar genotypes, Urmaz (CuV) and Ozgen (NvO) also had attracted genotypes in their cluster according to FRAP activity.

These results demonstrate that the use of phenolic content and antioxidant assays can be a tool to assess which genotypes come forward with regard to high total phenolic content and antioxidant activity in order to help animal welfare and meat quality. However, in order to identify, to isolate, and to characterize these bioactive constituents from extracts and to illustrate their structure and to elucidate their exact antioxidant mechanism against various disorders are needed to research in further studies.

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SEDIMENTARY CHARACTERISTICS AND EVOLUTION OF THE UPPER PALEOZOIC COAL-BEARING STRATA IN THE XISHAN COALFIELD, EASTERN ORDOS BASIN, CHINA

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ABSTRACT

The Upper Paleozoic strata in the eastern North China is an important stratigraphic system of deep oil and gas exploration. For a long time, this field has not received much attention, and there are few studies on the sedimentary characteristics of the Upper Paleozoic coal-bearing strata in this area. In this paper, through the observation and measurement of the Upper Paleozoic profiles in the southwestern part of the Xishan coalfield, eastern Ordos Basin, combined with the previous research results, the sedimentary facies types and sedimentary evolution of the Upper Paleozoic in this area were systematically analyzed. The results show that the Upper Paleozoic strata in this area mainly developed sedimentary systems such as the barrier coastal facies, carbonate platform facies, delta facies, meandering facies and paroxysmal fluvial facies. The Benxi Formation and the Taiyuan Formation mainly developed the barrier coastal facies and carbonate platform facies deposits. The Shanxi Formation developed delta facies deposits, the Lower Shihezi Formation and Upper Shihezi Formation developed meandering river facies deposits, and the Shiqianfeng Formation developed paroxysmal fluvial facies deposits. The sedimentary evolution of the Upper Paleozoic strata mainly undergoes the process of transformation from marine sediments to marine-continental transitional sediments, which eventually transforms into continental sediments.

KEYWORDS:
Sedimentary evolution, sedimentary facies, Upper Paleozoic, Xishan Coalfield, eastern North China

INTRODUCTION

The eastern part of North China is an important oil and gas exploration area in China. The long-term exploration horizon has been concentrated in the shallow Cenozoic strata, and there has been little attention to the exploration of the Upper Paleozoic strata in this basin [1-7]. As the exploration of shallow oil and gas in this region gradually entered a highly mature stage, the deep upper Paleozoic in the region has become a realistic field of oil and gas exploration.

As the exploration work progressed, the Upper Paleozoic in this area did show great exploration potential. For example, the Huagu 102 well in the Jiyang Depression received an industrial oil flow in the Permian system, and the Hugu 2 well in the Dongpu Sag received an industrial gas flow in the Upper Permian Shihezi Formation and Shiqianfeng Formation [8-13]. However, the understanding of the sedimentary facies types and sedimentary evolution of the Upper Paleozoic strata in this area has not been clear, which restricts the in-depth exploration work. The Xishan area has a complete exposure of the Upper Paleozoic strata [14-16]. Therefore, through the detailed observation of the Upper Paleozoic outcrops in the Xishan area, the sedimentary facies types and sedimentary evolution processes of the Upper Paleozoic strata can be identified. This study is of great significance for the Upper Paleozoic oil and gas exploration in the coverage area of the eastern North China.

MATERIALS AND METHODS

Geological background. The Upper Paleozoic outcrops in the southwestern part of the Xishan area are located in the middle section of Luliang Mountain, southwestern part of Xishan Coalfield (Fig. 1). The Upper Paleozoic strata are well developed in the area, and the sedimentary boundaries between different strata are clear and continuous, which provides favorable conditions for the research.

Through the outcrop observation and the identification of the marker layers, and combined with the regional geological data, the Upper Paleozoic strata were divided into the Benxi Formation, the Taiyuan Formation, the Shanxi Formation, the Lower Shihezi Formation, the Upper Shihezi Formation and the Shiqianfeng Formation, from bottom
to top (Fig. 2) [17-20]. The Taiyuan Formation and the Shanxi Formation have developed several sets of stable thick coal seams, while other formations mainly developed some coal lines. The Upper Paleozoic strata in the study area are typical coal-bearing strata.

Methodology. In this paper, we conducted a field survey of the Upper Paleozoic strata in the southwestern part of Xishan area, eastern Ordos Basin [21-28]. There are several basic principles for the survey of field outcrops: (1) First, we need to find a complete outcrop profile; (2) Second, we should collect detailed geological information reflected in the field stratigraphic profile; (3) Finally, the sedimentary facies characteristics of different sites should be compared, and the sedimentary evolution law of the outcrops of the Upper Paleozoic strata in the Xishan area is summarized.

Through the field investigation of the outcrops, the color, mineral composition and rock type, rock structure, rock stratum structure and rock formation signs can be determined. These characteristics can help us determine the sedimentary environment, sedimentary facies types and their evolution laws of the Upper Paleozoic strata in the study area.

RESULTS

Sedimentary characteristics of the Benxi Formation. The bottom boundary of the Benxi Formation and the top of Ordovician are parallel unconformity. The top boundary of the Benxi Formation is the bottom of the Jinci sandstone, while the bottom is developed with iron mudstone and bauxite (Fig. 3a, b). Gray-black, dark-gray mudstone and silty mudstone are developed in order from the bottom of the Benxi Formation. The Benxi Formation mainly develops barrier coastal facies deposits. The barrier coastal facies of the Benxi Formation mainly develop lagoon facies deposition. The lagoon facies is a limited bay sedimentary environment influenced by the Ordovician bedrock paleotopography. Its lithology is mainly dark mudstone (still water environment) (Fig. 3c), in which iron nodules and paleontological fossils can be seen.
FIGURE 2
Comprehensive sedimentary histogram of the Upper Paleozoic strata in the study area.
Lithology and sedimentary characteristics of the Benxi Formation and the Taiyuan Formation.

Notes: (a) Iron mudstone, Benxi Formation; (b) Bauxite, Benxi Formation; (c) Gray-black silty mudstone, Benxi Formation; (d) Mud flat facies mudstone interbedded with thin sandstone, Taiyuan Formation; (e) Peat flat coal seam, Taiyuan Formation; (f) Mixed flat facies thin sand-mud interbed, Taiyuan Formation; (g) Sand flat facies sandstone interbedded with veined mudstone, Taiyuan Formation; (h) Lagoon facies gray black mudstone, Taiyuan Formation; (i) Tidal facies quartz sandstone, Taiyuan Formation; (j) Tabular cross-bedding in the tidal facies, Taiyuan Formation; (k) Mud interlayer between tidal facies single sand bodies, Taiyuan Formation; (l) Tidal facies sand body with positive sequence structure, Taiyuan Formation; (m) Barrier island facies quartz sandstone, Taiyuan Formation; (n) Barrier island sand body with anti-sequence structure; (o) Paleontological fossils developed in the carbonate platform facies, Taiyuan Formation.

Sedimentary characteristics of the Taiyuan Formation. The Taiyuan Formation in this area takes the bottom of the Jinci sandstone as the bottom boundary, and takes the top surface of the No. 6 coal as the top boundary. Its lithology includes sand-shale interbeds (clipping coal seams) and limestone [20, 29-30]. The Taiyuan Formation developed a barrier coastal facies and carbonate platform facies deposits.

Barrier coastal facies. The barrier coastal facies of the Taiyuan Formation in this area include the tidal flat facies, the lagoon facies, the barrier island facies and the tidal channel facies.

(1) Tidal flat facies. The tidal flats in this area include mud flat, peat flat, mixed flat and sand flat. The mud flat facies are developed above the average high tide line, and the lithology is dominated by dark gray mudstone, while the thin lenticular sand body is visible. The lenticular bedding is also developed in the rock (Fig. 3d), and the biological borehole structure can be seen. The peat flat facies is developed in a restricted environment with terrestrial debris input near the average high tide. They mainly develop of salt-tolerant plants similar to those of modern mangroves. After the plants die, they accumulate peat on the ground and form coal after burial (Fig. 3e). The mixed flat facies are developed between the average high tide line and the average low tide line, and its lithology is dominated by dark gray mudstone and fine sandstone interbed (Fig. 3f). The single layer thickness of mudstone and fine sandstone is about 20
cm, and there is a tidal rhythm bedding. Horizontal bedding is developed in mudstone. The sand flat facies are developed near the average low tide line, and it mainly developed fine sandstone interbedded with thin mudstone. The single layer thickness of sandstone is 15-20cm, and the thickness of single layer of mudstone is generally less than 5cm. A veined bedding is visible in the rock (Fig. 3g). The mud flats, mixed flats and sand flats appear in combination on the profile. It can be seen that the upward transition from the sand flat to the mixed flat and to the top mud flat reflects a slow sea receding process.

(2) Lagoon facies. The lagoon facies in the Taiyuan Formation are mainly restricted bay environments obscured by barrier islands, and their lithology is dominated by dark gray and gray-black mudstones [31]. Horizontal bedding and bioturbation structures are developed in the rock (Fig. 3h), and iron nodules and fossils can be seen. With the siltation of the lagoon, the top of the lagoon often has coal seams.

(3) Tidal channel facies. The tidal facies sandstone is repeatedly selected by the high tide and low tide, and its composition has high maturity. The lithology is dominated by quartz sandstone (Fig. 3i), and the bottom of the sand body develops a flushing surface. The bedding types of the tidal facies include plate-like cross-bedding (Fig. 3j), fluted cross-bedding and bimodal cross-bedding. The single tidal facies sand body has a lens-like shape, and it can be seen that a plurality of sand bodies is stacked vertically, and a thin layer of argillaceous interlayer is visible between the sand bodies (Fig. 3k). The composite sand body has a distinct positive grain order in the vertical direction (Fig. 3l).

(4) Barrier island facies. The barrier island sandstone is repeatedly selected by the action of tides and waves, and its compositional maturity is also high. The lithology of the barrier island facies is mainly quartz sandstone (Fig. 3m), which developed low-angle wedge-shaped cross-bedding and plate-shaped cross-bedding (Fig. 3n).

Carbonate platform facies. The lithology type of the carbonate platform facies is mainly bioclastic limestone, which is thin and generally does not exceed 1.5 m. Meanwhile, scorpions, brachiopods and echinoderms fossils can be seen in the rocks (Fig. 3o).

Sedimentary characteristics of the Shanxi Formation. The bottom of the Shanxi Formation is the top surface of the No. 6 coal, and its top boundary is the bottom surface of the camel neck sandstone. It mainly developed sandstone and mudstone interbedded with coal seams [18, 32-33]. The Shanxi Formation developed delta facies deposits, in which the delta plain and the delta front are more developed, and has a distinct anti-granular structure in the vertical direction (Fig. 4a).

1) Delta plain facies. The delta plain facies mainly consists of a distributary channel and a distributary bay. At the bottom of the distributary channel, the flushing surface can be seen, and the Sand grain cross-bedding (Fig. 4b), the plate-shaped cross-bedding, the trough-like cross-bedding and the plant stem fossils can be seen (Fig. 4c). The distributary bay includes natural levees and flood plains. Natural levees mainly developed thin interbedded fine sandstone and mudstone, and the thickness of a single thin layer does not exceed 3 cm (Fig. 4d). The flood plain mainly developed dark gray, gray-black mudstone. Moreover, the plant charcoal is developed in the rock and it is often interbedded with coal seam (Fig. 4e).

2) Delta front facies. The delta front facies include a distributary channel and a distributary bay. The distributary channel facies mainly developed thin sandstones, and the thickness of the sandstone is generally less than 1 m, which is often trapped in the mudstone (Fig. 4f). The distributary bay facies mainly developed dark gray, gray-black mudstone (Fig. 4g). Plant charcoal is developed in the rock, and the lens-like overflow sedimentary sandstone is visible (Fig. 4h).

Sedimentary characteristics of the Lower Shihize Formation and the Upper Shihize Formation. The bottom boundary of the Lower Shihize Formation is the bottom surface of the camel neck sandstone, and the top boundary is the top surface of the peach flower mudstone. The bottom boundary of the Upper Shihize Formation is the top surface of the peach flower mudstone, and its top boundary is the bottom surface of the K8 sandstone. The lithology of the Lower Shihize Formation and the Upper Shihize Formation are interbedded sandstone and mudstone rocks, and the sedimentary facies is a meandering river facies, including riverbeds, embankments, and flood plains.

1) River bed facies. The riverbed mainly developed thick-layer medium-grained sandstone (Fig. 5a), which forms a distinct binary structure with the floodplain mudstone (Fig. 5b). The scouring surface is developed at the bottom of the sand body, and the confined conglomerate deposits are visible, and the gravel carried by the underlying strata is often washed (Fig. 5c). There are various types of bedding in sandstone, including plate-like cross-bedding, trough-like cross-bedding and parallel cross-bedding. Plant stems and fossils can be seen (Fig. 5d). The sand body particle size generally has a thick bottom and a fine upper structure.
Notes: (a) Delta facies has an anti-grain structure, Shanxi Formation; (b) Sand cross-bedding developed in the distributary channel, Shanxi Formation; (c) Plant stem fossils developed in the distributary channel, Shanxi Formation; (d) Thin interbedded sand and mudstone in natural levee facies, Shanxi Formation; (e) Flood plain facies dark mudstone interbeded with coal seam, Shanxi Formation; (f) Distributary channel facies thin sandstone, Shanxi Formation; (g) Distributary bay facies dark mudstone, Shanxi Formation; (h) Distributary bay facies deposits and lens-like sandstone, Shanxi Formation.

**FIGURE 4**

Sedimentary characteristics of the Shanxi Formation.

Notes: (a) Riverbed facies thick sandstone, the Lower Shihezi Formation; (b) Riverbed facies and floodplain facies constitute a binary structure, the Upper Shihezi Formation; (c) Gravel in riverbed facies sandstone, the Lower Shihezi Formation; (d) Plant stem fossils in riverbed facies sandstone, the Lower Shihezi Formation; (e) Thin interbedded sand and mudstone in natural levee facies, the Upper Shihezi Formation; (f) Flood plain facies mudstone, the Upper Shihezi Formation; (g) Flood plain facies, Lenticular flood fan sand body, the Upper Shihezi Formation; (h) Floodplain lake facies dark mudstone, the Lower Shihezi Formation.

**FIGURE 5**

Sedimentary characteristics of the Lower Shihezi Formation and the Upper Shihezi Formation.
Sedimentary characteristics of the Shiqianfeng Formation.

Notes: (a) Riverbed facies conglomerate, the Shiqianfeng Formation; (b) Riverbed facies gravel-bearing coarse sandstone, the Shiqianfeng Formation; (c) Thin interbedded sand and mudstone in natural levee facies, the Shiqianfeng Formation; (d) Calcareous tuberculosis interbeded in the flood plain facies mudstone, the Shiqianfeng Formation.

(2) Embankment facies. The embankment facies deposits are usually developed in the riverbed facies sandstones. Its lithology is the interbedded thin sandstone and mudstone (Fig. 5c). This lithological combination reflects frequent alternating periods between flood and dry seasons. The thickness of the single layer of sandstone and mudstone is not more than 15 cm, and the climbing bedding can be seen in the sandstone.

(3) Flood plain facies. The floodplain facies is dominated by the over-shore mudstone during the flood period (Fig. 5f), and sometimes a thin layer of lens-shaped breaching fan sandstone (Fig. 5g) is visible. In the humid climate, floodplain can accumulate water to form floodplain lakes, and its lithology is dominated by dark gray and gray-black mudstone (Fig. 5h). At the same time, plant charcoal and aquatic fossils are visible in the rock.

Sedimentary characteristics of the Shiqianfeng Formation. The bottom boundary of the Shiqianfeng Formation is the bottom of the K8 sandstone, and its top boundary is the bottom boundary of the Liujiagou Formation. Its lithology is dominated by sand-shale interbeds. The most important feature is that both sandstone and mudstone are red by oxidation. During the sedimentary period of the Shiqianfeng Formation, due to the influence of the giant monsoon climate, the climate is extremely dry, and the seasons of precipitation are significantly different. Rivers are dominated by paroxysmal water flow, which develops paroxysmal rivers, including riverbeds, embankments, and flood plains.

(1) River bed facies. The paroxysmal water flow has strong handling capacity, resulting in coarse sediments in the riverbed. It mainly developed conglomerate, pebbly coarse sandstone and coarse sandstone (Fig. 6a, b), and has a large plate-like cross-bedding (Fig. 6b), trough-like cross-bedding and parallel cross-bedding. The paroxysmal water has a high shale content. After the rainy season, the river is dry and the mud in the sediment is oxidized, making the sandstone appear oxidized red.

(2) Embankment and flood plain facies. The embankment facies are appeared as an interbed between thin sandstone and mudstone (Fig. 6c), and the thickness of the monolayer generally does not exceed 20 cm. The floodplain facies deposits mainly developed brick red mudstone. Due to the climatic drought, evaporation is strong, and in some local areas, calcareous tuberculosis formed by evaporation was observed (Fig. 6d).

Sedimentary evolution analysis. After the Middle Ordovician, the North China Plate experienced a 138 Ma uplift and erosion, and then settled in the Late Carboniferous. During the sedimentary period of the Benxi Formation, the transgression occurred from the northeastern part of the basin to the interior of the basin, and a restricted epicontinental sea was developed in the North China plate. At this time, due to the influence of the ancient topography of the bedrock, the ancient lagoon was developed in the study area.
During the sedimentary period of the Taiyuan Formation, the basin pattern changed to a trend that sloped southward from the northern uplift. The seawater invades into the basin from the southeastern part of the basin, and the barrier coastal facies and the carbonate platform facies were deposited. The developed sand body includes the tidal flat facies, the tidal channel facies and the barrier island facies sand body.

During the sedimentary period of the Shanxi Formation, the seawater retreated to the south, and the delta facies are developed in this area. The sand body type is dominated by the distributary channel. During the deposition period of the Lower Shihezi Formation and the Upper Shihezi Formation, the seawater completely exited from this area. At this time, the meandering river facies in the background of the inland depression were developed in this area, and the sand body type was mainly the riverbed facies sand body. During the sedimentary period of the Shiqianfeng Formation, due to the influence of the giant monsoon climate, the seasonal variation of precipitation was significant, and the river has a strong burst effect. At this time, the sand body type was mainly conglomerate and pebbly coarse sandstone formed by paroxysmal water flow.

CONCLUSIONS

(1) The Upper Paleozoic in the southwestern part of the Xishan Coalfield developed the Benxi Formation, the Taiyuan Formation, the Shanxi Formation, the Lower Shihezi Formation, the Upper Shihezi Formation and the Shiqianfeng Formation. The sedimentary facies of the formation include the barrier coastal facies, carbonate platform facies, delta facies, meandering facies and paroxysmal fluvial facies.

(2) The Benxi Formation and the Taiyuan Formation mainly developed the barrier coastal facies and carbonate platform facies deposits. The Shanxi Formation developed delta facies deposits, the Lower Shihezi Formation and Upper Shihezi Formation developed meandering river facies deposits, and the Shiqianfeng Formation developed paroxysmal fluvial facies deposits.

(3) The Benxi Formation and the Taiyuan Formation developed marine sediments in the epicontinental sea background, the Shanxi Formation developed the marine-continental transitional sediments in the residual epicontinental sea background, and the Lower Shihezi Formation to the Shiqianfeng Formation developed terrestrial fluvial facies sediments. The sedimentary evolution of the Upper Paleozoic strata mainly undergoes the process of transformation from marine sediments to marine-continental transitional sediments, which eventually transforms into continental sediments.

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PHYTOPLANKTON OF BOZTEPE RECAI KUTAN DAM LAKE LOCATED IN UPPER EUPHRATES RIVER BASIN (EASTERN ANATOLIA)

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ABSTRACT

Seasonal changes in phytoplankton community of Boztepe Recai Kutan Dam Lake were investigated on samples taken from three stations between January 2014 and December 2014. The phytoplankton of Boztepe Recai Kutan Dam Lake consisted of 20 taxa belonging to Bacillariophyta (12 taxa), Chlorophyta (1 taxa), Chrysophyta (1 taxon), Cyanobacteria (2 taxa), Dinozoa (2 taxa) and Euglenozoa (2 taxa). Members of Bacillariophyta was commonly observed in January and August whilst Chlorophyta grew well in September. Chrysophyta only occurred and recorded in high numbers in September. Cyanobacteria became dominant in spring, at the end of summer and also in autumn. However, Dinozoa was remarkably conspicuous in spring and fall as well as in winter in terms of prevalence. Excessive growth of Cyanobacteria, which is a characteristic aspect of mesotrophic lakes, was observed in lake in both April and August. Euglenozoa also occurred at the end of summer and early autumn. Phytoplankton composition was appeared to be affected mainly by temperature, phosphate and nitrate. Secchi disk depth, concentrations of plant nutrients (TN, TP), chlorophyll-a analyzed in the present study supported the view that the Boztepe Recai Kutan Dam Lake could be included in mesotrophic lake category.

KEYWORDS:
Phytoplankton, nutrient, Boztepe Recai Kutan Dam Lake, Malatya, Turkey

INTRODUCTION

The genuine physicochemical and biological characters of dam lakes are formed due to seasonal, periodical filling, emptying by humans and fluctuations on the surface levels based on these. These water movements are generally defined as unstable environments [1, 2]. The changes taking place in the lake ecosystem affect the phytoplankton community first. These changes lead to a transformation in the species composition of phytoplankton and dominance/abundance of algal species as phytoplankton reacts to the physical and chemical changes in the structure of a lake ecosystem most quickly. These changes naturally influence a set of living being up to the ones on the highest levels of the food pyramid [3, 4]. In fact, phytoplankton communities may show changes based especially on the physicochemical (water temperature, pH, nutrients, etc.) changes in the water [5-8]. Particularly chlorophyll-a is an important parameter that can indicate the total biomass of phytoplankton and the progress of primary production. Additionally, it was reported that the hydrological factors of dam lakes such as water level, water flow and water-holding capacity are highly significant in phytoplankton development [9].

With their reaction characteristics/abilities to environmental changes quickly, phytoplankton is an important group in determination of the environmental pollution and trophic levels of lakes and also large rivers. Several researchers accept dominant phytoplankton communities as indicators in determination of the trophic levels of lakes. Presence, absence or abundance of phytoplankton species in a region are based on certain ecological conditions. Thus they may be used as an indicator of environmental variables [10].

Studies on algae occurring in dam lakes and reservoirs [11-19] have particularly received attention after 80’s. However determination of water and ecological quality of surface waters has gained special importance through identification of diatoms (particularly planktonic forms) in parallel to European Union the Water Framework Directive [20]. In parallel to this taxonomical and ecological studies carried out on the phytoplankton of lakes have rapidly increased in Turkey [21-33]. It is a well-known fact that monitoring of phytoplankton is a useful tool for investigating eutrophication and environmental degradation both in natural and dam lakes. However, total phytoplankton alone is not generally enough to conduct a full investigation for environmental changes and eutrophication in lakes [3, 6, 7]. Therefore, such studies should include physicochemical properties of the studied ecosystem as well.
The present investigation studied the effects of environmental factors on the seasonal growth dynamics and succession of phytoplankton species in Boztepe Recai Kutan Dam Lake that is located in the province of Malatya, Turkey (Figure 1). The dam was constructed in 1997 and water retention was started in 2011. Reservoir has a surface area of 4.9 km² with a maximum depth of 81 m and total lake volume of 116.1 hm³ [34]. The mean depth of the lake is determined 23.7 m. The lake is mainly fed by Kurucay Stream. It is commonly used for the purposes of irrigation, energy production and flood prevention.

First manuscripts belonged to Boztepe Recai Kutan Dam Lake appears to belong to Saler et al. [35] who studied the zooplankton and Alpaslan et al. [36] who studied water quality. However the present study has a special importance to be the first account on monthly changes in species composition and biomass of phytoplankton in the Boztepe Recai Kutan Dam Lake located in Upper Firat Basin of East Anatolia Region. In addition the study is hoped to contribute to the understanding of the relationship between occurrence of planktonic algae and ecological quality based on different seasonal environmental conditions. The study is also expected to contribute to the planktonic algal species list of Turkish freshwater habitats.

**MATERIALS AND METHODS**

Water and phytoplankton samplings were conducted at three stations (Fig. 1) for a period of a year (January 2014 - December 2014).

The water temperature, pH, dissolved oxygen, oxygen saturation and electrical conductivity were measured directly by means of an oxygen meter (YSI 52) and a pH meter (YSI 63), while a Secchi disk depth was used to measure light transmittance. Suspended solid matter concentration was determined by filtration of sample through fiber glass filter. Filtrate was dried at 105 ºC and weighed. Total alkalinity was determined through titration analysis whilst spectrophotometric method was employed for the analysis of chloride, sulfate, nitrate, total nitrogen, total phosphorus (Hach-Lange DR 6000 spectrophotometer). Chlorophyll-a was measured by fluorometric determination of the acetone extract of a Whatman GF/C filter, through which a known volume of the sample was filtered [37].

Phytoplankton sampling was carried out on the surface of lake water by using plankton net of the brand Hydro-Bios with a brim diameter of 25 cm and pore width of 55 µm. The samples were placed in sterilized bottles and fixed by adding 4% formaldehyde for qualitative and quantitative analyses.

An Olympus CX21FS1 microscope was used to identify the species and Olympus CKX41 inverted microscope was used for counting individuals. The samples were shaken before being transferred into counting chamber to achieve homogenous distribution, and 1 ml of sample was taken to determine the number of individuals. Average values were obtained out of five repetitions [38]. The following formula was used to determine the individual numbers of algal species:

\[ \text{Lt} = \frac{250 \times (2cc \text{ phytoplankton number})}{2 \pi r^2 h} \]

Mainly Krammer and Lange-Bertalot, John et al. [39-43] were used for the identification of phytoplankton species. The accuracy of the current names of the species was achieved through the website www.algaebase.org [44].
TABLE 1

Range, mean values, median and standard deviation of physical and chemical variables of Boztepe Recai Kutan Dam Lake during the study period.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Station I</th>
<th>Station II</th>
<th>Station III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature (°C)</td>
<td>Min 5.8 Max 24.0</td>
<td>Min 6.2 Max 16.4</td>
<td>Min 5.6 Max 16.5</td>
</tr>
<tr>
<td>pH</td>
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<td>7.4-9.2</td>
<td>7.4-9.2</td>
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<td>Saturation (%O₂)</td>
<td>71-138</td>
<td>75-142</td>
<td>67-135</td>
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<tr>
<td>Electrical conductivity (µS/cm)</td>
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<td>260-432</td>
<td>241-442</td>
</tr>
<tr>
<td>Suspended solid matter (mg L⁻¹)</td>
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<td>0.1-4.5</td>
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<tr>
<td>Total alkalinity (mg CaCO₃ L⁻¹)</td>
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<td>312-451</td>
<td>287-451</td>
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<tr>
<td>Chloride (mg Cl⁻ L⁻¹)</td>
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<td>4.5-6.6</td>
</tr>
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<td>Sulfate (mg SO₄²⁻ L⁻¹)</td>
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<td>95±11</td>
<td>93±11</td>
</tr>
<tr>
<td>Nitrate (mg NO₃⁻ L⁻¹)</td>
<td>0.03-1.67</td>
<td>0.01-1.65</td>
<td>0.02-1.67</td>
</tr>
<tr>
<td>Total nitrogen (mg N L⁻¹)</td>
<td>0.10-0.99</td>
<td>0.10-0.95</td>
<td>0.10-0.89</td>
</tr>
<tr>
<td>Total phosphorus (µg P L⁻¹)</td>
<td>5-50</td>
<td>5-30</td>
<td>6-70</td>
</tr>
<tr>
<td>Chlorophyll a (µg L⁻¹)</td>
<td>1.3-18.7</td>
<td>1.3-14.4</td>
<td>3.2-17.3</td>
</tr>
<tr>
<td>Secchi disk depth (m)</td>
<td>0.5-2.8</td>
<td>0.6-3.8</td>
<td>0.8-3.5</td>
</tr>
</tbody>
</table>

PHOTOPLANKTON OF BOZTEPE RECAİ KUTAN DAM LAKE

FIGURE 2

Percentage (%) distribution of phytoplankton groups in Boztepe Recai Kutan Dam Lake

RESULTS

Physico-Chemical Parameters. Table 1 shows the physico-chemical properties of Boztepe Recai Kutan Dam Lake. The mean values of surface water temperature measured at stations were found to vary in the range of 5.6-25.8 °C through the year. The mean pH values were in the range of 7.4-9.3. The amount of dissolved oxygen was measured in the range of 6.7-14.0 mg L⁻¹ and oxygen saturation was changed between 67-142%. Electrical conductivity was measured between 241-442 µS/cm. The monthly amounts of total alkalinity and chloride varied between 279-451 mg CaCO₃ L⁻¹ and 3.7-6.6 mg L⁻¹ respectively. For sulfate (SO₄²⁻) and nitrate (NO₃⁻ -N) values changed between 77-108 mg L⁻¹ and 0.01-1.67 mg L⁻¹. Changes for total nitrogen (N) and total phosphorus were determined as 0.10-0.89 mg L⁻¹ and 5-70 µg L⁻¹ respectively. Chlorophyll-a was calculated between 1.3-18.7 µg L⁻¹. Secchi disk visibility in Boztepe Recai Kutan Dam Lake changed in seasons. Secchi disk depth at stations was measured between 0.5-3.8 cm (Table 1) and mean values varied in the range of 1.8 m and 2.0 m. The highest visibility (3.8 m) was reach at Station II.

Phytoplankton Community. A total of 20 algal taxa were recorded during the study. Bacillariophyta was represented by more taxa (12 taxa) than other algal group. Chlorophyta and Chrysophyta were represented only by one taxon whilst recorded taxon number were two in Cyanobacteria, Dinoflagelates and Euglenozoa (Fig. 2).
**FIGURE 3**
Number of taxa of determined species in Boztepe Recai Kutan Dam Lake according to the months and stations

**TABLE 2**
The list of algae recorded in the phytoplankton of Boztepe Recai Kutan Dam Lake

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Station I</th>
<th>Station II</th>
<th>Station III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BACILLARIOPHYTA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asterionella formosa Hassall</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cymatopleura solea (Brébisson) W.Smith</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gyrosigma balticum (Ehrenberg) Rabenhorst</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Iconella hibernica (Ehrenberg) Ruck &amp; Nakov</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitzschia linearis W.Smith</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pinmularia viridis (Nitzsch) Ehrenberg</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhopalodia gibba (Ehrenberg) Otto Müller</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Stauroneis nobilis Schumann</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Surirella ovalis Brébisson</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ulnaria amphihrynchus (Ehrenberg) Compère &amp; Bukhtiyarova</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ulnaria delicatissima var. angustissima (Grunow) Aboal &amp; P.C.Silva</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Ulnaria ulna</em> (Nitzsch) Compère</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>CHLOROPHYTA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closterium aciculare T.West</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>CHRYSOPHYTA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinobryon sociale (Ehrenberg) Ehrenberg</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>CYANOBACTERIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aphaniizomenon flos-aquae Ralfs ex Bornet &amp; Flahault</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oscillatoria limosa C. Agardh ex Gomont</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>DINOA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceratium hirundinella (O.F.Müller) Dujardin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peridinium cinctum (O.F.Müller) Ehrenberg</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>EUGLENOZOA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepocinclis oxyurus (Schmarda) B.Marin &amp; Melkonian</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phacus pleuronectes (O.F.Müller) Nitzsch ex Dujardin</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Taxa Number</strong></td>
<td>15</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>
Number of taxa started to increase in January and continue to increase gradually during spring and summer. The highest number of taxa recorded in August at all stations. Taxa numbers gradually decreased in autumn. Lowest number of taxa was determined in January (Fig. 2).

Distribution of algal species at stations were listed at table 2. As seen from the same table number of species occurring at stations I, II and III were 15, 11 and 14 respectively. Five taxa from Bacillariophyta, two taxa both from Cyanobacteria and Dinoflagellates were recorded at all stations. These algae were: Iconella hibernica, Gyrosigma balticum, Ulnaria amphiphrychnus, Ulnaria delicatissima var. angustissima and Ulnaria ulna (Bacillariophyta), Aphanizomenon flos-aquae and Oscillatoria limosa (Cyanobacteria) and Ceratium hirundinella and Peridinium cinctum (Dinofla) (Table 3). The highest number of algal species (12 taxa) at all stations was found in August, while the lowest taxa were (5 taxa) recorded in January (Fig.2). Pinnularia viridis (Nitzsch) Ehrenberg, Staurosirella pinnata Schumann and Sarrella ovalis Brébisson and Phacus pluerozentes (O.F.Müller) Nitzsch ex Dujardin (station I) were noticeable algae occurring only at station I. However occurrence of Dinobryon sociale (Ehrenberg) Ehrenberg only at station II and Rhopalodia gibba (Ehrenberg) Otto Müller, Closterium aciculare T.West and Lepocinclis oxyuris (Schmarda) B.Marin & Melkonian only at station III were distinguishable.

Seasonal growth dynamics of algae at stations. Station I. A total of 15 taxa belonging to Bacillariophyta (10 taxa), Cyanobacteria (2 taxa), Di-
Differences of the spatial and temporal change in the parameters among stations of study were not statistically significant, and this showed that there was spatial homogeneity in the dam lake (Table 3).

### DISCUSSION

Alkalinity values of Boztepe Recai Kutan Dam Lake were high varying between 279-385 mg CaCO$_3$ L$^{-1}$. These values clearly show that the dam lake is quite alkaline thus it can be classified as an alkaline lake due to high alkalinity. Alkalinity values for lakes are preferred to be ranged between 20-250 mg CaCO$_3$ mg L$^{-1}$ and lakes with higher alkalinity values more than 300 mg CaCO$_3$ L$^{-1}$ are usually considered to be unproductive [45, 46]. This may be one of the major reasons for low taxon number and individual numbers of algae (low biomass) in Boztepe Recai Kutan Dam Lake. Low concentrations of nitrate and ortho-phosphate might have played a critical role for biomass of phytoplankton in the lake.

The light transmittance values measured in Boztepe Recai Kutan Dam Lake were lower in comparison to those of oligotrophic lakes [47, 48]. Mean Secchi disk depth and chlorophyll a concentration at stations were measured and analyzed as 2.0-2.3 m, and 5.4-6.5 µg L$^{-1}$ respectively. These values indicate that Boztepe Recai Kutan Dam Lake has mesotrophic features according to Nürnberg [49] who reported Secchi disk depth as 2-4 m and Chlorophyll-a concentration as 3.5-9.0 µg L$^{-1}$ for mesotrophic lakes. This view is also supported by Hakanson ve Jansson [50] who suggested Secchi disk depth to be 3-6 m and chlorophyll a concentration to be within 2-8 µg L$^{-1}$ for a lake to be considered as mesotrophic. These values are also in harmony with the report of Sakamoto [51] and Håkanson [52] who reported the concentration of chlorophyll-a as 5-140 µg L$^{-1}$ for eutrophic lakes, 1-15 µg L$^{-1}$ for mesotrophic lakes and 0.3-2.5 µg L$^{-1}$ for oligotrophic lakes.

Nürnberg [49] reported that total nitrogen and total phosphorus concentrations in mesotrophic lakes may vary as 350-650 µg N L$^{-1}$ and 10-30 µg P L$^{-1}$ respectively. Total nitrogen (490-560 µg N L$^{-1}$) and total phosphorus (20-29 µg P L$^{-1}$) concentrations analyzed in the present study also supported the view that the Boztepe Recai Kutan Dam Lake could be included in mesotrophic lake category. According to Hakanson ve Jansson [50], Boztepe Recai Kutan Dam Lake also displays mesotrophic features in their trophic classification of lakes. They define a lake as mesotrophic with total N and total P to be in the range of 300-500 µg N L$^{-1}$ and 8-25 µg P L$^{-1}$ respectively.

The mean amount of dissolved oxygen was 9.3 mg/L during the study. While tropholytic reactions that consume oxygen take place in a wide hypolimnion layer in deep lakes, they take place in the entire water column in shallow lakes. This simply explains that shallow lakes with the same surface area store less oxygen than deep lakes do [53]. However, mean depth of Boztepe Recai Kutan Dam Lake is calculated as 23.7 m at original water level showing that the lake cannot be considered as a shallow lake. Mean dissolved Oxygen concentrations at three stations never fell below 9 mg/L showing that surface water column is always in rich oxygen.

The largest share of the phytoplankton belonged to Bacillariophyta (12 taxa) in Boztepe Recai Kutan Dam Lake. The highest number of taxa (10) was found at 1st station, while the lowest number (6) was recorded at 2nd station. The species composition of the phytoplankton of Boztepe Recai Kutan Dam Lake showed similarities to those of many lakes and reservoirs in Turkey [11-19, 21-35].

The species composition and seasonal succession of the phytoplankton were almost similar at three stations during the study. The reasons for the similarities might be attributed to the similar environmental conditions at the stations. Seasonal values of almost all physicochemical parameters measured and analyzed showed similarities at stations. This may explain that in different parts of the same ecosystem, phytoplankton display similar growth characteristics under similar environmental conditions.

The increase both in species composition and algal individual numbers phytoplankton during the spring and summer months in Boztepe Recai Kutan Dam Lake were noticeable in the period of increasing water temperature. This finding may suggest that water temperature is one of the main factors affecting the species composition and abundance of planktonic algae in the dam lake. A strong relationship between phytoplankton composition and water temperature was also reported in some studies [54-56]. However, it is difficult to mention any relations between phytoplankton growth and pH since high and low individual numbers were observed at similar pH levels in the present study. However, there was a noticeable correlation between the growth of phytoplankton and transparency in Boztepe Recai Kutan Dam Lake since the larger populations of algae occurred during summer when transparency was high. In contrast low individual numbers coincided with low transparency in winter.

It is also hard to establish a relation between plant nutrients (TN and TP) and seasonal occurrence of phytoplankton species at stations. Although TN and TP concentrations were similar at stations seasonal occurrence of algal species varied from one station to another. Some species occurred at only one station and absent at others. Contrary, some species

| TABLE 3  |
| --- | --- | --- | --- |
| Stations | Station I | Station II | Station III |
| Station I | 1 | 0.69 | 0.69 |
| Station II | 1 | 0.80 | 1 |
| Station III | 1 |  |  |
occurred at all stations. These findings suggest that occurrence of algal species in the Boztepe Recai Kutan Dam Lake might be affected not only by the nutrients, but also by other factors. However, it was noticeable to observe that high chlorophyll a concentrations coincided with high amount of nitrogen and phosphorus in lake water.

The species diversity of Bacillariophyta was the highest in spring followed by summer, and the lowest in winter and fall. _Gyrocomma attenuatum_ and _Iconella hyberrica_, which were reported to be found in eutrophic waters by Reynolds _et al._ [57], were identified in Lake Boztepe Recai Kutan in almost all seasons. According to these findings, it may be possible to express that _Gyrocomma attenuatum_ and _Iconella hyberrica_ may occur in mesotrophic as well as eutrophic lakes depending on the conditions.

_Ulnaria delicatissima_ var. _angustissima_ is accepted by various authors as the characteristic indicator of both oligotrophic and mesotrophic lakes [58-60]. It was also reported that _Ulnaria delicatissima_ var. _angustissima_ strongly related to the trophic status of the lakes and announced to be the specific alga of oligotrophic lakes [61]. In contrast, Wetzel [62] reported that _Ulnaria delicatissima_ var. _angustissima_ may be dominant in intermediate-sized eutrophic lakes. However, the present study is partly in harmony with these studies. Considering insistent occurrence of this diatom at three stations in the Boztepe Recai Kutan Dam Lake, one may suggest that _Ulnaria delicatissima_ var. _angustissima_ may incline to occur in lakes with various trophic status.

Occurrence of Chrysophyta species in Boztepe Recai Kutan Dam Lake generally coincided with increasing temperature. However, occurrence of _Dinobryon sociale_ with highly numbers was observed only in September when water temperature was already high. Studies [57, 63] revealed that phosphate and nitrate have an influence on the development of _Dinobryon sociale_ could have developed in Boztepe Recai Kutan Dam Lake with the support of nitrate and phosphate that were always in sufficient concentrations to support the growth of plankton species.

_Aphanizomenon flos-aquae_ and _Oscillatoria limosa_ were only representatives of Cyanobacteria in Boztepe Recai Kutan Dam Lake. Cyanobacteria species were observed mostly in summer and autumn. They were encountered with much fewer individual numbers in spring. Excessive Cyanobacteria development in summer may be explained by sufficient amount of nutrients and high water temperature [57, 63, 64]. Moreover, in cases where water movements are stable and lake is rich in nitrate, these species are known to reproduce rapidly [65] Boztepe Recai Kutan Dam Lake. _A. flos-aquae_ was recorded at all stations in spring and autumn, while _O. limosa_ occurred only in summer.

REFERENCES


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FRESH GAS FLOW RATE MANAGEMENT IN ANESTHESIA INDUCTION WITH SEVOFLURANE FOR ECOLOGICAL BALANCE: 1-1-5

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2Department of Anesthesiology and Reanimation, Meltem Hospital, Istanbul, Turkey

ABSTRACT

In anesthesia applications, inhaler anesthetic drugs used in operating rooms may cause damage to the stratospheric ozone layer of the atmosphere. The development of methods in which the consumption of these gases is reduced can contribute to the maintenance of ecological balance.

A randomized study was performed on 70 ASA I-II patients aged 18-70 years who underwent elective surgery. LFA was administered to both groups, and sevoflurane was used as inhaler anesthetic. The group in which N2O is used as a carrier gas is Group N; group A was named as the group that was not used. In this study, we used the 1-1-5 principle instead of high flow at the beginning of anesthesia. Induction and maintenance of anesthesia 1 L / min. Fresh gas flow was used. Sevoflurane was initially given at 5%. We determined the time required for alveolar sevoflurane concentration (FS) to increase from 1% to 2.2% and recovery characteristics with or without N2O. After intubation of the patients, gas samples taken from the Y piece were monitored. O2, N2O, CO2 and inhalation anesthetics drug concentrations and MAC values of the inspiratory gas and post-respiration were recorded. Differences between the two groups were examined. P <0.05 was considered statistically significant.

Cases included in the study; The mean age of Group N was 51.00 ± 12.76 years, 25 men and 6 women, and the mean age of Group A was 49.40 ± 12.62 years, 21 men and 14 women. The differences between the groups in terms of demographic data were not statistically significant (p >0.05). O2 values between the two groups were statistically significant (p <0.01). The difference between Alderete score duration values was not statistically significant (p > 0.05).

When sevoflurane is used with a 1-1-5 wash-in scheme, the required sevoflurane concentration can be achieved faster without the use of N2O by applying low flow anesthesia. In this way, fluorocarbon emission from the operating room will be reduced, and ecological balance will be supported.

KEYWORDS:
Anesthesia, Ecological Balance, Global Warming, Greenhouse Gas, Low Flow Anesthesia, N2O, Sevoflurane

INTRODUCTION

It has been shown in many studies that human activities in daily life are associated with global warming and consequently, climate change [1, 2]. Increasing greenhouse gas production in the 21st century has been recognized as one of the biggest health threats [3]. Greenhouse gas is reported to be effective in global warming [4]. Achieving a reduction in greenhouse gas production, which affects the atmosphere and threatens people's health, has been accepted as an emergency action plan all over the world [5].

Volatile anesthetic agents with halogenated chlorofluorocarbon derivatives such as halothane, enflurane, sevoflurane, desflurane, isoflurane used in the operating room during anesthesia applications and nitrogen oxide (N2O) can cause damage to the stratospheric ozone layer of the atmosphere [6, 7]. However, this situation was seen as a necessity for health and was ignored. Inhaler anesthetic drugs are powerful greenhouse gases, and this is measured by a global warming potential (GWP). GWP is a scale that shows how much heat a particular gas heats in the atmosphere compared to a similar CO2 mass [8].

With the spread of environmental awareness in societies, among anesthesiologists, "Is it possible to reduce the consumption of these gases?" question came up. Thus, the use of regional anesthesia, total intravenous anesthesia, and low-flow anesthesia (LFA) has increased among anesthetists [9].

The term LFA is used to describe inhalation anesthesia techniques with a semi-closed re-inhalation system where the re-inhalation rate is at least 50% [10].

Environmentally sensitive approaches and the development of more sophisticated anesthesia systems have expanded the use of this technique in anesthesia.
Since LFA reduces waste gases, atmospheric contamination is less, and the health risks of operating room personnel are reduced, and ecological balances are preserved [2, 9].

N₂O, an inorganic inhalation agent, has been in use for the last 150 years for its analgesic and anxiolytic properties. N₂O use is the most common inhaler anesthetic drug with the most greenhouse gas effect [11, 12]. The availability of modern inhalation anesthetic drugs such as isoflurane, desflurane, and sevoflurane has been considered a revolution in this sense [13, 14].

When the literature review is performed, it is seen that LFA studies are mostly performed with desflurane [15, 16]. In fact, in a 1997 article, desflurane was particularly indicated for LFA administration because of its low solubility and negligible metabolism [17]. LFA studies performed with the use of sevoflurane as an inhaled anesthetic drug are less [3].

This is because sevoflurane interacts with carbon dioxide absorbers containing sodium and potassium hydroxide to produce compound A, which may have detrimental effects on kidney function in laboratory animals [18]. However, subsequent studies in humans have shown that sevoflurane has no significant effect on renal function [19, 20]. In the same publications, it was emphasized that sevoflurane could be used easily with the fresh gas flow (FGF) of 1 L/min up to 2 MAC dose levels. The lack of data that compound A is harmful to humans renders the fresh gas flow limitations insignificant when using sevoflurane.

The GWP was adopted as an indicator in the Kyoto Protocol of the United Nations Framework Convention on Climate Change (UNFCCC). Comparing the amount of heat held by inhaler anesthetic drugs over 20 years compared to a similar CO₂ mass; this number, 6810 for desflurane, 1800 for isoflurane and 440 for sevoflurane [21]. The higher the number, the higher the greenhouse gas effect. As seen, sevoflurane is the most innocent in terms of greenhouse effect among the last inhaler anesthetic agents.

To reach the minimum alveolar concentration (MAC) value in schemes using the LFA method, it was necessary to wash with high flow amounts and a 10-15 minute period at the beginning of anesthesia application [22]. In this study, we used the 1-1-5 principle instead of high flow at the beginning of anesthesia. We determined the time required for alveolar sevoflurane concentration (FS) to increase from 1% to 2.2% and recovery characteristics with or without N₂O.

Our proposed 1-1-5 wash-in scheme is a fast and simplified wash-in technique that starts with an FGF of N₂O: O₂ at 1:1 L/min and a vaporizer concentration of sevoflurane (FS) of 5%.

In this wash-in scheme, we start with 1 L flow N₂O / air and 1 L flow O₂ and adjust the vaporizer evaporator concentration to 5%.

Maintaining a high dial concentration at 5% initially helped to achieve the required FS in a short time.

FS decreased according to MAC values so that the measured value of sevoflurane went to the patient, and there was no unnecessary waste of gas and pollution.

Therefore, using LFA, our program aims to achieve each stage FS from 1% to 2.2% earlier and more easily, more effectively and safely without N₂O. Thus, the greenhouse gas production of inhaler anesthetic drugs will be reduced.

**MATERIALS AND METHODS**

After obtaining the written approval of the Institutional Ethics Committee and the patients, a randomized study was performed on 70 ASA I-II patients aged 18-70 years who were scheduled for elective surgery.

Patients with obstructive pulmonary disease, unregulated diabetes mellitus, hunger duration of more than 12 hours, history of malignant hyperthermia in own history or family; morbidly obese patients, alcohol or drug addiction, chronic obstructive pulmonary disease, coronary artery disease, congestive heart failure, significant anemia, liver or kidney disease, women in pregnancy or lactation, allergic to thiopental or halogenated drugs, Hypotension, hypovolemia and systematic disease were excluded.

Two groups of patients, each containing thirty-five, were formed. Groups were randomly assigned due to carrier gas and inhaler anesthetic agent. Each patient received a randomization number and was put in the group according to his/her number.

LFA was applied to both groups. The group that N₂O-guided (O₂: 50%; N₂O:50%) sevoflurane was used as the carrier gas and was named as Group N. Group A was named as the group of patients using sevoflurane by air (O₂: 50%; Air 50%).

Before each operation, leakage control of anesthesia circuits and gas monitors were calibrated. A single-use anesthesia circuit and bacterial filter were used for each case. The inspiration CO₂ ratio was 0. When the ratio was 1, it was immediately changed to soda-lime. The fasting time of the patients was adjusted to be at least 8 hours. Patients were given venous vascular access from the back of the hand or antecubital fossa with 22G angiocath 30 minutes before the operation and I.V. premedication with 1.5 mg midazolam. After being taken to the operating table, routine electrocardiographic, noninvasive blood pressure and peripheral oxygen saturation monitoring were applied to patients via a monitor (Drager Infinity Delta, MS13466E539D, 2005, Drager Medical Systems, Inc. 16 Electronics...
Avenue Denver MA 01923 USA) and the values were recorded. Before induction, all patients were given O2 mask at a rate of 4 L / min for 2 min. After being taken to the operating table, routine electrocardiographic, noninvasive blood pressure and peripheral oxygen saturation monitoring were applied to patients via a monitor (Drager Infinity Delta, MS13466E539D, 2005, Drager Medical Systems, Inc. 16 Electronics Avenue Denver MA 01923 USA) and the values were recorded. Before induction, all patients were given O2 mask at a rate of 4 L / min for 2 min. In anesthesia induction 1 µg / kg I.V. 1 minute following fentanyl, 5-7 mg/kg thiopeptil I.V. 20-30 sec. Was given inside. Following the loss of eyelash reflex, 0.6 mg/kg rocuronium I.V. In 90 seconds, endotracheal intubation was performed with cuff tube of appropriate diameter for body and age. All patients were manually ventilated with 100% O2 until endotracheal intubation was performed after thiopental. After intubation, ventilation was started in volume control mode by adjusting Drager-Primus anesthesia ventilator with a tidal volume of 6-8 ml/kg, respiratory frequency of 12, and PEEP of 5 cmH2O.

Group N received LFA using volatile anesthetic sevoflurane with N2O (O2 50%; N2O 50%), whereas Group A received LFA using volatile anesthetic sevoflurane with air (O2 50%; air 50%).

After intubation, gas samples taken from the Y piece were inspected and expired O2, N2O and inhalation agent concentrations, end-tidal CO2 and MAC values were monitored. Values were recorded in the first 10 minutes of operation and 15, 30, 60. Minutes.

Time to reach alveolar FS was recorded at 1-2.2%. After performing 2.2% FS, the O2: N2O or O2: Air GFG was reduced to 0.5: 0.5 L / min and the FS was adjusted to maintain the MAC value around 1% for sufficient anesthesia depth.

Hemodynamic parameters were monitored every 5 minutes until the end of surgery. The inhalational anesthetic agent was closed 5 minutes before the end of the operation. N2O was then stopped in Group N, the air was stopped in Group A, and all patients were ventilated with oxygen only at a rate of 6 L / min.

In the last 20 minutes of the operations, no additional dose of rocuronium was given. 0.01 mg/kg atropine, 0.02 mg/kg neostigmine I.V. was performed to reverse the neuromuscular block.

Patients were extubated after criteria such as the resumption of regular breathing regimen, adequate minute ventilation, oxygen saturation >95%, and continuation of respiratory reflexes.

The patient was then transferred to the postanesthesia care unit (PACU). Eye-opening, extubation, response to verbal order, Aldrete Scoring System according to a recovery profile (Table 1) Score of 9-10 times were recorded.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrete recovery assessment system</td>
</tr>
<tr>
<td>RESPIRATORY</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Deep breathing and coughing</td>
</tr>
<tr>
<td>Dyspneic and limited</td>
</tr>
<tr>
<td>Apneic</td>
</tr>
<tr>
<td>Blood pressure before anesthesia ≥%50</td>
</tr>
<tr>
<td>Pink</td>
</tr>
<tr>
<td>Pale</td>
</tr>
<tr>
<td>cyanotic</td>
</tr>
<tr>
<td>Awake conscious</td>
</tr>
<tr>
<td>The stimulus can only be awakened</td>
</tr>
<tr>
<td>Purpose-oriented</td>
</tr>
<tr>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics of patients</td>
</tr>
<tr>
<td>Group N (n=35)</td>
</tr>
<tr>
<td>Gender n (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Age (year) Ort.±SD</td>
</tr>
<tr>
<td>Weight (kg) Ort.±SD</td>
</tr>
<tr>
<td>ASA n (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Anesthesia time (min) Mean±SD</td>
</tr>
</tbody>
</table>
### TABLE 3
Mean end-tidal concentration of inhalational anesthetic agent (sevoflurane)

<table>
<thead>
<tr>
<th>Time</th>
<th>Group N (n=35)</th>
<th>Group A (n=35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>1 min after intubation</td>
<td>0.79±0.03</td>
<td>0.78±0.06</td>
<td>0.38</td>
</tr>
<tr>
<td>2 min</td>
<td>1.65±0.06</td>
<td>1.63±0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>3 min</td>
<td>1.86±0.12</td>
<td>1.83±0.12</td>
<td>0.29</td>
</tr>
<tr>
<td>4 min</td>
<td>2.23±0.15</td>
<td>2.20±0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>5 min</td>
<td>2.06±0.07</td>
<td>2.04±0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>6 min</td>
<td>2.19±0.13</td>
<td>2.15±0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>7 min</td>
<td>2.22±0.01</td>
<td>2.20±0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>8 min</td>
<td>2.21±0.10</td>
<td>2.18±0.11</td>
<td>0.23</td>
</tr>
<tr>
<td>9 min</td>
<td>2.12±0.12</td>
<td>2.09±0.12</td>
<td>0.29</td>
</tr>
<tr>
<td>10 min</td>
<td>2.07±0.23</td>
<td>2.04±0.23</td>
<td>0.58</td>
</tr>
<tr>
<td>15 min</td>
<td>2.01±0.30</td>
<td>1.98±0.29</td>
<td>0.67</td>
</tr>
<tr>
<td>30 min</td>
<td>1.88±0.29</td>
<td>1.84±0.28</td>
<td>0.55</td>
</tr>
<tr>
<td>60 min</td>
<td>0.44±0.02</td>
<td>0.35±0.16</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### TABLE 4
Mean minimum alveolar concentration of inhalational anesthetic agent (sevoflurane)

<table>
<thead>
<tr>
<th>Time</th>
<th>Group N (n=35)</th>
<th>Group A (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>1 min after intubation</td>
<td>0.36±0.04</td>
<td>0.35±0.05</td>
<td>0.36</td>
</tr>
<tr>
<td>2 min</td>
<td>0.75±0.05</td>
<td>0.74±0.03</td>
<td>0.31</td>
</tr>
<tr>
<td>3 min</td>
<td>0.85±0.06</td>
<td>0.83±0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>4 min</td>
<td>1.02±0.07</td>
<td>1.00±0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>5 min</td>
<td>0.94±0.06</td>
<td>0.93±0.04</td>
<td>0.42</td>
</tr>
<tr>
<td>6 min</td>
<td>1.00±0.06</td>
<td>0.98±0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>7 min</td>
<td>1.02±0.06</td>
<td>1.00±0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>8 min</td>
<td>1.01±0.04</td>
<td>0.99±0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>9 min</td>
<td>0.96±0.03</td>
<td>0.95±0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>10 min</td>
<td>0.94±0.03</td>
<td>0.92±0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>15 min</td>
<td>0.92±0.05</td>
<td>0.90±0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>30 min</td>
<td>0.85±0.08</td>
<td>0.83±0.04</td>
<td>0.19</td>
</tr>
<tr>
<td>60 min</td>
<td>0.20±0.05</td>
<td>0.18±0.05</td>
<td>0.10</td>
</tr>
</tbody>
</table>

### STATISTICS

Statistical analysis of the data was performed using IBM SPSS for Windows 23.0 (USA) software, and statistical significance was accepted as p < 0.05. Variables of qualitative data were expressed as numbers and percentages, while variables of quantitative data were expressed as mean or minimum-maximum ranges. According to the normality test, t-test was used in groups independent of parametric tests and Mann Whitney U test, one of the nonparametric tests, was used for data showing normal distribution.

### RESULTS

Cases included in the study; The mean age of Group N was 51.00 ± 12.76 years, 25 men and 6 women, and the mean age of Group A was 49.40 ± 12.62 years, 21 men and 14 women. The operation time was 44.00 ± 8.34 min in Group N and 45.30 ± 9.34 min in Group A. The difference was not statistically significant (p > 0.05). Demographic characteristics are shown in Table 2. The differences between the groups in terms of demographic data were not statistically significant (p < 0.05). Hemodynamic parameters were also statistically insignificant (p > 0.05).

In Group N, end-tidal FS increased rapidly and linearly to 1.6 in 1 minute and 2.2 in 4 minutes (Table 3). In Group A, it rose to 2.2 within 4 minutes. As shown in Table 3, the all-time FS and its values were not statistically significant between the two groups (p > 0.05).

The dial concentration from the vaporizer was changed to maintain MAC concentration around 1% in both groups throughout the operation. The MAC values determined in detail in time are given in Table 4.

In Group N, N₂O concentration was increased during the anesthesia during the first 10 minutes faster. The mean end-tidal N₂O concentration ranged from a minimum of 20.90 ± 5.74 to 60.03 ± 2.56 in 60 minutes. O₂ levels in Group N ranged from 35.56 ± 1.85 to 71.23 ± 2.13%, and in Group A ranged from 57.73 ± 19.45% to 82.10 ± 1.35%. O₂ values between the two groups were statistically significant (p < 0.01). The times during wake-up and recovery were as in Table 5.
The duration of the Aldrete score was 14.84 ± 3.98 minutes in Group N, and 13.68 ± 6.77 minutes in Group A. The difference between the two groups was not statistically significant (p> 0.05).

**DISCUSSION**

Significant savings can be achieved with low N2O / air and O2 flows. However, new generation inhaler anesthetic drugs such as sevoflurane and desflurane should be used to save more [23].

First stage high FGF is required for LFA. In the initial period of induction of anesthesia, high FGF provides the necessary concentration of denitrogenation and O2 and inhaler anesthetic in the ventilation system. This period is called a washing circuit. The inhaler anesthetic drug used in LFA during this period was ignored, and saving during maintenance was aimed. Anesthesia maintenance is the best opportunity to reduce FGF because circuit gas concentrations are relatively more stable and often the longest phase of the procedure. However, in recent years, methods of managing fresh gas flow, which can reduce the amount of inhaled anesthetic drugs wasted during induction, have been developed [24]. In anesthesia devices where it is possible to monitor oxygen and anesthetic gas concentrations, it is possible to implement these strategies safely and effectively. The use of high FGF in induction eliminates the main purpose of the LFA method [17]. Thus, we do not evaluate the opportunities offered by sophisticated anesthesia devices used in operating theaters. The application of LFA with closed-loop system breathing circuits, which are widely used today, provides almost zero waste.

As FGF increases in respiratory systems, gases entering the cleaning system increase. Finally, inhaler anesthetic drugs that increase the greenhouse gas load are given to the atmosphere [24]. The amount of waste given to the atmosphere by FGF is directly proportional.

Nowadays, where global warming is a major problem, the increase in environmental concerns has been a compelling reason for using applications that minimize or eliminate inhaled anesthetic drugs discharged into the environment during the washing cycle in induction [6 , 25].

Jeffrey M. Feldman, in his study with the GasMan simulation system (Med-Man Simulations, Chestnut Hill, MA), calculated the efficiency of the inhaler anesthetic agent [26].

The efficiency was 15% at FGF 8 L / min, 24% at 2 L / min, 29% at 1 L / min [24].

As it is seen, as FGF increases, less portion of inhaler anesthetic drug is given to the system, and most of it is released to atmosphere. The desired inhaler anesthetic drug concentration in the breathing circuit is determined by the vaporizer concentration set from the vaporizer dial and the total FGF. The amount of waste that pollutes the environment and the factor that increases the amount of waste is high FGF, as stated above. Increasing the concentration of anesthetic drug in the inhaler instead of FGF during induction gives the same result. This contributes to the reduction of environmental pollution. This principle is called overpressure [26].

It should be noted that the anesthetist applying this technique should be very careful and should reduce the inhaler anesthetic setting when the desired MAC is reached.

The 1-1-5 scheme proposed in our study is based on this principle. After intubation, we administered 5% sevoflurane with 1 L / min FGF. Our goal was to create a 1-1-5 wash-in scheme to ensure that FS can reach 2.2% safely and with less damage to the environment. 2.2% of FS concentration was reached in 4 minutes in both groups.

In previous years, wash-in schemes according to the overpressure principle for desflurane have been proposed by different authors [16, 27]. A recommended wash-in scheme for sevoflurane was not found in the literature.

In our study, 0.78, 1.63, 1.83, 2.2 FS levels in Group A were determined as respectively 1., 2., and 3., and 4 minutes. In Group N, FS levels of 0.79, 1.65, 1.86, 2.23 were determined as 1, 2, 3,., and 4 minutes. The difference between FS levels between the two groups was not statistically significant.

Sevoflurane MACs were measured during anesthesia in both groups. Although MAC was higher in Group N, the dial concentration in both groups was changed to keep MAC at 1%.

The mean end-tidal N2O concentration was measured in Group N for 1 to 60 minutes. The first rise in N2O was when N2O entered the circle system after intubation. In both groups, the initial O2

**TABLE 5**

<table>
<thead>
<tr>
<th>Evaluation of Recovery between Group N and Group A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Extubation time (Minute Mean ± SD)</td>
</tr>
<tr>
<td>Eye-opening time (Minute Mean ± SD)</td>
</tr>
<tr>
<td>Response to verbal order (Minute Mean ± SD)</td>
</tr>
<tr>
<td>Time to get Aldrete score 9-10 (Minute Mean ± SD)</td>
</tr>
</tbody>
</table>
level was 100% due to preoxygenation. With the introduction of air into the respiratory system in Group A and N\textsubscript{2}O in Group N, the O\textsubscript{2} level began to decrease. The O\textsubscript{2} concentration never fell below 30%. Since Group N, N\textsubscript{2}O: O\textsubscript{2} and Group A contained air: O\textsubscript{2}, end-tidal O\textsubscript{2} concentration was higher in Group A. The difference between the two groups was statistically significant (p <0.01). This difference was not clinically significant because the O\textsubscript{2} ratio did not decrease below 30% at any time during the operation. O\textsubscript{2} tended to fall during anesthesia. It has been shown in previous studies that O\textsubscript{2} tends to decrease over time as it is consumed during metabolism in the body [28].

When LFA is used, some gases (nitrogen, carbon monoxide, methane, acetone) may accumulate in the respiratory system [29]. In our study, N\textsubscript{2}O levels increased during anesthesia in Group N.

None of the patients had a history of intraoperative wakefulness. Completion times were similar in both groups. Although the duration of the Aldrete score in PACU was slightly higher in Group N, there was no statistically significant difference between the two groups (p > 0.05). A score of 14.84 min for Group N, 13.68 min for Group A was. The use of sevoflurane with or without N\textsubscript{2}O showed no change in recovery properties.

In studies, it is reported that when N\textsubscript{2}O: O\textsubscript{2} mixture is used instead of air: O\textsubscript{2} as the carrier gas, greenhouse gas emissions can increase up to 65% for isoflurane and up to 900% for sevoflurane [6, 30].

N\textsubscript{2}O is a gas that contributes to about 5% of the ‘greenhouse’ effect in the atmosphere [31]. Its medical use contributes 2% to the total amount of N\textsubscript{2}O released [32]. N\textsubscript{2}O used in anesthesia affects 15 to 20 times the climate of sevoflurane [32]. Using LFA, we can reduce it to insignificant levels or reset it with new wash-in techniques and never use it.

In 2012, awareness was raised in 7 hospitals in a university in Canada to use sevoflurane instead of desflurane and prefer LFA because of the greenhouse effect. In their article, they state that they achieved a 66% reduction in greenhouse gas emissions between 2012 and 2016 [3]. If we express with an analogy what this difference means; The annual carbon emissions generated by 1,700 personal vehicles driving an average of 22,000 km per year were avoided [32].

The use of closed circuits and LFA can reduce the volume of anesthetic gases consumed by 80 to 90% [21]. In a study, it was stated that the use of these techniques resulted in a 10-20% reduction in CO\textsubscript{2} equivalent emissions in one year [33].

We preferred sevoflurane with lower greenhouse gas effect as an inhaler anesthetic in LFA cases. Sevoflurane usage has been increasing in recent years with increasing environmental awareness. In a survey on anesthesiologists in Australia and New Zealand, 72% of the users stated that they preferred sevoflurane, and 12% preferred desflurane [34].

In studies with various CO\textsubscript{2} absorbers; No compound A was produced using KOH, NaOH [35]; N When NaOH and Ca(OH)\textsubscript{2} were compared, it was shown that there was no difference in the amount of compound A produced [36].

Kennedy et al., in a recent article, concerns about LFA and sevoflurane are irrelevant; stated that they believe that there is no reason to avoid FGF <1 L / min when using sevoflurane [37].

The advantages of our wash-in program (1-1-5) include less FGF requirement (no first stage of high flow anesthesia required), simple application, directing the use of air instead of N\textsubscript{2}O, the usability of sevoflurane with less greenhouse gas effect, minimum operating room pollution, protection of ecological balances.

With this wash-in technique, the respiratory system was washed rapidly according to the programs using high flow rates, and sevoflurane was provided to reach the MAC value required for the depth of anesthesia. In our study with air, it can be concluded that the time taken to obtain 2.2% FS is the same as for N\textsubscript{2}O. This means that the use of N\textsubscript{2}O, which has a very high greenhouse gas effect, is irrelevant. Our results showed that 1-1-5 wash-in program performance is not dependent on N\textsubscript{2}O usage. The presence of N\textsubscript{2}O did not affect the rise time of FS and alveolar uptake.

**LIMITATIONS**

Although the results of our study are sufficient, we believe that the larger sample size will be more useful for establishing future guidelines. Furthermore, the fact that the amounts of CO\textsubscript{2} absorbent consumed have not been monitored is a weak aspect of the study.

**CONCLUSION**

When sevoflurane is used using the 1-1-5 wash scheme, it can be advantageous to quickly reach the required FS without N\textsubscript{2}O, and the initial high FGF phase of LFA.

In this way, fluorocarbon emission from the operating room will be reduced, and ecological balance will be supported. Scales should be developed to monitor the use of toxic gases, and awareness should be raised among users about these medically beneficial environmental pollutants. This study is a step to raise awareness of reducing the environmental impact of medical gases used in clinical centers and especially in the field of anesthesiology.
REFERENCES


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THE EFFECTS OF THYME ESSENTIAL OIL ON ENZYME ACTIVITIES OF RHIZOCTONIA SOLANI

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1Isparta University of Applied Sciences, Faculty Agricultural Sciences and Technologies, Department of Plant Protection, Isparta, Turkey
2Aglasun Directorate of District Food Agriculture and Livestock, Burdur, Turkey

ABSTRACT

In this study, determination of the effects of different concentrations of essential oil of Origanum onites L. (Izmir Thyme) on mycelial development of Rhizoctonia solani in liquid medium, total protein amount, pectin-methyl-esterase and cellulase activity produced in culture filtrate and mycelial extract were aimed. In the study, spectrophotometric analyses were carried out to determine the substances of protein structure. As a result of HPLC analysis, carvacrol and thymol were detected at higher rate in thyme oil added media. In this study, determination of the effects of different concentrations of thyme oil (20-100%) compared to the control and completely inhibited at 1000 ppm concentration. Analysis of the culture filtrate and mycelial extracts obtained from the development of R. solani under certain conditions in the thyme oil added media resulted in a reduction in the total protein content. Thyme oil reduced pectin-methyl-esterase activity of R. solani at increasing concentrations. Mycelial development of R. solani was inhibited at 1000 ppm concentration but the Pectin methyl-esterase activity was stopped at 750 ppm of thyme oil. Thyme oil reduced cellulase activity at increasing concentrations and stopped completely at 1000 ppm concentration. The effects of thyme oil on the enzyme activities of R. solani were found to be caused by the higher rates of carvacrol and tymol. Our study has promising results in terms of thyme oil, which is known to be effective against R. solani, and to provide a more detailed explanation of the efficacy of different concentrations on the pathogen control and more conscious use.

KEYWORDS:
Thyme, Rhizoctonia solani, Carvacrol, Total protein, Pectin methyl esterase, Cellulase

INTRODUCTION

Rhizoctonia solani Kühn (teleomorph: Thanatephorus cucumeris) is an important and prevalent plant pathogenic fungus worldwide. It is a destructive soil-borne plant pathogen and causes symptoms in more than 142 plant species in agricultural production all around the world [1, 2]. Potato, tomato, bean, cucurbitaceous plants including cucumber, melon, pumpkin and muskmelon; sugar beet, peanut, clover, lettuce, corn and strawberry can be counted among most important hosts [1]. R. solani usually effects seed and seedlings causing damping off. R. solani causes symptoms such as seed, hypocotyl and root rot. At subsequent levels of plant development, it causes black scurf disease by forming sclerotia on tuber and stolon cancer in potato. Pathogen may be transferred to new areas via diseased plant materials or diseased soils [3].

Cool and moist soil with optimal temperature of 18°C are favorable conditions for disease development. Beside this, R. solani uses enzymes like pectinase cellulase, hemicellulase, protease, lipase and lignolytic enzymes like laccase to form symptoms. Pectin is one of the most important structural parts of middle lamella and primary cell wall. Poly saccharides, shivered by pectolytic enzymes, are produces by phytopathogenic enzymes [4, 5]. Plant pathogenic fungi can produce enzymes destructing cell wall. Pectolytic enzymes that are secreted by pathogens, are considered to play a key role at tissue maceration. Still enzymes are suggested to be an important factor for colonisation of plant tissue [6]. Pectin esterase, pectin lyase and polygalacturonase are most important of those enzymes. Production and activity of host cells, that are sensitive to plant pathogen fungus, on cell walls may vary under various chemical and biological stress [7, 8, 9]. Poligalacturonase play a key role at pathogenic property and virulence [9]. Poligalacturonase moving before other pectic enzymes and is first proteolytic enzyme secreted by pathogenic fungus when acculturated on isolated cell walls [10]. At the same time polygalacturonases forces phytoalexin synthesis at plants and activates plant defence response [11]. Polygalacturonases are hydrolases having important effect on pectins. Enzymes are suggested to have a different role during pathogenicity. Endo polygalacturonases are more effective at tissue maceranation and necrosis and forms oligalacturonides which are revealers of plant defence reactions [12]. Exo-polygalacturonases dissolves high polymeric substrates and secretes low molecular weight oligalacturonides, these cells can enter other cells where catabolyzed and act.
as inductors of other pectic enzymes [9]. Many fungus may form polygalacturonase enzyme. Verticillium albo-atrum [13], Rhizoctonia fragariae, Rhizoctonia solani [14], Fusarium oxysporum f.sp radicis lycopersici [15], Sclerotinia borealis [16], Sclerotium cepivorum [17] are characterized among of them as biochemicals. Cellulase is another important enzyme produced by phytopathogenic fungi. Cellulases catalyzes decomposition of β-1,4 glucosidic bonds in cellulose. They are divided into three types; endoglucanases, exoglucanases and β-glucanases. Endoglucanases, causes speed decrease at polymer length and gradual increases in reduction of sucrose concentration by randomly hydrating internal glucosidic bonds. Exoglucanases, hydrates cellulose bonds by removing cellobiose from reductive and non-reductive ends, and this causes fast release of reductive sucrose but it causes minor changes in polymer length. Endoglucanases and exoglucanases synergically affect to produce cellobiose on cellulose and afterwards those decompose to β-glucosidase and glucose. Plant pathogenic fungus produce a series of cellulase [18]. It has been showed that cellulase formed by plant-pathogenic fungus has role on pathogenicity but effects of cellulase on pathogenicity still continues compared with chitinase, pectinase and xylanases as other cell wall destructive enzymes. For example; Macrophomina phaseolina is claimed to use endocellulase for pathogenicity. But importance of cellulase in aspect of plant-pathogenic fungus pathogenicity can be put forth after detailed researches [19].

R. solani management performed by cultural and chemical controls methods. In addition, researches have been conducted on alternative or supportive methods additional to traditional methods for controlling plant diseases. As harmful effects of pesticides to environment and human were considered, plant extracts come into the focus [20]. Many studies were performed aiming to determine the effects of essential oils and plants extracts on pathogens in vitro and in vivo. As result of these studies some essential oils are found effective on pathogens in vitro and some others are found to decrease severity of plant pathogens or ineffective in vivo. Many oils and extracts of medicinal and aromatic plants including savory, coral kiosk, capers, mint, thyme, lavender and rosebud, almond, marjoram, and lavender are determined to be effective to reduce R. solani [21, 22, 23, 24]. Thyme (Thymus spp.) known as one of the most effective plant againts diseases among them [25, 26, 27, 28, 29, 30]. This study was performed to determine the effects of different concentrations of thyme oil on mycelial development and enzyme activities of Rhizoctonia solani in liquid medium.

### MATERIALS AND METHODS

**Materials.** Rhizoctonia solani isolated from potato with high virulence was maintained from culture collection of Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Plant Protection, Mycology Laboratory.

*Origanum onites L.* (Izmir Thyme) essential oil was supplied from Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Fields Crops.

**Determination of components in thyme oil.** The determination of components was performed using the modified procedure of Caponio et al. [31]. Chromatographic analysis was carried out using reverse-phase high-performance liquid chromatography (Shimadzu Corp., Kyoto, Japan) equipped with a SCL-10A VP system controller, a SIL-10ADVP auto sampler, a DGU-14A degasser, a CTO-10ADVP column heater, a LC10ADVP pump and a diode array detector set at a wavelength of 278 nm. A 250 ·4.6-mm i.d. 5-mm XDB-C18 column was used with flow rate: 0.8 mL/min; sample injection volume: 10 mL and the column temperature: 30°C (Agilent Eclipse, Walborn, Germany). For gradient elution, mobile phase A containing acetic acid and water (2 : 98, V/V); solvent B absolute methanol. The data were integrated and analysed using the Shimadzu Class-VP Chromatography Laboratory Automated Software System (Chiyoda-ku, Tokyo, Japan). The oil, standard solutions and mobile phase were passed through a 0.45-mm pore size membrane filter (Vivascience AG, Hannover, Germany). The instrument was calibrated based on carvacrol and thymol standards (Sigma, St Louis, MO, USA) and then the components in the samples were determined in percent.

**Effects of thyme essential oil on mycelial development of Rhizoctonia solani in liquid medium.** Modified liquid PD medium (glucose 20 g L⁻¹, NaNO₃ 1 g L⁻¹, K₂HPO₄ 1 g L⁻¹, MgSO₄ and NaCl 0.5 g L⁻¹, FeSO₄ 0.01 g L⁻¹) was used to add thyme essential oil at different concentrations.

100 mL of liquid medium was added to erlen mayer. Media were autoclaved for 20 minutes at 121 °C, 1 atm kPa. After cooling the media to 45 °C; thyme essential oil was added at 50 ppm, 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm concentrations. In control flasks, essential oil was not added. After addition of essential oil concentrations, 10 mm diameter mycelial discs from fresh culture of R. solani culture grown on PDA (Potato Dextrose Agar) were inoculated. Experiment were designed as completely randomized design with 3 replicated. Cultures were incubated for 2 weeks at 25 °C without shaking. At the end of incubation period, mycelial biomasses were collected and filtered via
vacuum pump and separately weighed to determine fresh weight.

**Sample preparations for enzyme analysis.** Filtered mycelial masses were washed 3 times with 35 mL of cold distilled water and were left on two layers of filter paper to remove excess water. Mycelium was homogenized with liquid nitrogen. Mycelial powder was put into 2 mL eppendorf tubes. 100 mg mycelial powder were weighed from homogenized samples and 1.25 mL 10 Mm phosphate buffer (pH 6) including 1 % ascorbic acid was added. Tubes were vortexed at 20,000 g for 10 minutes at 4°C. The supernatant including mycelial proteins and filtrate including metabolites occurring during growth of pathogen in liquid medium were stored at -20 °C to use for further studies [32].

**Determination of total protein amounts.** Total protein amount was determined in mycelium extract and culture filtrates according to Bradford [33] is based on the absorbance shift observed in an acidic solution of dye Coomassie Brilliant Blue G-250. A standard curve was made of BSA and absorbance was read at 595 nm.

**Determination of pectin-methyl-esterase activity.** Pectin-methyl-esterase activity were determined by Hagerman and Austin [34]. Measurement was performed at 620 nm wavelength at spectrophotometer. Measurements were performed with 3 replicates from culture filtrates and mycelial extract.

**Determination of cellulase activity.** Di-nitrosalicylic acid (DNS) method was used to determine cellulase enzyme amounts in culture filtrate and mycelial extract. 1000 µl sample, 1 mL buffer solution and 2.5 mL carboxymethyl cellulose solution were added to reaction solution and incubated at 37 °C for 1 hour. Two mL of DNS was added to 1 mL sample from supernatant and filtrate to obtain mixture to determine enzyme amount and incubated at 90°C for 15 min. Afterwards, 1 mL Rochelle salt was added and vortexed. After tubes were cooled at room temperature, 5 mL pure water were added to every sample and vortexed. Samples were measured at 540 nm absorbance (T-80± UV-VIS Spectrophotometer, PG Instruments Ltd.) [32]. Measurements were performed with 3 replication from culture filtrates and mycelial extract.

**Statistical analysis.** The data were evaluated by using variance analysis technique using Minitab 17 packet program. The differences between the averages were determined by the Tukey test (P<0.05). The effects (%) are calculated according to the Abbott formula [35].

**RESULTS**

**Composition of thyme oil.** Basic components of thyme oil was determined. The results of the chromatography analysis of the thyme oil showed a high amount of carvacrol and thymol. Carvacrol and thymol amounts in thyme oil were 43.86% and 1.91%, respectively.

**Effect of thyme oil on mycelial weight in liquid medium.** Effects of different concentrations of thyme oil on mycelial weight (g) of *Rhizoctonia solani* in liquid medium was determined. Thyme oil added between 50-1000 ppm concentrations in liquid medium reduced the mycelial weight of *R. solani* with increasing concentration compared to control and completely inhibited at 1000 ppm (Table 1).

**TABLE 1**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Mycelial weight (g)*</th>
<th>Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10,576 a</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>8,439 b</td>
<td>20.2</td>
</tr>
<tr>
<td>100</td>
<td>7,516 b</td>
<td>28.9</td>
</tr>
<tr>
<td>250</td>
<td>4,187 c</td>
<td>44.3</td>
</tr>
<tr>
<td>500</td>
<td>1,226 d</td>
<td>70.7</td>
</tr>
<tr>
<td>750</td>
<td>0,234 e</td>
<td>80.9</td>
</tr>
<tr>
<td>1000</td>
<td>0,000 f</td>
<td>100</td>
</tr>
</tbody>
</table>

*Means shown with different letters in the same column are statistically different according to Tukey (P<0.05) test.

**Effect of thyme oil on total protein production (µg/mL) of *Rhizoctonia solani***

**TABLE 2**

<table>
<thead>
<tr>
<th>Thyme oil concentration (ppm)</th>
<th>Total protein amount (µg/mL)*</th>
<th>Culture filtrate</th>
<th>Mycelial extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31,6±0,002 a</td>
<td>26,8±0,002 a</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>24,2±0,001 b</td>
<td>19,1±0,002 b</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>22,6±0,003 b</td>
<td>18,2±0,001 b</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>18,3±0,003 c</td>
<td>16,6±0,001 c</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>15,6±0,001 c</td>
<td>8,9±0,002 c</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>8,1±0,002 d</td>
<td>7,5±0,001 c</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>2,4±0,002 e</td>
<td>1,0±0,001 d</td>
<td></td>
</tr>
</tbody>
</table>

* Means shown with different letters in the same column are statistically different according to Tukey (P<0.05) test.

**Effects of thyme oil on total proteins of *Rhizoctonia solani***. Effect of different concentrations of thyme oil on total protein production of *R. solani* in mycelial extraction and culture filtrate were determined. Thyme oil decreased total protein amount after mycelial extraction and culture filtrate (Table 2). Decreases in protein amount were observed at total protein production amount after mycelial extraction and in liquid medium where *R. solani* were grown with adding of different concentrations of thyme oil. It is determined that some protein-structured materials may be observed at highest
concentration levels of thyme oil at 1000 ppm. Those proteins are estimated to be present because of some metabolites secreted from very low amounts and the ones composed of vital activities of fungus at establishment in culture.

**Effects of thyme oil at liquid medium on pectin-methyl-esterase activity of *Rhizoctonia solani***. Effects of different concentrations of thyme oil on pectin methyl esterase activity (U/mL) of *Rhizoctonia solani* in liquid medium were determined. Thyme oil reduced pectin methyl esterase production of *R. solani* both in filtrate and after mycelial extraction with increasing concentration. No measurable value has been obtained at 750 vs 1000 ppm concentrations of thyme oil (Figure 1).

**Effects of thyme oil on cellulase activity of *Rhizoctonia solani***. Effects of different concentrations of thyme oil on *Rhizoctonia solani* cellulase activity (U/mL) in liquid medium was determined. Cellulase production and extracted mycelium of *R. solani* decreased with increasing concentration of thyme oil. Any measurable value has been obtained from 1000 ppm concentrations of Thyme oil (Figure 2).
DISCUSSION

Carvacrol and tymol ratios were found to be higher in thyme oil used in our study. Ozkaya et al. [36] obtained carvacrol and thymol ratios in their aromatic water 99.43% and 0.57%, respectively. Similarly, Kirimer et al. [37], found that some of the aromatic water of Origanum onites obtained by commercial firms contained the higher carvacrol (82% - 92.7%) and thymol (0-12%).

In our study, different concentration of thyme oil was reduced the mycelial weight of R. solani with increasing concentrations or completely inhibited at highest concentration. Similar studies have results showing thyme oils reduced and/or inhibited the mycelial growth of R. solani at solid and liquid mediums [38, 39]. Yanar et al. [22], researched effects of some plant essential oils on mycelial developments of soil borne pathogens and used Mediterranean thyme (Thymus citicus Boiss et Bal.) in their study. Thyme extract was added at 4, 8, ve 12 g/l dosages and have limited effect on mycelial growth of R. solani. In another study antifungal effects of some plant extracts and essential oils against root rot of cotton were studied in vitro [24]. In this study, the effect of different doses of thyme extract (%0.5, 1, 2, 4, 8) and oil (1, 2, 3, 5, 10 µl mL⁻¹) was tested on mycelial growth of R. solani. Thymus vulgaris L. inhibited the colony growth of R. solani at increasing concentration. The highest fungitoxic effect on colony growth of R. solani was determined at highest dosage (8 %) of thyme extract. All tested concentrations of thyme essential oil has been highly (100 %) antifungal effective and this effect was fungicidal.

Production of extracellular enzymes may be observed at culture filtrate and fungus mycelium. Chemicals and other ingredients added to medium may increase or decrease production of protein based materials. Biomass and extracellular protein production of Rhizoctonia solani A79 race in medium including Validamycin A and cellulose including medium, decreased [32]. Almost in a study of Ozgonen et al. [40] total protein production was researched at culture filtrate and mycelial extract of three Alternaria mali isolates. Protein content in nutritious media were determined both in culture filtrate and fungus mycelium via spectrophotometric analyses. As result of study total protein content in mycelial extract was determined higher. Total protein content was determined 100 times more in mycelial extract. But in our study total protein amount decreased with increase of concentration because of thyme oil was used as growth inhibitor.

In our study, decreasing effects of thyme oil was put forth when being added at different concentrations to medium conditions on Pectin-methyl-esterase production of R. solani. Before performed studies issues that enzyme acivity of R. solani differ according to medium conditions, growth conditions and anastomosis groups of Rhizoctonia spp. [41, 42, 43]. In additional to this, there are similar studies that some substances and chemicals added to medium decreased extracellular metabolites production of fungi. In a study of Robson et al. [32] reported Validamisin antibiotic stopping growth of R. solani, to decrease polygalacturanase activity that is one of an pectin degrading enzymes.

In our study, cellulase production of R. solani were decreased at increasing concentrations of thyme oil. In another study, enzyme production of R. solani was decreased in presence of Validamisin A with the presence of carboxymethyl cellulase (CMCase) and cellobiose in vitro [32].

CONCLUSION

In conclusion, essential oil of Origanum onites L. (İzmir Thyme) primarily decreases protein amount, pectin-methyl-esterase and cellulase activities of R. solani and stops at most effective concentration levels because of the effects of carvacrol and tymol.

In recent years, alternative and supportive methods are conducted to control of plant pathogens. Plant extracts and essential oils are popular ones of those studies and important outputs were obtained. More detailed studies are being performed with plant extract and essential oils against some diseases. Nanotechnological products are produced and launched to market among of them. In vitro ve in vivo effects of thyme species among medical and aromatic plants to control of plant pathogens were determined. Thyme essential oils are observed to have fungistatic and fungitoxic effect on fungal and bacterial pathogens. As previous studies reviewed, some of them are concerned with effects of thyme oil and extracts against pathogens and effects on durability parameters of plants in vivo. But, studies on direct effects of plant extract and essential oils are in small number. In our study, pectin-methyl-esterase and cellulase, that are two important enzymes produced by R. solani were observed to decrease at increasing concentration of thyme oil. Enzymes produced by plant pathogens are one of virulence factors while at entrance and spreading in plant. In this sense, it is a new study about effects of herbal products on enzyme production of R. solani. In the study, effects on only two of enzymes as pectinase and cellulase were researched. Some other enzymes like polygalacturase, protease and laccase are also important as pathogens entrance and spread in plants. Prospective studies aiming to determine effects of thyme extracts and essential oils may be important. Also, effects of thyme species to pathogens may vary. Also, the effects of different thyme species may be researched in those aspects for further studies. In this sense, our study provided review of effects of thyme oil on pathogen activity. Our study will enlighten future studies. Similar studies about effects of extracts and essential
oils of medical and aromatic plants which known beneficial effects against pathogens may be the subject of research.

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NUMERICAL ANALYSIS ON FLOW CHARACTERISTICS OF WATER IN UPWARD DIP SECTION OF LONG DISTANCE CRUDE OIL PIPELINE

Guiyin Zhang*, Dong Li, Dongsheng Yao, Liqiang Xue, Hui Gao, Zhenya Chen, Zhongxiao Niu

The No. 3 Oil Production Plant of Huabei Oilfield, PetroChina, Hebei Province, Hejian 062450, China

ABSTRACT

In order to avoid the corrosion risk caused by the internal water in the pipeline, the transnational long distance crude oil pipeline carries out the accumulated water in the low-lying part of the topographic undulating pipeline through the flow of the crude oil itself. According to the characteristics of oil-water two-phase flow state and oil-water interface fluctuation, when the flow rate of crude oil exceeds the critical speed of contact angle θ and crude oil velocity, and the wettability of the wall. When the contact angle is 30° (hydrophilic wall surface), the oil-water interface appears wave shape, and when the contact angle is 120° (hydrophobic wall surface), the water accumulates into water mass on the upward inclined pipe. The flow rate of water increases with the increase of contact angle θ and crude oil velocity, and when the flow rate of crude oil exceeds the critical velocity of Kelvin-Helmholtz interface fluctuation, it will lead to the accelerated movement of water.

KEYWORDS:
Crude oil pipeline, Water accumulation, Wall wettability, Water carrying flow, Numerical simulation

INTRODUCTION

In the past few decades, the two-phase flow in pipelines has been extensively studied because it occurs in many industrial pipeline transportation processes, especially in the oil industry, such as crude oil gathering and transportation pipelines on offshore platforms and production fluid transportation in desert oil fields [1-3]. In the oil-water two-phase mixed conveying process, the interface of the oil-water two-phase can exhibit different characteristic structures due to the different flow patterns. However, the pavement of the oil pipeline is often dominated by hills and the plains are less. Therefore, the pipeline consists of horizontal sections, ascending sections and descending sections and is connected by elbows. The oil-water flow in the slope pipelines is much less concerned than in the straight pipeline section. Because of the higher density of water compared to the oil phase inside the pipeline, water tends to deposit at the bottom of the pipeline, especially during shutdown period, where the water phase in the fluid medium deposits and accumulates at the bottom of the pipeline [4-6]. The water phase deposited at the bottom of the pipeline is in contact with the metal matrix on the inner wall of the pipeline, and under the condition that the pipeline fluid medium contains corrosive substances such as CO₂, H₂S and bacteria, it is easy to cause corrosion of the pipeline. The resulting corrosion products can cause blockage of critical components such as valves, further causing pipeline safety problems. From a practical point of view, accurately predicting the distribution and flow mechanism of trapped water, and the minimum oil flow required for oil displacement are critical to the process of carrying water in crude oil [7]. By means of crude oil flow, the water is taken away from the straight pipe section or the low section, which can not only solve the problem of water accumulation but also save management costs. However, due to the influence of the complex flow state of oil-water two-phase flow in upward dip section, there is still a lack of in-depth research on the interface morphology of oil-water two-phase flow and the process of water accumulation movement [8-10].

Computational fluid dynamics (CFD) simulations provide a very intuitive and clear understanding of the mechanism for controlling interface flow. In this case, experimental techniques are limited by the inherent three-dimensional nature of the flow. Flow states with complex interfacial motions such as gas-liquid and liquid-liquid are now accurately solved using CFD calculation method [11-14]. Among many CFD calculation methods, the fluid volume (VOF) method is widely used to simulate oil-water two-phase flow, and the VOF algorithm uses a single-fluid model to directly capture interface motion.
Therefore, the numerical simulation method is used to analyze the flow characteristics of water in upward dip section of long distance crude oil pipeline, and the continuity equation and momentum equation based on VOF model are solved. The specific morphology of the oil-water interface was analyzed, and the two-phase flow characteristics under different up-angle conditions were compared.

SIMULATION METHODS AND PARAMETERS DESIGN

In order to simulate and analyze the flow characteristics of water in upward dip section, a mathematical model of oil-water two-phase flow was established [15-17]. OpenFOAM software was used to simulate the flow state of the water-carrying process. To capture the flow state and interface shape of the oil-water interface, the InterFoam solver based on VOF model is used to solve the dynamic equation of oil-water interface.

**Governing equation.** In this paper, the oil-water two-phase is used as a mixed fluid, and a volume fraction $\alpha$ (0<\(\alpha\)<1) is defined in the cell to identify the oil phase and the water phase [18]. The expression of the Navier-Stokes equation of the mixed fluid can be obtained as follows:

$$ \frac{\partial \alpha}{\partial t} + \nabla \cdot (\alpha \mathbf{u}) + \nabla \cdot \left[ \alpha (1-\alpha) \mathbf{U}_r \right] = 0 \quad (1) $$

Where $t$ is time, $s$; $\mathbf{u}$ represents the fluid velocity vector, m/s; the last term in the equation is the compression term which is used to prevent the divergence of the alpha value at the oil-water interface [18-19].

Continuity equation and momentum equation are shown as follows:

$$ \nabla \cdot \mathbf{u} = 0 \quad (2) $$

$$ \frac{\partial (\rho \mathbf{u})}{\partial t} + \nabla \cdot (\rho \mathbf{u} \mathbf{u}) = -\nabla p + \nabla \cdot \left[ \mu \left( \nabla \mathbf{u} + (\nabla \mathbf{u})^T \right) \right] + \rho g + \sigma n k \nabla \alpha \quad (3) $$

Where $p$ is the pressure, Pa; $\rho$ is the density of the mixed fluid, Kg/m$^3$; $\mu$ is the dynamic viscosity of the mixed fluid, Ns/m$^2$; $g$ is the acceleration vector of gravity, $\sigma$ is the surface tension coefficient, $n$ is the normal vector of the interface, and $k$ is the curvature.

The volume fraction equation and the momentum equation are solved discretely by using the first-order explicit format and the first-order implicit format respectively. The spatial derivative of the equation is solved by the second-order TVD format. In order to obtain a better numerical solution of the oil-water interface, in this paper, the last item of the volume fraction equation is solved discretely using the interface compression format. The pressure-velocity coupling equation is calculated using the PISO solution format.

**Geometric model and physical parameters.** As the pipeline is temporarily stopped, the water phase in the internal medium of the pipeline will accumulate at the bottom of the pipeline under the action of gravity settlement, and the liquid in the upward dip section of the pipeline will slide down with the pipeline [20-23]. The water will eventually gather at the lowest point of the pipeline. In order to improve the actual fluid situation at the simulated site, this paper established the pipeline model based on the actual pipeline of an oilfield, and designed the physical parameters of the oil phase and the water phase according to the nature of the fluid medium inside the pipeline. The geometric model and meshing results are shown in Fig. 1. The physical properties of the oil and water phases are shown in Table 1.

**FIGURE 1**

3D geometric model structure and meshing results
When amount of water accumulation taken as the initial calculation conditions (The tion is carried out, the experimental conditions are the end of the pipeline. When the numerical simulation results based on the crude oil carrying capacity data at simulation results are compared with the experimental results of this paper, the numerical simulation results are shown in Fig. 2. It can be seen from Fig. 2 that under the condition of oil phase and water phase velocity in the lateral surface of the pipeline, and the crude oil flow rate is asymmetrically distributed on the lateral surface of the pipeline, and the crude oil flow rate is the largest at the oil-water interface. This is due to the existence of the upward elbow leading to the flow state of crude oil is not completely stable after changing the flow direction, and the existence of shear stress at the oil-water interface leads to the promoting effect of crude oil on the upward displacement of stagnant water. However, even under the current conditions, the water phase located in the upward dip pipeline still has a downward flow trend [17, 22-23]. The strong shear stress caused by the upward flow of crude oil makes the accumulated water eventually flow upward and gradually flow out of the pipeline outlet. Two-phase flow characteristics of upward dip pipeline. It can be seen from Fig. 3 (a) that the crude oil flow rate is asymmetrically distributed on the lateral surface of the pipeline, and the crude oil flow rate is the largest at the oil-water interface. This is due to the existence of the upward elbow leading to the flow state of crude oil is not completely stable after changing the flow direction, and the existence of shear stress at the oil-water interface leads to the promoting effect of crude oil on the upward displacement of stagnant water. However, even under the current conditions, the water phase located in the upward dip pipeline still has a downward flow trend [17, 22-23]. The strong shear stress caused by the upward flow of crude oil makes the accumulated water eventually flow upward and gradually flow out of the pipeline outlet. It can also be seen from the figure that when the contact angle $\theta$ is $30^\circ$, the oil-water interface in the upward dip pipe is in a wave form, and the flow rate

### RESULTS AND DISCUSSIONS

**Verification of numerical simulation results.** In order to verify the correctness of the numerical simulation results of this paper, the numerical simulation results are compared with the experimental results based on the crude oil carrying capacity data at the end of the pipeline. When the numerical simulation is carried out, the experimental conditions are taken as the initial calculation conditions (The amount of water accumulation $V_w$ is 15ml, 25ml, 40ml; the wall condition is hydrophobic wall, and the contact angle $\theta = 120^\circ$). The results of the calculation are shown in Fig. 2.

It can be seen from Fig. 2 that under the conditions of different pipeline liquid volume, the experimental value and simulation value of the effect of crude oil velocity on the water carrying capacity of pipeline outlet are in good agreement with each other. At the same time, both numerical simulation and laboratory experimental studies have predicted that the minimum crude oil velocity causing the minimum water carrying effect of crude oil is $U_{w_{\text{min}}}=0.08\text{m/s}$. When $U_{w_{\text{min}}}$ is in the range of $0.08\text{m/s} \sim 0.12\text{m/s}$, the numerical simulation results are in good agreement with the experimental results, but the numerical simulation results are obviously lower. This is due to the slip between oil and water caused by the inconsistency of oil phase and water phase velocity in VOF model. At the same time, in the process of numerical simulation, the capillary effect of the inner wall surface of the actual pipeline cannot be reproduced very well, and the existence of capillary effect will make the water droplets more easily condense into a whole and promote the aggregation of water molecules. Thus, the separation of droplets and the carrying of water phase are promoted. When $U_{w_{\text{min}}} > 0.12\text{m/s}$, the water at the bottom of the pipeline is gradually taken out of the pipeline, which is as consistent with the experimental results. Therefore, the mathematical model selected and the numerical simulation method adopted in this paper can accurately simulate the water-carrying flow of crude oil.

### Table 1: Physical properties of fluid medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Density ($\text{kg/m}^3$)</th>
<th>Dynamic viscosity ($\text{mPa s}$)</th>
<th>Surface tension ($\text{mN/m}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude oil</td>
<td>856</td>
<td>3.43</td>
<td>18.33</td>
</tr>
<tr>
<td>Water</td>
<td>997</td>
<td>0.895</td>
<td>/</td>
</tr>
</tbody>
</table>

**FIGURE 2**

Water carrying capacity of crude oil at pipeline outlet under different crude oil flow rates

### Table 1: Physical properties of fluid medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Density ($\text{kg/m}^3$)</th>
<th>Dynamic viscosity ($\text{mPa s}$)</th>
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<td>997</td>
<td>0.895</td>
<td>/</td>
</tr>
</tbody>
</table>

9570
appears the maximum near the oil-water interface, and the flow rate decreases at the top of the pipeline. This is due to the influence of the volume extrusion of the accumulated water and the force of oil-water interface at the oil-water interface. The velocity of crude oil increases to a certain extent. Moreover, the position near the top of the pipeline is weakly affected by the extrusion effect of the volume of the accumulated water. At the same time, it is obviously hindered by gravity and pipeline wall, so the flow rate of crude oil from the oil-water interface to the top of the pipeline shows a decreasing trend, and when the contact angle $\theta$ is 120°, the water phase in the upward dip pipeline accumulates in the shape of water mass. Because the formation of water mass reduces the effective flow area in the pipeline, the flow rate of crude oil increases obviously [11-14].

Comparing Fig. 3(a) and (b), it can be found that the maximum flow rate of the water phase appears near the bottom of the pipeline. This is due to the less shear stress from the oil-water interface at the bottom of the pipeline, which is not enough to overcome the gravity of the water phase. At this time, the water phase shows an obvious downward movement trend. But in the process of continuous downward movement, the thickness of the water phase continues to become thinner. The shear stress from the oil-water interface is increasing. When the critical point is crossed, that is, the action of shear stress is greater than that of gravity, the water phase tends to move upward. This process constitutes a complete cycle, and the accumulated water continues to move upward and upward in the process of this cycle.

**Local flow pattern analysis.** Fig. 4 shows the pressure field and streamline diagram at the elbow and the upward dip section of the pipeline. It can be seen from the figure that when the flow rate of crude oil is lower, the shape of the oil-water interface changes dynamically. The oil-water interface is wavy and tends to move upward due to the shear stress. It can be seen from Fig. 4(a) that the accumulated water tends to flow upward near the oil-water interface due to the shear stress of the oil-water interface. However, near the bottom of the pipeline, because the shear force at the oil-water interface is not enough to drive accumulated water upward under the condition of overcoming gravity, the stagnant water at the bottom of the pipeline tends to flow downward. This is consistent with the phenomenon of downward flow near the wall in the stratified oil-water flow in the inclined pipe.

For the case of $\theta=120^\circ$ shown in Fig. 4(b), a similar dynamic process is observed in the interior of
the pipeline. It can be seen from the figure that the water accumulation mass is located at the elbow of the pipeline, and the obvious wave phenomenon can be found on the water phase interface of the pipeline. From the streamline of the Fig. 4, it can be found that only a small part of the water in the water mass tends to flow downward. On the other hand, an obvious circulation of accumulated water can be found at the bottom of the water accumulation mass. When the shear stress at the oil-water interface is not enough to overcome the gravity and drive the water to flow upward, the accumulated water at the bottom of the pipeline tends to flow downward. Because in the lower half of the water mass, the thickness of the water mass gradually decreases and the shear force at the oil-water interface gradually increases. This again forms the phenomenon that the shear stress at the oil-water interface is greater than the gravity, so that the accumulated water shows a trend of continuous upward movement. As a result, the circular movement of the water accumulation mass is formed. The pressure gradient distribution in Fig. 4 shows the characteristics of equivalent distribution, which indicates that the shear stress at the oil-water interface and the movement of water masses have no obvious effect on the pressure distribution in the pipeline. At the same time, because the pressure in the figure shows horizontal distribution, this shows that in the interior of the upward dip pipeline, the pressure gradient line is horizontal. This shows that the pressure gradient in the pipe is affected by gravity.

**FIGURE 5**
Schematic diagram of the accumulated water flow in upward dip section of pipeline

**Influence of crude oil flow rate on the moving speed of accumulated water.** Fig. 5 and Fig. 6 show the principle of the accumulated water flow in the upward dip section and the relationship between the moving velocity of the accumulated water and the velocity of crude oil under different wall wettability conditions.

**FIGURE 6**
The relationship between the flow rate of the accumulated water and the velocity of crude oil
It can be seen from Fig. 6 that the influence of the amount of water accumulated at the bottom of the pipeline on the flow rate of the accumulated water can be ignored in the process of water carrying flow in the upward dip section. But the influence of wall wettability and crude oil velocity on the flow rate of the accumulated water is more obvious. The overall performance is that the larger contact angle \( \theta \) and the larger crude oil velocity promote the flow of the accumulated water [16]. At the same time, when the flow rate of crude oil continues to increase from 0.16 m/s at the contact angle \( \theta = 30^\circ \), there is an obvious acceleration phenomenon of the accumulated water movement (Kelvin-Helmholtz interface fluctuation phenomenon). When the Kelvin-Helmholtz interface fluctuation phenomenon appears, the shear action at the oil-water interface increases sharply, which leads to the acceleration of the accumulated water movement.

The influence of crude oil flow rate on the movement speed of the accumulated water is caused by directly affecting the shearing force of crude oil on the accumulated water. The wall wettability affects the moving speed of the accumulated water indirectly by affecting the effective hydraulic diameter in the pipeline. In order to further analyze the effect of wall wettability on the effective hydraulic diameter of the pipeline, the corresponding simulation calculation is carried out, and the calculation results are shown in Fig. 7.

It can be seen from Fig. 7 that \( h_w \) increases with the increase of contact angle \( \theta \), and slightly increases with the increase of water accumulation. The difference of \( h_w \) is small when the contact angle \( \theta \) is 90° or 120°. This is consistent with the phenomenon that the moving velocity of the accumulated water has a similar changing trend under this condition [15] (Fig. 6). However, when the contact angle \( \theta \) is 120° or 150°, \( h_w \) decreases gradually with the increase of crude oil flow rate. This is due to the internal oil-water interface shear stress to overcome the capillary effect of the inner wall of the pipeline. When \( \theta \) is 30°, the initial height \( h_w \) of the accumulated water at the bottom of the pipeline increases with the increase of the volume of stagnant water. This is due to the existence of a curved part of the pipeline, which limits the axial development of the accumulated water. When \( \theta \) is 120° or 150°, the shape of the accumulated water at the bottom of the pipeline is uncertain. The initial height \( h_w \) is independent of the amount of water accumulated.

**FIGURE 7**

The relationship between the flow rate of the accumulated water and the flow rate of crude oil
When \( \theta \) is 30°, the slope of the relation curve between \( U_{w} \) and \( U_{os} \) changes obviously at \( U_{os} = 0.16m/s \). In addition, the height of the water mass shows the maximum at a similar oil speed, as shown in Fig. 6. This is because at the \( U_{os} > 0.16m/s \), the fluctuation of Kelvin-Helmholtz interface occurs at the oil-water interface. The fluctuation of the oil-water interface leads to the decrease of the effective cross-section area of the oil flow, which leads to the sharp increase of the accumulated water velocity observed in Fig. 6.

On the premise of considering the viscous effect, Brauner and Moalem derived the unstable conditions for the fluctuation of Kelvin-Helmholtz interface in oil-water two-phase flow.

\[
U_{os} > \sqrt{\frac{12(\rho_{w} - \rho_{o}) g \cos \theta A_{o}^{3}}{\pi^{2}D^{4} \rho_{o} \left( dA_{w} / dh_{w} \right)}} \tag{4}
\]

Where \( U_{os} \) is the crude oil flow rate, m/s; \( \rho_{w}, \rho_{o} \) are respectively the density of water and crude oil, kg/m³; \( \theta \) is the contact angle; \( A_{o}, A_{w} \) are respectively the surface area of water and crude oil, m²; \( D \) is the inner diameter of the pipe, m.

When \( \theta \) is 30° and 90°, the critical flow rate of the Kelvin-Helmholtz interface fluctuation phenomenon is respectively 0.135m/s and 0.118m/s. Since these values are lower than the crude oil flow rate studied in Fig. 6 and Fig. 7, the oil-water interface is wavy in the whole range of \( U_{os} \). However, the fluctuation of Kelvin-Helmholtz interface is not observed in the results.

**CONCLUSIONS**

In this paper, the flow characteristics of the accumulated water in the upward dip section of long distance crude oil pipeline are studied. The numerical simulation analysis is carried out by the method of establishing mathematical model, and the numerical simulation results are compared with the experimental results. The effects of two-phase flow characteristics, local flow pattern and crude oil flow rate on the movement of the accumulated water in the pipeline are evaluated. The following conclusions are drawn:

1. The method of using OpenFOAM to simulate and analyze the water-carrying flow is feasible, and the simulation results are in good agreement with the experimental results.

2. The morphology of the oil-water interface of the upward dip pipeline is related to the wettability of the wall. When the contact angle is 30° (hydrophilic wall), the oil-water interface is wavy. When the contact angle is 120° (hydrophobic wall), the stagnant water aggregates into a water mass on the upward dip pipeline.

3. The moving velocity of the accumulated water increases with the increase of contact angle \( \theta \) and the flow rate of crude oil. When the flow rate of crude oil exceeds the critical flow rate of Kelvin-Helmholtz interface fluctuation of the accumulated water, it will lead to the phenomenon of accelerated movement of the accumulated water.

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CHARACTERISTICS AND MECHANISM OF CHEMICAL STRUCTURE CHANGES IN MICRO-DEFORMATION OF MEDIUM COAL GRADE COAL UNDER SECONDARY HIGH TEMPERATURE AND HIGH PRESSURE DEFORMATION

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ABSTRACT

The self-developed TRTP-2000 high temperature and high pressure deformation experimental system is used to test the deformation of coal under sub-high temperature and high pressure deformation conditions. Micro-Raman and Micro-FTIR in-situ test methods are used to observe the microscopic deformation of coal after deformation. The typical brittle and ductile deformation points in the domain are analyzed for macromolecular structure. The results show that with the decrease of strain rate, the toughness deformation phenomenon in the sample develops gradually, which promotes the increase of secondary structural defects and the disorder of molecular structure. The brittle deformation is produced by the coupling of mechanical force and frictional heat. The oxygen-containing functional groups of coal macromolecules break and fall off with side chains, resulting in increased disorder and secondary structural defects. The ductile deformation acts on the coal macromolecular structure through the continuous accumulation of strain energy, due to the difference in strain rate. The deformation time changes. The high strain rate ($\geq 1 \times 10^{-6}$) toughness deformation leads to an increase in the total amount of aromatic carbon, a decrease in molecular structure disorder, and a relative decrease in secondary structural defects; a deformation at a low strain rate ($=2 \times 10^{-7}$) There is sufficient time to accumulate a large amount of strain energy in the process, which leads to dislocation slip of the aromatic layer. The accumulation of dislocations causes the aromatic ring to disintegrate, the total amount of aromatic carbon is relatively reduced, and the secondary structural defects are increased.

KEYWORDS:
Microscopic deformation, Chemical structure, Brittle deformation, Ductile deformation

INTRODUCTION

In the process of structural stress causing deformation of coal body, the physical structure of pores and fractures of coal body is not only modified [1-3], but also the chemical structure has regular changes [4-8]. Pan et al. and other studies have shown that the degree of secondary structural defects of coal molecules under strong and weak deformation is different from the degree of coalification [9-14]. Cao Daiyong proposed stress polycondensation and stress degradation to explain the chemical effects of structural coal, and believed that stress promotes the advanced evolution of coal [15-16]. Xu Rongting et al. believe that stress can promote polycondensation through experimental research [17]; The weak toughness deformation and toughness deformation have different effects on aromatization and defect formation on the basis of studying natural sequence tectonic coal, resulting in different degrees of the conclusion that the effect of coal macromolecular polycondensation is opposite [18-20]. It can be seen that the mechanism and effect of different deformation mechanisms on the structure of coal macromolecular structure are still unclear and further research is needed.

High temperature and high pressure experiments are very effective methods for studying the formation mechanism of structural coal. However, the previous high temperature and high pressure experiments tend to concentrate the temperature conditions above 200 °C, which is higher than the pyrolysis temperature of the sample, and cannot eliminate the pyrolysis effect on the coal structure. Impact. The sub-high temperature conditions set the experimental temperature below 200 °C for the above problems, and try to eliminate the chemical structure change of the coal caused by pyrolysis. In this paper, the sub-high temperature and high pressure experiments were designed by self-developed high temperature and high pressure deformation experimental instruments. The in-situ Micro-FTIR and Micro-Raman detection methods were used to analyze the sub-high temperature and high pressure of the chemical structure of the coal under the experimental
The heating system uses a graphite tube as a heating medium, and the upper and lower indenters serve as electrodes, and the temperature is increased by electric heating. The thermocouple is inserted into the middle of the pressure bar from the bottom to detect the sample temperature, and the temperature control system uses the SRS temperature controller.

**MATERIALS AND METHODS**

**Sample selection and preparation.** According to the national standard, samples of the original structural massive coal in the Xishan mining area of Shanxi Province were collected (Fig. 1). The coal sample is Triassic native structural coal, and $R_o$-max is between 0.59% and 0.81%, belonging to medium-low coal grade coal. The components of the block sample were uniform, the layering was clear, the endogenous fissures were sparsely developed, and they were cut by two sets of vertical layers. It can be seen that calcite and pyrite minerals are mostly filled in endogenous fissures. The sample is bright coal, and the content of mirror coal and bright coal is high. The coal sample was drilled by the drilling sample, and a total of 5 samples (numbered 1~5) were drilled. Each sample size was φ=25mm, H=45mm cylinder (Fig. 1). Samples 1~5 are located in the same coal seam, and the influence of the primary sedimentary micro-environment and microscopic composition on the chemical structure is negligible in the same structural part.

**Experimental instrument and methods.** The deformation experiment was completed by the self-developed "High Temperature and High Pressure Deformation Experimental System TRTP-2000". The experimental system includes an experimental deformation system, a pressure system, a cooling system, a heating system, and a temperature control system. The partial pressure medium of the loading reaction system is a compacted analytically pure NaCl or salt sleeve (Fig. 2). The experimental instrument realizes the constant strain rate deformation experiment by controlling the moving speed of the lower pressing head by the digital hydraulic servo. The heating system uses a graphite tube as a heating medium, and the upper and lower indenters serve as electrodes, and the temperature is increased by electric heating. The thermocouple is inserted into the middle of the pressure bar from the bottom to detect the sample temperature, and the temperature control system uses the SRS temperature controller.

The open system is used in the experimental process, and the gas evolution in the deformed coal body only affects the mechanical properties of the coal rock indirectly, so it will not be considered in the subsequent discussion [21]. In the experiment process, the actual burial process of the coal sample is simulated by synchronous warming and boosting to the burial condition. After the pre-set condition is reached, the constant strain rate is set to carry out the deformation experiment. A total of 4 sets of experimental samples and 1 set of control samples were set. The experimental conditions of the samples are shown in Table 1.
**Methods.** The sample for the deformation experiment was borrowed from the treatment method of Korolev, Y. and Panigrahi, D. [22-23], and the sample was consolidated by injecting epoxy resin. After the consolidation, the sample was cut perpendicular to the bedding plane and the sample was cut. The sample section was polished by a number of incremental sandpapers, and finally the cut surface was polished with an Al2O3 colloidal solution. Under the optical microscope, the microscopic deformation characteristics of the polished sample surface were observed and described. At the same time, the test points in the typical brittle and tough microscopic deformation domain were selected and marked to prepare for the Micro-FTIR and Micro-Raman tests.

1. Micro-Raman is a Raman Spectroscopy Senterra micro-convex Raman spectrometer manufactured by Bruker. The in-situ test method of Yandoka, B. and Wang, G [24-25], is used to test the representative points of the mark under the optical microscope. The test wavelength is 532nm laser, the laser power is 5 mW, 2s integration time, 10 cumulative times, the resolution of this test is 9-18cm⁻¹.

2. Micro-FTIR is a Fourier transform infrared spectrometer (Fourier Transform Infrared Spectroscopy Vertice 80V) equipped with a HYPERSION 2000 infrared microscope manufactured by Bruker to perform the spot test in the reflection mode. In the process of detection, Liu et al. used the method of reflection in situ Micro-FTIR to detect the position of Micro-Raman detected in situ. The test resolution is 4 cm⁻¹ and the number of scans is 128.

The infrared spectral characteristics of different micro-coal components indicate that the vitrinite group is affected by the adjacent coal and rock components and its heterogeneity, and its chemical structure shows a certain degree of heterogeneity [36-38]. However, the chemical structure characteristics of the same coal rock component in the small scale are relatively small. Therefore, the in-situ test points select a square region (with a side length of 300 μm) with a uniform internal microstructure of the broad vitrinite strip away from other components. The test is repeated several times of the selected area, and the waveform representing the average level is selected to characterize the final test result in the square area [26]. At the same time, the same sample is selected to select the points in the same vitrinite strip, and the same sample is selected for different samples to eliminate the influence of the original factors and improve the contrast.

**RESULTS**

**Microscopic deformation characteristics of samples.** In this experiment, the original structure of the original structure control sample 1 was preserved intact, and the short cracks of the vertical bedding plane were sparsely developed, which was more stable and flat (Fig. 3(a)). The experimental samples showed different microscopic deformation phenomena with the change of experimental conditions. The experimental samples with high strain rate mainly developed brittle deformation phenomenon. With the decrease of strain rate, the plastic deformation phenomenon of samples from No.1 to No.5 gradually became significant.

The microscopic deformation characteristics of the sample are typical. Take the No. 2 and No. 5 samples as an example. The No. 2 sample experiment uses the highest strain rate of this experiment, 1×10⁻⁵ s⁻¹. The microscopic deformation phenomenon of the sample is mainly brittle deformation, and the development is typical. The micro-rupture or rupture zone, the fractures are more parallel to each other, and the angle between the principal stress is about 50°, the crack width is narrow, and even the “necking” phenomenon occurs (Fig. 3(b)); only the partial visible component strip. The small folds with strong bending deformation are typical microscopic fold plastic deformation phenomena (Fig. 3(c)). The strain rate of the No. 5 sample deformation experiment is the lowest, only 2×10⁻⁷ s⁻¹. The whole sample is mainly plastic deformation, and the angle between the main crack and the principal stress of the sample is about 30°, along the two groups of parallel main cracks. A typical SC microstructure is developed (Fig. 3(d)).

Due to the uneven distribution of stress in the same sample, the micro-mechanical deformation mechanism of different parts of the sample is different, so that brittle deformation and toughness deformation may occur inside the same sample. As the strain rate decreases, the plastic deformation phenomenon increases significantly, indicating that the low strain rate promotes the development of plastic deformation. The brittle deformation and toughness deformation of coal have different ways, mechanism and efficiency for the change of macromolecular structure of coal [12-14, 21], so the change of coal macromolecular structure in microscopic deformation domain with different deformation phenomena. There may also be differences, and accordingly,
a series of Micro-FT IR and Micro-Raman tests are performed for different deformation regions.

**Analysis of chemical structure change characteristics.** Raman spectroscopy is very sensitive to crystal microstructure and molecular structure, and is an effective means to study the microstructure and properties of graphite materials [27]. Raman spectroscopy graphite species characteristic peaks include primary and secondary mode regions. The primary mode region includes G and D peaks, and the D peak belongs to the vibration mode of amorphous graphite irregular hexagonal lattice structure, which is related to the vibration mode of A1g; G peak is in-plane C= The stretching vibration of the C double bond is generated, which is related to the E2g mode of the graphite aromatic layer. Peak displacement, peak area and full width at half maximum can be used as characteristic parameters of molecular structure. The peak displacements of the G and D peaks are selected as the characterization parameters. The transformation of the peak displacement is controlled by the molecular vibration level. Different chemical bonds or valence states have different vibration modes and determine the vibration energy between the energy levels. The D peak-to-peak displacement WD is affected by the secondary structural defects and the order of the molecular structure caused by the structural deformation; the G peak-to-peak displacement WG is mainly affected by the aromatic ring condensation degree and the total amount of aromatic carbon.

Firstly, the Raman spectral line is fitted. In order to ensure the best fitting effect, the fitting method (Fig. 4) adopted by pan JN is used to extract the peak displacements of the fitted G and D peaks.

Under 100°C high strain rate deformation (strain rate $=1 \times 10^{-6}$), the experimental samples are mainly brittle deformation, mechanical fracture and grinding of brittle deformation lead to enhanced mechanical chemistry, and friction heat generation during crushing and grinding. Under the joint action of the two mechanisms, the branches and functional groups of the macromolecules break and fall off into disordered units, resulting in an increase in the disorder of the molecular structure, and at the same time, microscopic shearing and other effects on the next structural defects are also increased; The strain rate (strain rate = $2 \times 10^{-7}$) deformation is gradually significant, and the mechanical energy of ductile deformation is mainly transformed into strain energy, which makes the coal body more susceptible to secondary structural defects. Therefore, the D peak displacement of each experimental sample increases with the decrease of the strain rate (average value), the disorder of molecular chemical structure increases, and the development degree of secondary structural defects also increases. The strain rates used in samples No. 3 and No. 4 were the same, but the overall value of WD (average value) of sample No. 4 was slightly higher than that of sample No. 3, indicating that the increase in temperature caused the WD value of the sample to increase, mainly due to the increase in temperature. The mobility of the molecules, the side chains and the functional groups are more likely to break and fall off under deformation (Fig. 5(a)).

**FIGURE 3**
Sample microscopic deformation picture
Notes: (a) sample Y microscopic picture; (b) sample 2 microscopic picture of brittle deformation zone; (c) sample 2 plastic deformation zone microscopic picture, (d) sample 5 plastic deformation zone S-C surface
Fig. 5(b) shows that the peak displacement of the G peak decreases with the strain rate, and the overall value (average value) increases first and then decreases. The total amount of aromatic carbon in the coal molecule increases first and then decreases with the decrease of the strain rate. Under the deformation of 100°C high strain rate, WG increases with the increase of strain rate [11, 19]. The fatty chain and oxygen-containing functional group in the molecular structure become free small molecules, which makes the total amount of aromatic carbon in coal molecules relatively increase. When the strain rate of sample 5 reaches $2 \times 10^{-7}$, the WG shows a downward trend, and even the individual point values are lower than the original. The results are similar to those of Pan, J. [14], mainly due to low strain. Under the action of rate toughness deformation, the strain energy accumulated in a sufficient amount of time, causing the aromatic layer of the molecular structure of the coal body to be dislocated and slipped. The disintegration of the aromatic ring under the action of a large amount of accumulated
dislocation energy leads to a decrease in the total amount of aromatic carbon. The experimental temperature difference between samples 3 and 4 resulted in a slight decrease in WG, indicating that the increase in temperature caused an increase in molecular mobility and a decrease in the amount of aromatic carbon under stress deformation.

According to the microscopic deformation characteristics of the area where the measuring point is located, it is divided into three sections: undeformed, brittle and ductile deformation. The absissa represents the intensity of the microscopic deformation phenomenon (Fig. 6). From the undeformed, brittle deformation to the ductile deformation zone, the overall WD (average value) shows an increasing trend, and the secondary structural defects of the coal body increase, which is consistent with the research results of Lin Hong and others. It is considered that the ability of tough deformation to produce secondary structural defects is higher than that.

The average value of WG and the whole are in a wave-fold change. The average value of WG in the ductile deformation zone is lower than the average value of WG in the brittle deformation zone, indicating that the brittle deformation causes the fracture of the side chain and the functional group to be separated from the aromatic layer, and the free small molecule makes the total amount of aromatic carbon relatively [13, 25]. The effect of toughness deformation depends on the strain rate. In this experiment, the point of high strain rate is selected more, and the increase of the amount of aromatic carbon under high strain rate toughness is higher than that of low strain rate. The deformation causes the degree of aryl carbon to decrease, so the WG value of the ductile deformation zone decreases only slightly relative to the brittle deformation zone, but is still higher than the original coal sample.

Mechanism of chemical structure change under the action of brittleness and toughness change of coal body. Through the analysis between samples and the chemical structure characteristics of the microscopic deformation domain inside the sample, it is found that the microscopic phenomena, chemical structure characteristics and mechanism of action between the samples and the internal brittle and ductile deformation of the sample are uniform. The brittle deformation effect and the ductile deformation effect are not only different in the microscopic deformation phenomenon, but also the macromolecular structure of the coal is changed by different action mechanisms. The ductile deformation effect increases the secondary structural defects of coal molecules by converting mechanical energy into strain energy. Correlation calculations show that the strain energy is much larger than the energy of molecular bond rupture [9, 24-26]. Therefore, it is easier for the coal to break and fall off the ductile deformation. The ability to structural defects is stronger. The ductile energy is converted into strain energy under different strain rates and the accumulated time is inconsistent.

The strain energy accumulation under high strain rate toughness deformation promotes the selective insertion of free small molecules into secondary structure defects, which reduces the disorder of molecular structure and increases the molecular stability. At the same time, the accumulation of strain energy can promote the occurrence of aromatic fused rings. Rearrangement and mutual tiling, as well as free small molecule chemical polycondensation, increase the amount of aromatic carbon. The ductile deformation at low strain rate is transformed into a large accumulation of strain energy, which causes the dislocation energy during sufficient deformation time. The aromatic ring disintegrates and the secondary structural defects of the coal molecule increase.

CONCLUSIONS

(1) The comparative analysis between the samples shows that the sample under the strain of higher strain rate is mainly characterized by brittle deformation, and the branch and the oxygen-containing functional group undergo fracture and shedding under the action of mechanical force and frictional heat, and the total amount of aromatic carbon Relative increase; the ductile deformation phenomenon is gradually developed with the decrease of strain rate, and the ductile deformation is converted into strain energy. It has sufficient time to accumulate and cause secondary structural defects, and the disorder of coal molecular structure continues to increase;

(2) The comparative analysis of sample brittleness and toughness microscopic deformation domain shows that under the brittle deformation, the fracture of the fracture is broken, the grinding action and the accumulation of frictional heat energy cause the oxygen-containing functional group and the side chain of the coal body to break, fall off or even escape, leading to the molecule. The disordered elements in the structure and the secondary structure defects increase, and the total amount of aromatic carbon increases.

(3) The ductile deformation acts on the coal macromolecular structure by transforming into strain energy. High strain rate toughness deformation. The proper amount of strain energy accumulates to promote the aromatic polycyclic rearrangement and polycondensation, so that the total amount of aromatic carbon increases, the disorder of molecular structure decreases, and the secondary structural defects decrease; the ductile deformation of lower strain rate Sufficient time is converted into strain energy and
accumulated in a large amount. When accumulated to a certain extent, the edge of the aromatic layer is destroyed and the aromatic ring is disintegrated, so that the total amount of aromatic carbon in the coal molecular structure is relatively reduced, and the secondary structural defects and disordered elements in the molecular structure increase.

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OIL SHALE FRACTURING EFFECT OF SHOCK WAVES GENERATED BY PLASMA-IGNITED ENERGETIC MATERIALS EXPLOSION

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ABSTRACT

Shock wave tests on oil shale samples were carried out in water medium by using the composite electro-thermal chemical explosion method through the shock wave generator, and strain measurement system was also introduced, and the cracking effect of energetic materials and copper wire to oil shale was analyzed. In the process, 1# oil shale sample was tested with energetic rods containing different mass of energetic materials, while 2# oil shale sample was tested with 0.3 mm copper wire explosion. The results show that the 0.3mm copper wire explosion has a small cracking effect, while the energetic rods have a much stronger cracking effect on the oil shale, and the impact pressure and the process impulse of the energetic mixture deflagration which produced strong shock wave are positively related to its mass. It also suggested that the strain time curve during the cracking process is the comprehensive effect of the strong shock wave produced by the copper wire and the energetic materials’ explosion. What’s more, the impact wave effect is mainly emerging the splitting failure, and a small amount of shear failure is mainly concentrated in the interlayer. Moreover, under the impact of shock waves, the physical properties of oil and gas reservoirs are changed, and its permeability is obviously enhanced which is helpful for the analysis and diffusion of oil and gas. The reduction of the mechanical strength parameters proves that the shock wave plays a definite certain damage to the physical structure of the reservoir. Experiments results show that shock wave technology can be used as pre-fracturing technology in in-situ development of oil shale.

KEYWORDS:
Oil shale development, electrohydraulic shock waves, energetic rods, energetic mixtures, shock wave cracking

INTRODUCTION

Oil shale, a kind of fine-grained sedimentary rock rich in organic matter, is listed as a very important substitute energy in the 21st century because of its rich resources and the feasibility of development and utilization [1-5]. And shale gas, commonly defined as gas trapped in shale formations with a low permeability, has become an increasingly important source of natural gas in the United States since the start of this century, and interest has spread to potential gas shales in the rest of the world, and U.S. government's Energy Information Administration predicts that by 2035, 46% of the United States’ natural gas supply will come from shale gas [6], indicating its huge potential. It has emerged as a relatively clean energy source which offers opportunity for many regions to reduce their alliance on energy imports, also serves as a transition fuel helping to reduce the emission of CO2 [7].

At present, in the international research on shale gas development technology, the commercial exploitation method of shale gas and the feasibility and the economy of the exploitation process have been the main consideration [6-8]. However, some conventional technologies such as hydraulic fracturing and horizontal drilling, make the extraction of tightly bound shale gas from shale formations economically feasible and have been the focus of much controversy because of their accompanying environmental risks, including gas migration and groundwater contamination due to faulty well construction, blowouts, above-ground leaks and accidental spills of waste water and chemicals used during drilling and fracturing [8-10]. In terms of these environmental issues, we consider using the electrohydraulic shock waves (ESWs) during the exploitation process, as a promising method to increase the shale gas production.

When hydraulic fracturing and electrical shock waves act together on oil and gas reservoir, shock wave propagates through the water medium in the form of limited amplitude wave. For weak links in reservoir, if the intensity of shock wave exceeds its failure limit, fracture may occur. When the shock wave acts on the micro-fracture of the reservoir,
because the impedance of the reservoir is interrupted, the shock energy will be concentrated at the micro-fracture and the micro-fracture will expand. In recent years, pulse shock wave technology has been successfully used in oil production wells, water injection wells and coalbed methane wells, and it is the first application in shale reservoir development in a new field [11-15].

As for the generate of electrical shock waves, a new method that shock waves by plasma-ignited energetic materials was used, which could reduce the ignition delay time, enhance the burning rate, and increase the muzzle exit velocity significantly [16-18]. During the process, a high-current breakdown could achieve an electrical explosion by forming a high-energy electrical arc which would rapidly vaporize the metal and insulation material [19], and the rapid phase transition and changing current could generate strong electromagnetic radiation and shock waves. In previous studies, a large-scale triaxial stress pressurizing equipment was applied to the shale sample and it aims at the test of large projectiles’ cracking effect (3 and 5g EMs load). Based on the aforementioned studies, in this paper and in terms of the well-hole, the pulse capacitor is charged at 25 kV to store energy, and a strong shock wave is generated in the geometric center of the cubic oil shale sample by the composite electrothermal chemical method [17]. In addition, dynamic strain measurement system is introduced to analyze and discuss the fracturing effect produced by plasma ignition detonating the energetic rods, we also introduced water infiltration and mechanical parameters’ test.

EXPERIMENT DEVICE AND MEASUREMENT SYSTEM

Shock wave generator. The shock wave generating device acts on the oil shale sample, and its device, as showed in Fig. 1, consists of a pulse current source (including a capacity of 6 μF, a rated working voltage of 50 kV pulse capacitors, coaxial field distortion switches and high voltage cables), shock wave transducers including coaxial reflux buckets and discharge loads, and measurement systems and other parts. The size of oil shale samples is 600 * 600 * 600 mm, and they are all taken from Fushun West open pit located in Liaoning province. The energetic rods detonating by metal wire explosion is carried out in the oil shale sample well-hole through the water medium. In the discharge experiment, the upper shock wave transducer is connected to the tower crane, and the transducer disc is fixed through the cable to ensure that it does not shift in the experiment.

The energetic materials (EMs) in the energetic rod (ER) used in this paper is a stable physical and chemical property under normal condition, high detonation requirement, and a mixture that can ignite only combustion and cannot explode under general conditions, which is consist of Al and ammonium perchlorate (NH4ClO4) with an average charge density of ~1.56 g/cm3. The mass ratio of Al and NH4ClO4 was 1:3, and the average powder diameter were 13 and 23 μm, respectively.

Dynamic strain measurement system. In this experimental dynamic strain measurement system, four single channel dynamic resistance strain gauges are used to evaluate the circumferential and axial strain at the outer surface of the experimental sample and the two measuring points locate at the horizontal position of the discharge center. The Wheatstone bridge corresponding to each strain gauge is connected by double arms, of which 1 and 2 posts are related to work pieces, and 2 and 3 columns are connected with compensating sheets. The measured strain waveforms are recorded in four channels by two tek3054 oscilloscopes. The strain gauge used in the experiment is BX120-8AA, with a resistance value of 120 ± 0.1 ohms and a sensitivity coefficient of 2.08 ± 1%. The measurement system is illustrated in Fig. 2.

![Diagram of shock wave generator](image)
Before actual measurement, the dynamic strain gauge should be calibrated and zeroed. In the process of zero adjustment, the automatic zero adjusting device of the strain meter is used to adjust zero. If the zero drift is serious and the trend is increasing, it is usually because the strain gauge is too wet to measure, and the vacuum silicone grease can be evenly distributed at the connection terminal and the principle line of the cable. After each shock wave, the drying conditions at the measuring points should be carefully checked to make sure the accuracy and reliability of the measurement results. If the strain gauge is loaded, indicating that the cable is broken, it is necessary to concentrate on the on-off condition of the terminals.

In order to avoid the influence of the strong electromagnetic interference in the discharge process, the cable with the silver-plated shielding layer should be used as the signal extraction line, and the space area between the cables is reduced as much as possible, and the high frequency electromagnetic interference is filtered by the low pass filter of the strain meter. In order to reduce the damage of the shock wave to the measuring system, the experimental cable is 11–16 m, so that the dynamic strain gauge and the oscilloscope are arranged in the safe area, and the isolation transformer is used to supply independent power supply for the measuring equipment to prevent the interference of the power frequency. During the discharge process, oscillograph waveform should be monitored at any time to avoid signal mis-operation due to incorrect operation of switch or trigger.

RESULTS

Comparison of shock wave shapes of different mass EC. Shock wave generation device was used in a cylindrical tank for experiments. Using the PCB138A11 pressure sensor 55 cm from the test point, the shock waves generated by several different mass energetic rods through the water medium were accurately measured, and the resulting waveforms and impact strength were shown in Fig. 3 below.

It can be seen from Table 1 that the shock wave peak pressures generated by 0.2, 0.3, 0.5 and 3 g energetic rods are around 3 MPa, and the shock wave peak pressure generated by 0.5 g energy mixtures is 3.78 MPa which is relatively high, followed by the shock wave pressure peak of 0.3g mass energy mixtures, followed by 3 g and 0.2 g respectively.
TABLE 1
Pressure peaks and impulse meters for shock waves of different mass ERs

<table>
<thead>
<tr>
<th>Quality/g</th>
<th>0.2</th>
<th>0.3</th>
<th>0.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak pressure/MPa</td>
<td>2.87</td>
<td>3.11</td>
<td>3.78</td>
<td>3.07</td>
</tr>
<tr>
<td>Impulse/MPa μs</td>
<td>55.2</td>
<td>63.7</td>
<td>74.18</td>
<td>126.63</td>
</tr>
</tbody>
</table>

Fracture Effect and Dynamic Strain. (1) Analysis of wire explosion on oil shale. At present, according to the mode of stress wave failure, the area of action of the rock can be divided into crushing area, cracking area and elastic area. The shale is a brittle material, and the columnar energetic rods will produce a strong impact effect of coaxial diffusion, and the medium near the wall of the borehole will feel strong radial compression. When the compressive stress is greater than the compressive strength of the material, the fracture will be formed. At the same time, the expansion of the ring resulting from the radial compression of the medium produces cyclic tensile stress. Because the tensile strength of rock is much less than its compressive strength, circumferential tensile stress is easily higher than its tensile strength, resulting in cracking [21-29].

For sample 2#, under the experimental conditions of shock wave generator and water medium, the copper wire (60 mm in length, 0.3 mm in diameter) was firstly used for twice exploding in the geometric center. At the same time of wire explosion, the strain waveforms at measuring points 1 and 2 are measured by the dynamic strain measuring system shown in Fig. 3 and recorded by the oscilloscope. The strain waveforms obtained are shown in Fig. 6 below, Fig. 5 (a) and (b) indicate the strain waveforms after the first and two times of the wire explosion.

Compared with the circumferential and axial waveforms corresponding to point 1 in the Fig. 5(a) and (b), the trend is roughly the same, and after a short time, both the circumferential and axial strains are approximately zero. The peak of axial strain oscillation at point 1 in Fig. 5(b) is larger than that at point 1 in Fig. 5(a), which is about 1.8 times of the peak value of axial strain at point 1 in Fig. 5(a). According to the theory of rock stress and strain, it suggests that cracks appear in the path of shock wave action after two times of wire explosion.
Comparing the circumferential and axial waveforms of the measured point 2 in the Fig. 5(a) - (b) diagram, it is found that both the circumferential and axial strain waveforms in the Fig. 5(b) diagram oscillate greatly. The peak values of the circumferential strain waveforms in the Fig. 5(b) diagram are smaller than those in the Fig. 5(a) diagram, and there are smaller peak values and more oscillations in the short time, and then tend to be gentle, and it means that the cyclic plastic deformation decreases after impact pressure action. Moreover, in Fig. 5(b), the peak value of the axial strain waveform decreases first, then increases and then decreases. It is speculated that the ring direction in the direction of measuring point 2 generated a large gap in the shock wave action path after 2 times’ wire explosion, leading to the refraction of the gap during the shock wave action and affecting the strain test waveform. In addition, the shock strain waveform is more harmful to the rock, and the sample volume continues to undergo slight deformation during the shock wave action, resulting in increased cracks.

As shown in Fig. 6 below, the drilling pictures of oil shale sample 2# were taken by camera during the test, including before the start of the experiment and after two times’ copper wire explosion. There are no cracks in the borehole before the test, and the cracks in the borehole increase after the two copper wire explosion tests. The axial cracks are marked with red lines and the circumferential cracks are marked with yellow lines. It is found that there are more axial cracks in the borehole and the longitudinal bending connection is present. Circumferential fissures usually occur at longitudinal fracture junctions. By comparing the process pictures before and after test, it indicates that the 0.3mm Cu electrical wire explosion had a small cracking effect on the wire explosion had a small cracking effect on the rock and form certain pores and cracks.

For the sample 1#, the strain waveforms after 1 and 2 times using 3g energetic rods are shown in Fig. 8(a) - (b) below. As the strain waveform of Fig. 8(a) is measured, the circumferential and the axial strain are almost zero, which shows that after the unloading of the shock wave, the sample volume microelement is recovered without residual volume deformation, and diffusion of oil and gas in the oil shale. The destructive effect of water on oil shale in the water infiltration test can be ignored in the test, when comparing to the strong shock waves, although water can dissolve and diffuse the cement and mud layer in the rock and form certain pores and cracks.

(2) Analysis of ERs shock wave effect on oil shale. Under the experimental conditions of shock wave generator and the water medium, the 0.2 g energetic rods were used for three times’ explosion in the geometric center of sample 1#. At the same time of explosion, the strain waveforms were measured by the dynamic strain measuring system shown in Fig. 7 and recorded by the oscilloscope.

In this experiment, after a short reaction time, both the circumferential and axial strain were close to zero, which indicates that after the shock wave unloading, the sample volume microelement recovered without residual volume deformation. Meanwhile, the maximum value of circumferential strain measured by using 0.2 g energetic rod is 4937.7 με, and the axial strain’s peak value is 4013.65 με, with a log of 21.4 μs. The sample first experienced a short time compression strain wave, attended by a large tensile strain wave and then a small fluctuation. It can be approximately inferred that at this point, the volume of the differential cylinder at the discharge level of the whole sample decreases, then increases, and finally decreases.

For the sample 1#, the strain waveforms after 1 and 2 times using 3g energetic rods are shown in Fig. 8 (a) - (b) below. As the strain waveform of Fig. 8(a) is measured, the circumferential and the axial strain are almost zero, which shows that after the unloading of the shock wave, the sample volume microelement is recovered without residual volume deformation,
Notes: (a) Sample before test, (b) 0.2 g rods operation 3 times, (c) 3 g rods operation 2 times
TABLE 2
The mechanical properties of oil shale rock samples test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compressive strength (MPa)</th>
<th>Average tensile strength (MPa)</th>
<th>Average modulus of elasticity (GPa)</th>
<th>Shear strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average drying value</td>
<td>Saturation mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>33.78</td>
<td>27.52</td>
<td>2.16</td>
<td>3.56</td>
</tr>
<tr>
<td>1#</td>
<td>24.76</td>
<td>19.92</td>
<td>1.56</td>
<td>2.28</td>
</tr>
<tr>
<td>2#</td>
<td>18.19</td>
<td>13.78</td>
<td>1.17</td>
<td>1.72</td>
</tr>
</tbody>
</table>

and the axial and circumferential stress peaks are close to 5132.76 um, and there is no lag in time. For example, Fig. 8(b) is a strain wave after 2 time of the action of a 3 g energetic concentrator. The cyclic strain is approximately zero at that time, and the dynamic strain of the axial strain in the 300–500 μs fluctuates greatly. The peak value of the fluctuating strain is about 1049.2 μ, and the latter strain wave has a shock attenuation. It is known that there are many defects inside the sample. These oscillatory waveforms essentially originate from the catadioptric shock waves at the fracture surface of the sample, that is, a great many of micro cracks have been generated inside the sample.

As showed in Fig. 9, oil shale borehole pictures were taken after the tests. From a series of processing pictures, it can be found that the energetic rods have cracking effect on the oil shale, and its breaking effect is greater than that of copper wire, and the effect of shock wave is mainly appeared as splitting failure, and the shear failure is mainly concentrated in the interlayer crack.

After working with the 0.2 g energetic rods for 1 and 2 times and before starting the operation, the water is injected into the hole and the scale is erected at the side, the initial level is 53 cm, then the remaining height of the liquid surface is measured after a short time, and the time is recorded. The initial average water penetration rate is 0.367 mm/s, shows that there is a very small crack in the sample, which affects water seepage, and the average water penetration rate is 2.15 mm/s after a 0.2 g energetic rod operation, and two times with 0.2 g energetic rods, and the average water penetration rate is 6.5 mm/s, indicating that the micro cracks inside the sample have increased or added after the 0.2 g energetic materials were deflagrated two times. Gap increases permeability of oil shale interlayer.

Analysis of mechanical parameters of sample. Samples of oil shale before and after impact test were sampled and delivered to a professional testing institution for mechanical parameters test, including the compressive strength, average tensile strength, modulus of elasticity, etc.

The original sample has not undergone impact test, the sample 1# is impacted by energetic rods test, and the sample 2# is impacted by wire explosion test. The test results are shown in Table 2.

The original sample, 1# sample for energetic rods’ explosion and 2# sample for copper wire explosion are compared. It is found that the compressive strength, tensile strength, modulus of elasticity and shear strength of the oil shale samples decrease significantly after the shock wave test.

According to the theory of tensile failure of shock wave, tensile failure occurs first in the process of shale breaking, and the impact wave acts on rock samples to produce a certain number of cracks or micro cracks, which reduces the mechanical strength of the rock. At the same time, cracks and micro cracks will increase and continue to expand with the increase of impact times [30]. By comparing the test data before and after the experiment, it is found that the compressive strength, tensile strength, shear strength and modulus of elasticity of the samples are reduced respectively, which is very useful for reducing the fracturing strength of hydraulic fracturing and improving the economy. The dynamic strain of oil shale samples and the physical parameters of samples before and after impact are detected and analyzed. The results show that the shock wave can improve the permeability of shale samples to a certain extent, make the samples produce macroscopic and microscopic cracks, and reduce the tensile strength, compressive strength and modulus of elasticity of the samples, and the effect of the energetic rods is remarkable. Further analysis shows that the physical properties of oil and gas reservoirs have changed under the impact of shock waves, and the enhancement of permeability helps to analyze and spread the oil and gas, and the reduction of mechanical strength proves that the shock wave has a certain damage to the physical structure of the reservoir, and then the partial effect of hydraulic fracturing is realized.

CONCLUSIONS

In this paper, the shock wave test of the oil shale samples was carried out by the shock wave generator through the water medium and strain measurement system was also introduced, and the cracking effect of energetic materials and copper wire to the oil shale were analyzed. In terms of the mass of the energetic rods, the impact pressure and impulse of the energetic mixtures did not increase linearly with the increase of mass, but the positive correlation trend was shown, and the impact time of
the whole energetic explosion is about 25 $\mu$s, and the strain time curve during the cracking process of the oil shale is the result of the comprehensive effect of the strong shock wave produced by the copper wire and the energetic material explosion. When it comes to its cracking effect, the stable and reliable strain signals obtained by using the reasonable anti-interference measures during the explosion process show that the 0.3 mm copper wire explosion has a small cracking effect, while the energetic load containing energetic materials have a much stronger cracking effect on the oil shale, and the cracking effect is positively related to the quality of the energetic mixtures and oxygen balance. The impact effect of shock wave mainly appears as splitting failure, and the shear failure mainly concentrates on the interlayer to make the fracture bigger. Under the impact of shock waves, the physical properties of oil and gas reservoirs have changed, and the enhancement of permeability helps to analyze and spread the oil and gas, and the reduction of mechanical strength proves that the shock wave plays certain damage to the physical structure of the reservoir, and then the hydraulic fracturing is partly achieved.

In the future, we will consider improving the strain measurement system and introducing stress measurement, acoustic emission to monitor the formation and development of cracks, under the condition of applying confining pressure. At the same time, we will further verify the impact effect of the energetic rods by improving the proportion of the energetic materials, besides, microcosmic interaction mechanism and energy transfer between the discharge plasma and the energetic rods should be thoroughly investigated.

ACKNOWLEDGEMENTS

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RESEARCH ON MOVEMENT PROCESS AND CHARACTERISTICS OF DEBRIS FLOW UNDER DIFFERENT SLOPE CONDITIONS BASED ON NUMERICAL SIMULATION

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ABSTRACT

Debris flow has become one of the most serious geological disasters. It has the characteristics of large area and serious loss. The study of the movement process of debris flow is the precondition for the prevention and control of debris flow. In this paper, the Massflow debris flow numerical model is used, and the Voellmy resistance model is used to simulate the movement process of debris flow under two different slope shapes and five different slopes. The movement process and accumulation process of debris flow are analyzed, and different slope conditions are carried out. Analysis of the movement process of the debris flow. The results show that the channel-shaped slope shape can reflect the movement characteristics of the debris flow more than the slope-type slope shape. With the gradual increase of the slope, the energy of the debris flow fluid provided by the topography is further increased, and the maximum speed and the maximum distance of the rushing are further increased. Large, the area of its corresponding deposit increases, but the maximum thickness and volume of the deposit tend to decrease. This paper discusses the relationship between different slope factors and debris flow accumulation and provides a scientific basis for predicting debris flow accumulation under different slope topographic conditions, which will provide some help for the next step of debris flow geological disaster management and geological environment ecological improvement.

KEYWORDS:

INTRODUCTION

As a complex multi-phase mixed medium, debris flow contains complex rheological characteristics and calculation modes. In the formation area, the main process of the sedimentation of the sediment is studied, so the mathematical model is a complex unsteady flow, which is used to simulate the instability process and starting conditions of the rock and soil. In the circulation area, especially the debris flow channel, it is a channel for high-speed movement of debris flow. It can be described by a one-dimensional mathematical model [1]. At present, there are many research results in this field. Some scholars in Japan, the United States, and China do. Relevant research work [2-4]; in the accumulation area, there are usually clusters of residential and human engineering buildings, which often bring huge losses. In this study, more emphasis is placed on the spread of the two-dimensional plane (diffusion movement), its mathematical model can be expressed in a two-dimensional stream [5]. Japan has a lot of researches in this area. Gao et al. pointed out in the book "Debris Flow" that the numerical simulation of debris flow is based on mass conservation and momentum conservation, and can simulate the two-dimensional flow characteristics of debris flow in the accumulation area [6]. It is assumed that the debris flow is an incompressible continuous fluid and the resistance characteristics of the fluid are assumed to be Bingham body characteristics [7].

In China, the numerical simulation study of debris flow started late. The research scholars all used the mathematical model of Gao et al. [1, 4-5]. On the basis of the resistance terms, the expressions are slightly different, and their computer simulation methods have their own characteristics [5-6]. In the 1990s, Tangchuan's two-dimensional unsteady flow equations and Wang Guangqian were also representative of the flow group model of Shao Songdong [7-8]. In 2013, Ouyang et al. began to derive the three-dimensional Naiver-Stokes equation, established a two-dimensional debris flow motion model, and developed the Massflow numerical model to simulate the red gully debris flow, the simulated structure and the actual situation is consistent [9-13].

In order to quantitatively study the movement process of debris flow and analyze the movement
process of debris flow under different conditions, this paper uses the Massflow debris flow motion numerical model to set up two basic slope shapes and five angles to analyze the movement process of debris flow under different slope conditions. The purpose is to explore the accumulation pattern of geological disasters in debris flow under different slope conditions.

MATERIALS AND METHODS

**Model Geometry.** In this paper, two kinds of basic slopes are set, one is the unrestricted slope shape, similarly simulates the slope type debris flow, which is called the slope type slope shape in this paper; the other is the limited slope shape, that is, the channel slope shape, similarly simulated the gully-type debris flow [14-16]. The model geometry is designed as a sloped slope. The slope represents the formation and circulation of the debris flow, giving the kinetic energy of the material movement. The slope is connected to the unsloped horizontal plane, which represents the accumulation area of the debris flow. Through a series of inclined channel-type slopes, slopes of 20, 25, 30, 35 and 40 are respectively set.

The slope type is composed of two faces, one is a sloped slope and the other is a horizontal plane. The slope represents the debris flow in the formation zone and the circulation zone, and the horizontal plane represents the accumulation zone of the debris flow. To investigate the influence of different slope shapes on the movement of debris flow, the slope type and the channel type slope shape are compared. The slope of the slope is 30 degrees, and other conditions are the same. For the slope type slope, the length of the entire slope is 50 meters, the width of the slope is 50 meters, the length of the water plane is 80 meters, and the width of the water plane is 50 meters. Use the Surfer software to create a grid file and use the conversion tool in ArcGIS software to convert the grid file into an ASC II file recognized by the simulation software. The same file format can be recognized by the surfer software, where the grid size is 1 meter.

The channel-shaped slope shape belongs to a restricted slope shape that limits the lateral movement of the source material, that is, the movement in the Y direction. The surfer software is used to add a channel on the slope-type slope shape [8, 17-18]. The slope of the channel bottom surface is the same as the slope type slope shape of 30 degrees. The geometric features of the slope surface and the horizontal plane are also the same as the slope type slope shape. During the study and analysis of the debris flow movement under different slopes, the slope was changed to 20 degrees, 25 degrees, 30 degrees, 35 degrees and 40 degrees, respectively, and the grid size was 1 meter. The slope morphology is shown in Figure 1(a)-(b).

The geometry of the source area is a rectangular parallelepiped. The length of the rectangular parallelepiped is 10 meters, the width is 4 meters, and the height is 2 meters. The cuboid is located at the top of the slope and is suddenly released on the slope and moves to a level on the slope.

![FIGURE 1](image)

**FIGURE 1**

Schematic diagram of different slope types

Notes: (a) refers to slope type, (b) refers to channel slope type.

**Massflow debris flow motion numerical model.** In the nineteenth century, Navier and Stokes corrected and improved the Euler equation. The improved equation is abbreviated as the Navier-Stokes equation. The N-S equation is derived from the fundamental principles of mass, momentum, and conservation of energy, and assumes that the fluid is continuous, that is, the interior of the fluid does not contain voids. The three-dimensional NS equation includes the mass conservation equation and the momentum conservation equation. The momentum conservation equations are the x-direction, the y-direction, and the z-direction. In the numerical simulation of debris flow, the general debris flow flooding range is much larger than the debris flow accumulation thickness. In particular, the scouring distance of the debris flow is much greater than the debris flow accumulation thickness. In the numerical simulation of debris flow, the general debris flow flooding range is much larger than the debris flow accumulation thickness.
Fluid velocity in the x, y, and z directions (m/s); the friction model mainly describes the process, the friction model mainly describes the non-Newtonian body during the movement. According to the above-mentioned geometric structure, parameters and initial conditions, the slope type and channel type slope shape are simulated. The selection of the debris flow simulation parameters directly affects the numerical simulation results, and affects the accuracy and stability of the results. The selection of the parameters depends on the nature of the fluid. To better simulate the different slope shapes and different slopes for the debris flow movement, the parameters used in the friction model (also called the resistance model or the rheological model) in the numerical model are shown in Table 1. The boundary condition of the model is set to the open boundary condition. The calculation time is 15s, and the calculation result is output every 0.5s. The output file is set to the format recognized by the surfer software and the tepclot software.

### Table 1. Friction model parameters

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Coefficient of friction/μ</th>
<th>Turbulence coefficient/μ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voellmy</td>
<td>0.3</td>
<td>200m/s²</td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

According to the above-mentioned geometric structure, parameters and initial conditions, the slope type and channel type slope shape are simulated. The movement process of debris flow fluid under two slopes is obtained. The depth and speed of the mud flow during the movement.

**Movement process of fluid under slope type.**

Figure 2 shows the movement of the fluid under the slope-shaped slope. The X-axis and Y-axis are in meters. The vertical line at 50 meters on the X-axis represents the boundary between the slope and the horizontal plane. For every 1 second of motion profile, the fluid experienced a speed of approximately equal to 0 m/s after 12 seconds, meaning that the fluid experienced approximately 12 seconds from start to stop. The fluid reached a maximum speed of 17 m/s at 4 seconds, a speed reduction at 8 seconds, and a speed of 0 m/s at 12 seconds. The median line graph is the thickness map at 12s, in meters, which is the final stacking state, with the front end being about 95 meters from the starting point. The length of the deposit in the x direction is 12.45 meters, the stacking length in the y direction is 39.36 meters, the highest point in the z direction is 0.42 meters, and the stacking area is 371.16 square meters.

**Movement process of fluid under channel-shaped slope.**

Figure 3 shows the movement of the fluid under the channel-shaped slope. The X-axis and Y-axis are in meters, and the vertical line at 50 meters on the X-axis also represents the boundary between the slope and the horizontal plane. This...

\[
\frac{\partial (h)}{\partial t} + \frac{\partial (hU)}{\partial x} + \frac{\partial (hV)}{\partial y} = P - Q 
\]

\[
\frac{\partial (h^2)}{\partial t} + \frac{\partial (h^2U)}{\partial x} + \frac{\partial (h^2V)}{\partial y} = 
\]

\[
k_{ap} \frac{h}{\bar{n}} \frac{\partial (z_h)}{\partial x} - \frac{(z_h)}{\bar{n}} = 
\]

\[
k_{ap} \frac{h}{\bar{n}} \frac{\partial (z_h)}{\partial y} - \frac{(z_h)}{\bar{n}} = 
\]

\[
k_{ap} \frac{h}{\bar{n}} \frac{\partial (z_h)}{\partial y} - \frac{(z_h)}{\bar{n}} = 
\]

\[\frac{\partial (h)}{\partial t} + \frac{\partial (hU)}{\partial x} + \frac{\partial (hV)}{\partial y} = P - Q \quad (1)\]

\[\frac{\partial (h^2)}{\partial t} + \frac{\partial (h^2U)}{\partial x} + \frac{\partial (h^2V)}{\partial y} = \quad (2)\]

\[k_{ap} \frac{h}{\bar{n}} \frac{\partial (z_h)}{\partial x} - \frac{(z_h)}{\bar{n}} = \quad (3)\]

\[k_{ap} \frac{h}{\bar{n}} \frac{\partial (z_h)}{\partial y} - \frac{(z_h)}{\bar{n}} = \quad (4)\]

\[\rho = \text{Debris fluid density (kg/m}^3\text{)}; \quad u, \ v, \ w = \text{Fluid velocity in the x, y, and z directions (m/s)}; \quad g_x, \ g_y, \ g_z = \text{Gravity components on each coordinate axis}; \quad Z = \text{Riverbed elevation (m)}; \ h = \text{Depth of mudstone fluid (m)}; \ t_0 = \text{Shear stress at the bottom of the debris flow (Pa)}; \ k_{wp} = \text{Earth pressure coefficient}; \ \varphi = \text{Internal friction angle}; \ \delta = \text{Base friction angle}; \ \rho = \text{Average density of debris flow (kg/cm}^3\text{)}; \ P = \text{Source and rainfall increase (m/s)}; \ Q = \text{Source and rainfall loss (m/s)}.

The Voellmy model assumes that the shear stress of the moving fluid is the sum of the frictional force of the fluid and the additional resistance generated by the turbulent flow. The turbulence term is added to the Coulomb friction model, which means that the model has friction terms and the turbulent flow component consists of two parts, which have been widely used in models of flow such as debris flow, and are expressed by the following formula:

\[
\tau_b = \sigma U + \frac{P Q V}{\xi} \quad (5)
\]

Among the formula:

\[\sigma = \text{Normal stress (Pa)}; \quad U = \text{Coefficient of friction}; \ \xi = \text{Turbulence coefficient (m/s}^2\text{)}.

**Parameter selection and initial conditions.**

The establishment of a numerical model has been introduced above, in which the friction model uses the Voellmy model. Since the debris flow belongs to the non-Newtonian body during the movement process, the friction model mainly describes the case where the fine particles are less and the coarse particles are more. The frictional resistance is proportional to the effective stress of the ground, and the Voellmy model is considered on the basis of the friction model [12-14]. The enthalpy of the debris flow is expressed by the parameter turbulence coefficient.
kind of slope stopped moving at 11.5 seconds. The fluid reached a maximum speed of about 17 m/s at 4 seconds, a speed reduction at 7.5 seconds, and a speed of 0 m/s at 11.5 seconds. The median line graph is the thickness map at 11.5 seconds, in meters, which is the final stacking state, with the foremost end being about 98 meters from the starting point. The length of the deposit in the x direction is 15.96 meters, the stack length in the y direction is 23.38 meters, the highest point in the z direction is 0.58 meters, and the stacking area is 276.83 square meters.

Through the above numerical simulation process, under the same numerical model, the channel type slope shape is not much different in maximum speed compared to the slope type slope shape, but the front end fluid can propagate farther, as shown in Figure 4. This is because the channel in the channel-type slope limits the diffusion movement of the fluid in the y-direction, so that the fluid has a greater total kinetic energy as a whole and thus travels farther. And the debris flow accumulation area under the channel type slope condition is smaller, and the relative maximum thickness is larger than the slope type slope shape, that is to say, the debris flow movement under the channel type slope surface condition is more concentrated in the final accumulation pattern. The calculation results also show that the topographical conditions of the underlying surface are important factors affecting the movement and accumulation of debris flow.

![FIGURE 2](image2.png)
Outline of the movement of debris flow under slope type

![FIGURE 3](image3.png)
Debris flow motion profile of a channel-shaped slope

![FIGURE 4](image4.png)
Maximum velocity over time in two slopes
Analysis of motion process under different slopes. According to the above numerical model, the debris flow motion under different slopes is simulated. The geometric model adopts a channel-type slope shape, which can better describe the movement process of the debris flow. Five slopes are set, which are 20 degrees, 25 degrees, 30 degrees, 35 degrees and 40 degrees, and other conditions are the same as before.

Movement time and maximum speed. Through a numerical simulation with a total duration of 15 seconds, the debris flow fluid passes through the starting, moving and stacking processes. The fluid starts from the channel and finally accumulates on the horizontal surface. After calculation, the smaller the slope, the longer the total time from the beginning to the end of the movement. When the slope angle is 20 degrees, after 14 seconds, the fluid stops moving on the horizontal plane; under the condition of 25 degrees of slope, the fluid stops moving on the horizontal plane after 12 seconds; when the slope angle is 30 degrees, after 10.5 seconds, The fluid stops moving on the horizontal surface; under the condition of the slope angle of 35 degrees, after 10 seconds, the fluid stops moving on the horizontal plane; under the condition of the slope angle of 40 degrees, after 9.5 seconds, the fluid stops moving on the horizontal plane. This is because, when the slope is small, the total kinetic energy obtained by the fluid is small, and although there is friction but not enough to stop the fluid from moving on the slope, the fluid can flow slowly. When the slope is large, the fluid has a large total kinetic energy, allowing the fluid to flow rapidly within the channel.

As shown in Figure 5, when the slope angle is 20 degrees, the maximum speed is 11.34m/s when the fluid moves to 4.5 seconds; the maximum speed is 14.11m/s when the fluid moves to 4.5 seconds under the condition of the slope angle of 25 degrees; Under the condition of 30 degrees, the maximum speed is 17.32m/s when the fluid moves to 4 seconds; the maximum speed is 20.29m/s when the fluid moves to 3.5 seconds under the condition of 35 degrees; the slope angle is 40 degrees. When the fluid moves to 3 seconds, it reaches a maximum speed of 22.31 m/s. As shown in the figure, the fluid continues to reach its maximum value after accelerating through the slope of the channel. After that, the maximum value will continue for a period of time. This is because the fluid in the channel has not completely entered the horizontal plane. The maximum speed during this time is the ditch. The fluid inside the road. When the fluid is all in the horizontal plane, the speed is rapidly reduced by the frictional force until the motion stops completely. By comparing the maximum speeds of different slopes, it can be found that the larger the slope, the larger the fluid can reach in a shorter time, the smaller the slope, the longer the maximum velocity is reached, and the longer the fluid stays in the channel.

![Figure 5](image-url)

**Maximum speed at different slopes**

Stacking profile of debris flow under different slopes. The debris flows under different slope conditions move to the horizontal plane and eventually accumulate, but due to the different slopes of the slopes, the characteristics of each deposit are also different, as shown in Table 2.

<table>
<thead>
<tr>
<th>Slope</th>
<th>20°</th>
<th>25°</th>
<th>30°</th>
<th>35°</th>
<th>40°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (m²)</td>
<td>351</td>
<td>382</td>
<td>477</td>
<td>555</td>
<td>624</td>
</tr>
<tr>
<td>Volume (m³)</td>
<td>37</td>
<td>39</td>
<td>40.4896</td>
<td>40.5548</td>
<td>40.5899</td>
</tr>
</tbody>
</table>

According to the table content, as the slope increases, the maximum thickness of the deposit decreases, and the corresponding area increases as the slope increases. Since the initial conditions (i.e., the amount of material) of the fluid simulated by the numerical values of the respective slopes are the same, when the volume of the stacked body is small, the corresponding maximum thickness is large. When calculating the volume of the deposit, it is calculated for the volume above the thickness of 0.005 m in the slope and the horizontal plane, so the volume of the deposit under each slope condition is slightly reduced. At 30, 35 and 40 degrees, the volume of the deposit differs by less than 1 cubic meter. Under this condition, there is almost no residual fluid in the channel, and the volume of the deposit at 20 degrees is only 37 cubic meters. The volume of the deposit under the condition is only 39 cubic meters, which means that compared with the slope of the steep slope, the slope of the small slope will leave some fluid. These fluids are accumulated in the channel due to frictional resistance.
Converging in the accumulation area.

Figures 6 shows the final stack profile at different slope conditions. The x-axis shows a horizontal plane of 50 to 130 meters, which is the accumulation zone. The thin black solid line in the figure shows the debris flow profile every 1 second during the movement, and the color contour map shows the final stacked thickness map. It can be seen from the figure that the common feature of the deposits is that the maximum thickness is located in the middle portion, the thickness of the surrounding deposits is small, and the overall shape is slightly stretched in the y direction. As the slope increases, the fluid travels farther away, and the footprint of the deposit is larger, but the maximum thickness of the deposit is smaller. From the aspect of stacking morphology, the deposits under the five slopes are similar to semi-circular, but when the slope is small, the distance between the highest points of the deposits is approximately equal. As the slope increases, the highest point and the front end of the stack. The distance gradually increases.
Debris flow motion profile with different slopes (unit: m)

TABLE 3
Maximum rush distance at different slopes

<table>
<thead>
<tr>
<th>Slope (°)</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum punching distance (m)</td>
<td>25.13</td>
<td>35.22</td>
<td>48.56</td>
<td>61.34</td>
<td>72.12</td>
</tr>
</tbody>
</table>
**Punching distance and stacking thickness.**

Table 3 shows the maximum punching distance under different slope conditions. It can be seen intuitively from the table that the larger the slope, the larger the punching distance.

Figure 7 below shows the cumulative thickness distribution of the deposits under different slope conditions. The horizontal axis is the distance from the fluid starting point, and the vertical axis is the thickness of the deposit. It visually shows the profile of the debris flow deposit. From the figure, the maximum rushing distance of the debris flow and the increase of the maximum thickness and the cumulative area of the maximum slope can also be interpreted.

**CONCLUSIONS**

(1) Regardless of the slope type or the channel type slope shape, as the slope gradually increases, the energy provided by the slope of the terrain increases one step further, the distance of the fluid movement further increases, and the corresponding average speed increases. The moving distance is further increased, and the corresponding average speed, maximum speed and total kinetic energy are further increased, and a process from slow creep to rapid increase is experienced.

(2) The topographical condition of the underlying surface is an important factor affecting the movement and accumulation of debris flow. The channel-shaped slope shape is more representative of the movement characteristics of the debris flow than the slope-shaped slope. Under the condition of channel-type slope, five kinds of slopes (20 degrees, 25 degrees, 30 degrees, 35 degrees, 40 degrees) are set.

(3) As the slope increases, the fluid movement time decreases, the maximum speed increases. The channel-type slope-shaped debris flow accumulation body increases with the increase of the slope, the maximum thickness decreases, and the volume decreases slightly. Through the simulation research of debris flow movement process, a further understanding of the process of debris flow disaster was obtained, which is helpful for the follow-up risk assessment, geological hazard prevention/control design and the improvement of ecological environment.

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ANALYSIS OF OIL-WATER SEDIMENTATION MECHANISM AND SEPARATION PROCESS BASED ON CFD-DEM MODEL

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ABSTRACT

Sedimentation separation is widely used in China’s petroleum industry. However, the theoretical research obviously lags the structural design and application research. In this paper, based on the CFD-DEM coupling model, taking the discrete phase oil droplet in the sedimentation tank as the research object, the mathematical model of sedimentation and separation of oil droplet in gravity field is established. By using the method of theoretical analysis and numerical simulation, the movement characteristics of oil droplet in the process of sedimentation are analyzed. The trajectory of the sedimentation and separation process of oil droplet is obtained. The changes of velocity and force in the process of oil droplet separation were understood. The mechanism of oil droplet sedimentation separation in gravity field was obtained and the time and influencing factors of oil droplet separation were mastered. The research results provide theoretical basis and data reference for revealing the mechanism of sedimentation separation and guiding the design of a new type of high efficiency sedimentation device.

KEYWORDS: Sedimentation separation, Discrete element model, Oil droplet, Separation mechanism, Movement characteristics, CFD-DEM model

INTRODUCTION

With the continuous development of oil fields, to ensure the stable production of oilfields, most of the oilfields in China are developed by water injection and polymer flooding. Up to now, most oilfields have entered the high water cut development period.

The moisture content of the produced liquid increases year by year. How to effectively realize the pre-separation of the produced liquid and sewage treatment has always been one of the main research directions of scholars in related fields [1-4]. At present, most of the oilfields in China use the sedimentation method to separate the produced liquid from the produced sewage. Among them, the vertical sedimentation tank has been widely used. It is also the most common oil-water separation equipment. It plays a great role in the actual production of oil field [5-8]. Zhou et al. [9-10] used Euler model to analyze the oil-water separation process in three-dimensional vertical dewatering sedimentation tank. The effects of temperature and oil phase volume fraction on the flow rate of liquid distribution hole were studied. The optimum temperature is 60-80 °C. Li et al. [10, 11-13] studied the effect of parameters of sedimentation tank on dehydration effect. The influence rule of oil concentration, liquid inlet velocity and interphase density difference on the separation performance was studied. Some scholars have carried on the optimization design to the sedimentation tank structure and used the numerical simulation method to verify and analyze the feasibility of the optimized structure [14-15]. The research on the sedimentation mechanism and separation process of oil-water separation is mainly focused on the state of oil-water distribution and separation time by means of theoretical derivation and numerical simulation [16-20]. There are few reports on the force change, movement path and the velocity change of single particle oil droplet in the process of gravity separation in sedimentation tank.

In the process of oil-water separation, the migration trajectory and floating velocity of oil droplet in the sedimentation tank are the important factors of the gravity sedimentation tank. It can reflect the separation ability of gravity sedimentation tank. Therefore, based on the discrete element model (EDEM), this paper constructed the mathematical model of the sedimentation and separation of oil droplet in the gravity field by taking the discrete phase oil droplet in the sedimentation tank as the research object. The migration trajectory of oil droplet in the sedimentation tank is obtained by numerical simulation. The movement characteristics of the floating process of oil droplet in the process of sedimentation are analyzed from the point of view of mechanism. This study can guide the improvement of structure and process of the sedimentation tank to improve the separation efficiency of the sedimentation tank. The purpose of this paper is to provide a theoretical basis for shortening the sedimentation time and enhancing the separation performance.
MATeRIALS AND METHODS

Research method. The CFD-DEM coupling method used in this paper is to use Fluent software to couple with EDEM [13-14, 21]. The discrete element is used to analyze the movement process of oil droplet in the gravity field. Firstly, a mathematical model is established by taking each oil droplet particle in the oil-water mixed medium as an independent unit. The size and physical properties of the oil drop unit are given. Contact collision and elastic separation exist between discrete phase particles and between discrete phase and fluid. When the collision contact occurs, the contact point will produce contact force and contact torque, which can be calculated according to the contact mechanics model. According to the characteristics of discrete phase oil droplet and continuous phase flow field in gravity field, it is determined that there is no sliding contact model and no bond contact model between oil droplet and device wall in gravity field [22-23]. In the process of calculation, the equation of movement of oil droplet in discrete phase is established according to Newton's second law. The central difference method is used to solve the problem. In the simulation calculation, contact force, contact torque, unbalanced force and unbalanced torque produced by the collision between adjacent discrete oil droplet are used to calculate the force and velocity of each particle at a specific time. The movement characteristics of oil droplet in the whole gravity separation field are reflected by the movement characteristics of single oil droplet.

Basic principle of gravity sedimentation. Gravity sedimentation is mainly based on the density difference between two or more media. For the oil-water two-phase medium studied in this paper, the buoyancy, inertia force and diffusion interception of the discrete phase oil droplet in the gravity field are the main factors affecting the sedimentation process. The Stokes Equation can be used to calculate the floating and sinking velocity of oil droplet in discrete phase [23].

\[ v = \frac{2(\rho_w - \rho_o)g r^2}{9 \mu} \]  

(1)

Where, \(v\) — Vertical Motion Velocity of Oil Droplets, m/s; \(\rho_w\) — Density of water, kg/m\(^3\); \(\rho_o\) — Density of oil, kg/m\(^3\); \(g\) — Gravity acceleration, m/s\(^2\); \(r\) — Radius of oil, m; \(\mu\) — Viscosity of Medium, mPa·s.

For the low speed laminar flow field in the sedimentation tube, the sedimentation velocity of the droplet can be further modified as follows:

\[ v = \frac{2gd_o(\rho_w - \rho_o)(\mu_w - \mu_o)}{3\mu_o(3\mu_w + 2\mu_o)} \]  

(2)

Where, \(d_o\) — Diameter of oil droplets, m; \(\mu_o\) — Viscosity of oil, mPa·s; \(\mu_w\) — Viscosity of water, mPa·s.

The sedimentation velocity of the discrete phase oil droplet in continuous phase medium (water phase) can be seen from formula (2), which is determined by the density difference, viscosity difference and oil droplet size of the two-phase medium.

Movement Model of Oil droplet in Gravity Field. When the oil droplet is precipitated and separated in the static flow field, it is assumed that there is no collision and aggregation between the particles for the single particle oil droplet. When floating or settling occurs in the continuous flow field, the external forces mainly include gravity, buoyancy and viscous resistance between continuous phase media. The direction of gravity and resistance is opposite to that of buoyancy.

Moreover, the following equilibrium relations are satisfied:

\[ \frac{du}{dt} = \frac{g(\rho_p - \rho_f) - C_D 3\rho_p u^2}{\rho_p \delta} \]  

(3)

Convert formula (3) into dimensionless quasi-number form:

\[ \frac{dN_{Re_p}}{dt} = \frac{N_{Go} - C_D N_{Re_p}^2}{24\pi} \]  

(4)

In the above formula: \(N_{Go} = \frac{4(\rho_p - \rho_f)\rho_p \delta^2 g}{3\mu^2}\); \(N_{Re_p} = \frac{\delta \rho_p \rho_f}{\mu}\) — Flow field Reynolds number, \(t = \frac{\rho f \delta^2}{\mu}\) — Oil droplet relaxation time; \(\rho_p\), \(\rho_f\) — Density of discrete and continuous phases, kg/m\(^3\); \(\delta\) — Oil droplet size, m; \(u\) — Relative velocity in the vertical direction between oil droplet and fluids, m/s; \(\mu\) — Dynamic viscosity of fluid medium, Pa·s.

From formula (4), it can be concluded that when \(t\) equals 0, \(w_{0m}=0\), \(N_{Re_p} = N_{Re_p0} = 0\).

The time required for the droplet to reach a particular value \(u\) or \(N_{Re_p}\) can be expressed as:

\[ t = 24\pi \int_0^{N_{Re_p}} \frac{dN_{Re_p}}{N_{Go} - C_D N_{Re_p}^2} \]  

(5)

Based on the relationship between speed and distance:

\[ u = \frac{dy}{dt} = \frac{N_{Re_p} \cdot u}{\rho_o \cdot \delta} \]  

(6)

Where, \(\rho_o\) — Relative density of medium, kg/m\(^3\).

It can be concluded that the distance equation for the buoyancy lift of oil droplet under the action of gravity field at \(t\) is as follows:

\[ y = \int_0^t \frac{4\rho_p \cdot \delta}{3 \rho_f} \frac{N_{Re_p}}{N_{Go} - C_D N_{Re_p}^2} dN_{Re_p} \]  

(7)
RESULTS

To obtain the movement characteristics of oil droplet in the sedimentation tank in continuous phase water, such as diffusion form, floating velocity, force change, migration time, movement trajectory and so on. Firstly, the initial flow field of continuous phase medium is constructed and analyzed by CFD method [24-27]. Then the discrete element method is used to inject the oil droplet particle swarm into the phase flow field. To realize the capture and marking of the oil drop movement process. The movement characteristics of discrete phase oil droplet in three-dimensional sedimentation gravity field are realized by CFD-DEM coupling. The medium parameters in the numerical simulation referred to the measurement results of the produced fluid in an oil field. The medium is a mixture of oil and water. The continuous phase of the mixture is water phase. The volume fraction is 98%. The aqueous phase density $\rho$ is $9.9982 \times 10^3$ kg/m$^3$. The dynamic viscosity $\mu$ is $1.005 \times 10^{-3}$ kg/m·s. The density of discrete phase oil is $0.889 \times 10^3$ kg/m$^3$. The dynamic viscosity $\mu$ is $1.06 \times 10^3$ kg/m·s. Oil droplet size is 0.15mm.

The separation process of oil droplet particle swarm under the action of gravity field is obtained by numerical simulation, as shown in Fig. 1. The oil droplet basically maintains the original distribution state within 0.6 seconds when the oil droplet enters the sedimentation tank. It moves horizontally along the entrance. There is no obvious settlement separation. When $t < 0.9$s, the oil droplet gradually begins to spread and move to the top of the sedimentation tank under the action of buoyancy. Through the distribution of oil droplet particle swarm, the velocity difference is large in the process of oil droplet group floating. The floating distance of different oil droplet is different. The oil droplet near the wall have a large floating velocity. However, the floating speed of the oil droplet near the center of the sedimentation tank is relatively slow. After the oil droplet swarm enters the sedimentation tank for 1.5 seconds, the floating velocity is basically the same and tends to be stable.

Through the particle swarm separation process of oil droplet shown in Fig. 1, it is known that the movement of oil droplet is realized under the combined action of buoyancy, resistance and gravity. After about 1 s, the oil droplet gradually diffused into the oil film and began to migrate upward. Fig. 2 shows the top view of the oil droplet group diffusion process after the oil droplet enter the sedimentation tank for 1 s. It can be found that the oil droplet spread to the axis of the sedimentation tank under the action of the initial inertia force. With the increase of floating speed, the diffusion behavior slows down.

FIGURE 1
Sedimentation and separation process of oil droplet particle swarm
Vertical to the top of the tank under the action of force as soon as the oil droplet enters the settling tank.  The vertical direction of movement in the fluid is not certain.  According to the migration trajectory of oil droplet ID120, the oil droplet continues to move horizontally under the action of inertia force as soon as the oil droplet enters the settling tank.  After a period, the oil droplet accelerates and move vertically to the top of the tank under the action of buoyancy, gravity and resistance.

Fig. 3 shows the trajectory of randomly selected single particle oil droplets (ID: 120) within the oil droplet swarm. According to the migration trajectory of the oil droplet ID120, the oil droplet continues to move horizontally under the action of inertia force as soon as the oil droplet enters the settling tank. After a period, the oil droplet accelerates and move vertically to the top of the tank under the action of buoyancy, gravity and resistance.

**FIGURE 2**
Sedimentation and separation process of oil droplet particle swarm

**FIGURE 3**
Trajectory of target oil droplet separation process

**FIGURE 4**
The force balance of oil droplet in the process of sedimentation

**Force Analysis of Oil droplet.** To explain the trajectory of oil droplet, it is concluded that the main forces of oil droplet in the sedimentation tank are gravity, buoyancy, liquid resistance and so on [19-20, 28]. The force balance diagram of oil droplet in the vertical direction of discrete phase oil droplet in the flow field was constructed as shown in Fig. 4. Then the forces in the process of separation were analyzed separately.

1. **Gravity.** Because of the weight of the oil droplet itself, the oil droplet float upward in the direction of gravity. Gravity has a great influence on the direction and trajectory of movement in the process of separation. If the density of oil droplet is \( \rho_0 \) and the diameter of oil droplet is \( d \), the gravity of oil droplet is:

\[
F_g = \frac{\pi}{6} d^3 \rho_o g
\]  

2. **Buoyancy of oil droplet.** The buoyancy of oil droplet comes from the pressure of the liquid around the sedimentation tank. The buoyancy increases with the increase of depth. We assume that the shape of the oil droplet is a standard microcube [18]. All surfaces of the oil droplet will be subjected to pressure from the continuous phase medium. The oil droplet is the same height on the left and right direction, so they have the same pressure. In the vertical direction, because of the different depth, the pressure on the upper and lower surfaces is different.

   The pressure on the bottom is larger, and the pressure on the top is smaller. There is a pressure difference in the vertical direction, which is the buoyancy from the continuous phase medium. The calculation method can be expressed as follows:

\[
F_b = \frac{\pi}{6} d^3 \rho g
\]  

3. **Fluid resistance of oil droplet.** When the oil droplet and the continuous phase medium produce relative movement, the oil droplet will be subjected to the force opposite to the direction of movement of the fluid. The force of the continuous relative discrete phase movement is the fluid resistance. The fluid resistance varies with the relative velocity between the oil droplet and the fluid, and the expression is as follows:
Equation (10) can be expressed as:

\[ F_d = \frac{\xi \pi d^2 \rho u^2}{2} \]  

(11)

For spherical oil droplet, \( A = 0.25 \pi d^2 \), so it can be concluded that:

\[ F_d = \frac{\xi \pi}{4} d^2 \rho u^2 \]  

(12)

In summary, the equation of motion of oil droplet in the sedimentation process is obtained as follows:

\[ F_g - F_b - F_d = ma \]  

(13)

Namely:

\[ \frac{\pi}{6} d^3 \rho g \]  

\[ -\frac{\pi}{4} d^2 \rho u^2 \]  

\[ \frac{\rho \pi}{6} d^3 \rho a \]  

(14)

In fact, the actual force acting on the oil droplet in the process of movement in the sedimentation tank is much more complex than the above process. However, due to the random characteristics of the flow field, there is no comprehensive and systematic mathematical model to describe the settlement process. Therefore, it is impossible to describe the sedimentation process of oil droplet comprehensively in theory [26-30]. In this paper, the force acting on oil droplet in sedimentation tank is studied by means of CFD-DEM coupling. The force curve of oil droplet (ID: 120) in the process of movement in the sedimentation tank is obtained, as shown in Fig. 5. When the oil droplet enters the initial stage of the sedimentation tank, the resultant force of the oil droplet fluctuates greatly. When the oil droplet is affected by both buoyancy and fluid resistance, the oil droplet begins to float upward. Currently, the resultant force of the oil droplet is increasing. In this process, because the oil droplet mainly moves in the horizontal direction, the fluid resistance increases gradually, resulting in the continuous increase of the resultant force. When the trajectory of oil droplet changes from horizontal to vertical, the relative velocity between oil droplet and fluid decreases gradually. The resultant force of the oil droplet began to decrease, and finally the resultant force of the stable motion of the oil droplet approached zero.

**Velocity Analysis of Oil droplet in Sedimentation Tank.** Oil droplet are mainly affected by gravity, buoyancy and fluid resistance in the vertical direction. If the oil droplet begins to move gradually from rest, the accelerated motion is first performed and gradually transformed into uniform motion under the action of fluid resistance [23, 29]. If the final velocity of the oil drop is \( u \), it can be calculated by \( du/dt = 0 \):

\[ u = \sqrt{\frac{4(\rho_p - \rho_f)g \delta}{3C_D \rho_r}} \]  

(15)

In this paper, it is assumed that the oil droplet is not affected by the interaction between adjacent oil droplet in the process of sedimentation. The effects of oil droplet breakage, aggregation and rotation are not considered. According to the study in this paper, the oil droplet with particle size of 150um and density of 886 kg/m³ are precipitated and separated in
the sedimentation tanks. It is assumed that oil drops settle in the retention zone when calculating by trial and error method

\[ u_t = \frac{d^2 (\rho_s - \rho) g}{18u} \]  

(16)

If the parameters of the continuous phase medium are the same as those of the numerical simulation method in this paper, the sedimentation velocity of oil droplet is:

\[ u_t = \left( \frac{150 \times 10^3}{101.0} \right)^2 \times \frac{(998 - 860) \times 9.81}{18 \times 1.005 \times 10^{-3}} = 1.7 \times 10^3 \text{ m/s} \]  

(17)

From the above theoretical calculation, the floating velocity of the oil droplet with the particle size of 150μm in water without considering the temperature is 1.7 mm/s. Formula 1 shows that the viscosity of the flow field, the particle size of oil droplet and the density difference between oil and water have great influence on the sedimentation velocity of oil droplet. The velocity variation of oil droplet in the sedimentation tank with the same parameters is obtained by numerical simulation, as shown in Fig. 6. The oil droplet accelerates after entering the sedimentation tank. When T is 0.3s, the oil drop velocity reaches the maximum 2.184mm/s. Then the oil drop velocity decreases gradually. When T is 0.6 s, the oil drop velocity tends to be stable. Then the droplet velocity moves steadily near 1.5mm/s. The stable speed is basically the same as that of the theoretical analysis.

CONCLUSIONS

(1) Through the trajectory simulation of the discrete phase oil droplet in the sedimentation tank, it is concluded that the oil droplet moves mainly in the horizontal direction when entering the sedimentation tank. The time is about 0.6 s. Then the movement gradually changes to vertical direction. After 0.9 s, the oil droplet particle swarm moves vertically as a whole.

(2) The resultant force of oil droplet varies greatly in the process of horizontal movement into the sedimentation tank. There is a great deal of volatility. The magnitude of the resultant force tends to be stable after 0.4 s.

(3) Through theoretical calculation, it is concluded that the velocity of oil droplet particles after stabilization is 1.7 mm/s. The numerical simulation result is 1.5 mm/s. It showed a good consistency. The velocity of oil droplet tends to be stable after 0.7 s.

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GEOLOGICAL CONDITIONS FOR SHALE GAS ENRICHMENT IN THE UPPER PERMIAN DALONG FORMATION, THE NORTHEASTERN CHONGQING AREA, CHINA

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ABSTRACT

Significant progress has been made during the exploration of shale gas in the Lower Paleozoic Wufeng-Longmaxi Formations and the Shuijingtuo Formation in the Northeastern Chongqing Area. However, less attention has been paid to the Upper Paleozoic Dalong Formation shale, in terms of its shale gas enrichment conditions. Accordingly, this study aims to characterize the Dalong Formation shale from perspectives of its distribution, organic chemistry, reservoir capacity and gas content through combination of field measurement, experimental tests and well gas content quantification. It is demonstrated that the Dalong Formation shale is featured by dominance of Type II organic matter, moderate thickness, high TOC content, high maturity, and large gas content, all of which favor the shale gas generation and accumulation. Moreover, the presence of brittle minerals such as quartz and feldspar provide possibilities for later hydraulic fracturing, while organic pores and micro-fractures act as important reservoir space for shale gas enrichment. Compared with the other shale successions that have been producing commercial shale gas flows, the Dalong Formation shale in the Northeastern Chongqing Area has good shale gas enrichment conditions, and is thus of great shale gas exploration potentials. More attention should be paid to it in the future exploration of unconventional gas in this area.

KEYWORDS:
Shale gas, enrichment conditions, the Upper Permian Dalong Formation, Northeastern Chongqing Area, new successions.

INTRODUCTION

Known as a type of unconventional natural gas hosted by organic-rich shales [1], shale gas has been increasingly highlighted due to its abundant resources. Its total recoverable resources throughout the world amount to 221×10^{12} m^3, and those in China account for 15%, ranking first in the world [2]. According to the prediction by the Ministry of Natural Resources of China, geological resources of shale gas in China are 134.40×10^{12} m^3, among which recoverable resources are 25×10^{12} m^3. Specific to the Chongqing Area, its geological resources of shale gas reach 12.75×10^{12} m^3, ranking third in China [3]. Major exploration breakthroughs have been achieved in the Wufeng and Longmaxi Formations in the Fuling, Nanchuan, Yongchuan, Pengshui and Qijiang Regions [4-5], which indicate tremendous prospects of shale gas exploration in the Chongqing Area. Specifically, the Northeastern Chongqing Area is of great potential, as it hosts three sets of thick shales, namely the Lower Cambrian Shuijingtuo Formation (E1s), the Upper Ordovician Wufeng Formation-Lower Silurian Longmaxi Formation (Ow-S1l) and the Upper Permian Dalong Formation (P3d). Recent studies have comprehensively investigated the hydrocarbon accumulation conditions [5-6], preservation conditions [7] and resource potentials [3,9,10] of shale gas in the Northeastern Chongqing Area and its peripheral areas. However, most of these studies highlight E1s and Ow-S1l, with insufficient attention paid to P3d.

The Upper Permian Dalong Formation has been demonstrated to be one of the most massively developed, regional marine shales that are rich in organic matter in the Southern China, accompanied by its strong hydrocarbon generation capacity. According to He et al. [11], the Dalong Formation in the Central Hunan Area should be deposited in a deep-water intra-platform depression, and it is fea-
tured by its large thickness, huge hydrocarbon generation potential and good physical properties, which are favorable for shale gas exploration and development. Yu et al. [12] evaluate the shale gas resource potential of the Dalong Formation in the Jianshi-Hefeng Regions in the Western Hubei Area, and demonstrate its good exploration prospects by showing its large effective black shale thickness and high on-site desorbed gas content up to 2 m³/t. Most of previous studies highlight the high organic abundance and large thickness of the Dalong Formation as a set of effective source rocks [13-14]. Nevertheless, whether it can act as reservoir rocks hosting shale gas is rarely investigated. Accordingly, this study aims to explore the shale gas enrichment conditions in the Dalong Formation of the Northeastern Chongqing Area based on field measurements, experimental tests, and drilling data. Results of this study are expected to provide important guidance for shale gas exploration in the near future.

GEOLOGICAL SETTING AND METHODS

**Geological setting.** The Northeastern Chongqing Area tectonically spans the Yangtze Paraplateform and the Qinling Synclinal Fold Belt, which are in the north of the Shashi Concealed Fault in the Yangtze Plate. Basically, it is in the northern part of the Upper Yangtze Block and the southern part of the Qinling Orogenic Belt. Multiple episodes of tectonic movements are superimposed here, including the Jinning, Chengjiang, Caledonian, Variscan, Indosinian, Yanshanian, and Himalayan Movements. Consequently, multiple tectonic patterns coexist, including the NE-extended Dabashan overthrust belt, the NNE-extended baffle-like fold belt, the NNE- and NW-extended arc structure, and the NW-extended brush-shaped structure, respectively from the north to the south (Fig. 1). There are significant differences in preservation conditions among different tectonic units, which result in the complex hydrocarbon accumulation patterns in the Northeastern Chongqing Area.

There are mainly two stratigraphic zones in the Northeastern Chongqing Area, namely the Yangtze Stratigraphic Zone in the south and the Qinling Stratigraphic Zone in the north, which are separated by the Chengkou-Zhenping-Fangxian Fault. Except for the Devonian, the Carboniferous and the Tertiary, all the other strata from the Sinian to the Quaternary are outcropped. The Lower Paleozoic strata are widely distributed in the study area. Magmatic rocks are exclusively developed in local areas in the north of the Chengba Fault Belt, and they are mainly intrusive diabase veins, with a small amount of volcanic clastic rocks. The large tectonic uplift in the northern part of the study area leads to strong erosion and outcropping of the Lower Paleozoic strata. In comparison, the southern part of the study area is of relatively stable tectonic setting and consequent weak uplift and erosion, with main outcropping of the Mesozoic.

**FIGURE 1**
Tectonic setting of the Northeastern Chongqing Area
**Stratigraphy.** The Dalong Formation is speculated to be formed in the deep-water marine trough environment [15-17], and it hosts a set of high-quality black source rocks, which is coeval with the Changxing Formation carbonate deposits in the periphery platform. The Dalong Formation is mainly distributed in the Chengkou, Wuxi, Wushan, and Fengjie Regions, with a small part of it deeply buried (~6000 m) in the Kai County [18]. Lithologically, the Dalong Formation in the study area mainly consists of black thin-layered carbonaceous shale, siliceous shale, interbedded siliceous shale and gray-black thin-medium layered marl, siliceous limestone and limestone lens, with high abundance of ammonite (Fig. 2). The lower part of the Dalong Formation is a set of black siliceous and carbonaceous shale intercalated with limestone, which overlies the Wujiaaping Formation, while the middle and upper parts of the Dalong Formation are siliceous shale and calcareous shale interbedded with thin carbonaceous shales. The content of siliceous rocks gets reduced in the Chengkou Region and the Sichuan-Hunan provincial boundary, while the black shales intercalated with thin-layered limestone become dominant.

Extensively denuded in the northern part of the study area, the Dalong Formation is only slightly outcropped in the southern part. Through field measurement and drilling data calculation, the Dalong Formation is speculated to have a moderate burial depth (generally less than 4000 m, mainly ranging between 500 and 3000 m). Huge lithofacies change is seen within the Upper Permian strata across the study area. In this context, the Dalong Formation is also featured by its large variation of lithology and thickness in the study area. Its thickness dominantly ranges between 10 and 60 m, with a westward thinning tendency. In the very western part of the study area, namely the Kai County-Yunyang Regions, the organic-rich shale becomes less developed and gradually evolves into the Changxing Formation platform limestones. Large thickness values are mainly seen in the Fengjie-Wushan Regions. Specifically, the shale thickness in the Huoshaopo Section in the southeast of the Fengjie County reaches 50 m, and the lower organic-rich shale interval is about 30 m thick.

**RESULTS**

**Organic geochemistry.** (1) **Organic matter abundance.** According to the total organic carbon (TOC) contents of 120 shale samples from 10 outcrop sections and 2 wells in Manyue, Tianbao, Hexi and Kongjiacun Regions, the Dalong Formation shales in the study area are of huge TOC contents, largely ranging from 0.17% to 13.70%, with an average of 4.05%. According to the classification criteria for hydrocarbon generation potentials of marine source rocks in the Chinese Paleozoic [19], the Dalong Formation shale in the study area is a set of good source rocks with strong hydrocarbon generation potentials. The TOC content is found to vary with lithology, with high contents mainly seen in the lower and middle parts. In the map view, the TOC content increases eastward, with high contents mainly occurring in the Wushan and Fengjie Regions, averaging 2.88%–7.05% (Fig. 4).

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**FIGURE 2**

Photos of outcrops and cores of the Dalong Formation in the Northeastern Chongqing Area

Notes: (a) Black siliceous rocks in the middle of the Dalong Formation in the Tianjiawan Section; (b) Black carbonaceous shale in the lower part of the Dalong Formation in the Gaofeng Village Section; (c) Ammonite fossils in the Jiaodingyan Section; (d) Limestone lens in the middle of the Dalong Formation in the Gaofeng Village Section; (e) Gray calcareous shale in the upper part of the Dalong Formation in the Huangtuliangzi Section; (f) Carbonaceous shale core from Well X in the Fengjie Region.
The carbon isotopic composition of kerogen is mainly affected by that of its parent material, which is a reliable indicator of organic matter type [20]. According to the carbon isotope analysis of 36 representative kerogen samples from the study area, the δ13C content of the Dalong Formation shale in the study area varies between -29.5‰ and -24.7‰, with an average value of -26.01‰. Based on the classification criterion proposed by Huang [21], these kerogen samples should be categorized as Type II kerogens. Microscopic observation demonstrates presence of sapropelinite, vitrinite and inertinite (Fig. 4). Among them, sapropelinite is featured by the dominance of the amorphous body, the flocculent and blocky form, black colors under transmitted light, and no fluorescence. Therefore, kerogens in the Dalong Formation should be Type II based on analysis of both carbon isotopic compositions and microscopic compositions.
(3) **Organic matter maturity.** The maximum pyrolysis temperature ($T_{\text{max}}$) generally increases as organic matter maturity grows. The high $T_{\text{max}}$ range of 462–556 °C in the study area indicates high maturity. Specifically, organic matter should be in the high to over mature stage. Based on tests of 57 shale samples, the vitrinite reflectance ($R_o$) is demonstrated to range between 2.19% and 3.18%, with an average of 2.70%, further indicating high to over maturation. In the map view, there is no significance change in terms of $R_o$, with local high values in the Wushan-Fengjie Regions near the depositional center (Fig. 6).

**FIGURE 5**
Microscopic compositions of kerogens in the Dalong Formation shale of the Northeastern Chongqing Area

**FIGURE 6**
Contour map of $R_o$ of the Dalong Formation shale in the Northeastern Chongqing Area
Reservoir rock characteristics. (1) Mineral composition. Shale mineral compositions and contents not only control fracture development in shales, but also impact the shale gas content [22-23]. Detrital and clay minerals are the main compositions of the Dalong Formation shale in the study area, with a few carbonate and pyrite. In some samples, the content of carbonate minerals can be up to 50%, which indicates presence of calcareous shale and marl. Specifically, the content of clay minerals vary between 3.1% and 73.10%, with an average of 21.53%; the content of brittle minerals such as quartz, feldspar and pyrite is 12.9%~94.9%, with an average of 55.73%; and the content of carbonate minerals ranges from 0% to 82.9%, with an average of 21.02% (Fig. 7). The high content of brittle minerals in the Dalong Formation shale in the study area is comparable to those in the Wufeng-Longmaxi Formations as uncovered in Well JY1 [24], which is favorable for subsequent hydraulic fracturing.

(2) Reservoir space. According to scanning electron microscopy observation, there are mainly four types of pores in the Dalong Formation shale, namely the intergranular pores, intragranular pores, clay mineral interlayer pores, and organic pores (Figs. 8a-8d). All these pores are fine in diameter, and mostly in the nanometer scale. To be specific, micro organic pores are the most common type of pores in the study area, acting as the main reservoir space for shale gas [25-27]. The secondary most developed pores are dissolution intragranular pores, while the other types of pores are of limited occurrence. In addition, multiple episodes of tectonic movements in the study area contribute to the development of both macroscopic and microscopic fractures, which provide essential space for natural gas storage in shale (Figs. 8e and 8f).

(3) Physical characteristics. The effective porosity of the Dalong Formation shale in the study area is demonstrated to range between 0.23% and 18.37%, with an average of 2.56%. In some samples, the effective porosity can be up to more than 10%, which may be attributable to weathering of the samples. Generally, most of the effective porosity data falls in the range of 0%~3%. The permeability is between 0.0002×10⁻³ µm²~0.401×10⁻³ µm², with an average of 0.018×10⁻³ µm². Generally, most of the permeability data falls in the range of (0.0001~0.01)×10⁻³ µm². Therefore, the Dalong Formation shale in the study area is overall of low porosity and low permeability, which disfavors the accumulation of free gas. However, the much larger surface area of the Dalong Formation shale, averaging 21.02 m²/g, than the other shales, provides favorable conditions for shale gas adsorption [28].

Gas content features. Generally, the isothermal adsorption experiment is believed to be able to reflect the maximum shale gas adsorption capacity at certain temperature and pressure [28]. In this study, isothermal adsorption experiments are performed on 10 samples taken from wells and outcrop sections, which demonstrate the strong gas adsorption capacity of the Dalong Formation shale (2.16~8.01 m³/t, averaging 5.09 m³/t).
The obtained gas content following the industrial standard [29] can intuitively and accurately reflect the shale gas adsorption capacity. Specifically, the shale gas content mainly includes three parts, namely the content of gas loss in the rig-lifting process, the content of on-site gas desorption, and the content of residual gas after desorption. According to measurement in 13 core samples from Well Y in the Miaowan Village, Xinglong Town, Fengjie County, the shale gas content ranges between 0.73 and 4.44 m³/t, with an average of 1.91 m³/t. Moreover, there are 11 samples having the shale gas contents higher than 1.0 m³/t. Thickness of those high shale gas content layers are about 20 m. Generally, shale gas contents are higher in the base and top of the shale intervals (Fig. 9). In general, the Dalong Formation shale has a relatively high gas content, which exceeds the lower limit of commercial shale gas development in the North America (1.1 m³/t) [30] and thus is of great prospect in shale gas exploration and development.

**FIGURE 8**

Pores and fractures in the Dalong Formation shale in the Northeastern Chongqing Area

Comprehensive analysis of geological conditions for shale gas enrichment. Based on above analyses, the Dalong Formation shale in the Northeastern Chongqing Area is featured by its large
distribution area, moderate burial depth, medium thickness, high organic carbon content, high maturity, high brittle mineral content, and large gas content. Through comparison with shales of Wufeng-Longmaxi Formations where major breakthrough has been recently made (Table 1), the Dalong Formation shale has a relatively lower gas content than that in the Fuling Region, but is more or less similar to those in the Pengshui, Qijiang, and Wuxi Regions. In terms of the other parameters, the Dalong Formation shale has a much higher organic carbon content than the shales of Wufeng-Longmaxi Formations, which indicates good material basis for the formation of shale gas reservoirs. Therefore, the Dalong Formation shale can be listed as an alternative target layer in addition to shales of Wufeng-Longmaxi Formations and Shuijingtuo Formation in the Northeastern Chongqing Area.

![Gas content, m³/t](image)

**FIGURE 9**
Shale gas content against burial depth in the Dalong Formation shale in the study area

**TABLE 1**
Comparison between the Dalong Formation shale and Wufeng-Longmaxi Formations shales

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Central Chongqing</th>
<th>Southeastern Chongqing</th>
<th>Northeastern Chongqing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fuling</td>
<td>Pengshui</td>
<td>Well QY1</td>
</tr>
<tr>
<td>Stratigraphy</td>
<td>Os-w-S1</td>
<td>Os-w-S1</td>
<td>Os-w-S1</td>
</tr>
<tr>
<td>Buried depth (m)</td>
<td>1500–3500</td>
<td>1500–3000</td>
<td>800</td>
</tr>
<tr>
<td>Thickness (m)</td>
<td>80–120</td>
<td>100–110</td>
<td>70</td>
</tr>
<tr>
<td>TOC (%)</td>
<td>2.0–3.0/2.66</td>
<td>0.5–4.74/1.91</td>
<td>0.22–5.31/1.61</td>
</tr>
<tr>
<td>Ro (%)</td>
<td>1.38–4.14</td>
<td>2.31–3.09</td>
<td>2.24–3.38</td>
</tr>
<tr>
<td>Brittle mineral content (%)</td>
<td>55.9</td>
<td>54</td>
<td>65.5</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>4.53</td>
<td>2.35</td>
<td>3.23</td>
</tr>
<tr>
<td>Permeability (×10⁻³ μm²)</td>
<td>0.007</td>
<td>0.038</td>
<td>0.032</td>
</tr>
<tr>
<td>Average gas content (m³/t)</td>
<td>4.61</td>
<td>2.06</td>
<td>1.13</td>
</tr>
</tbody>
</table>
CONCLUSIONS

(1) The Dalong Formation in the Northeastern Chongqing Area mainly consists of carbonaceous shale and siliceous shale, with intercalated thinned-layered siliceous rocks and limestones. It primarily occurs in the Chengkou-Wuxi-Wushan-Fengjie Regions, with its thickness ranging between 10 and 60 m. Organic matter in the shale is featured by dominance of Type II kerogen, high TOC content (averaging 4.05%), and high maturity (average R0 reaching 2.70%), all of which are favorable for gas generation.

(2) Main mineral compositions of the Dalong Formation shale in the Northeastern Chongqing Area are quartz, feldspar, carbonate and clay minerals. The content of detrital minerals such as quartz and feldspar are high, which is beneficial to the later hydraulic fracturing. The development of pores (e.g. organic pores and intragranular pores) and micro-fractures provides important space for shale gas enrichment.

(3) The Dalong Formation shale in the Northeastern Chongqing Area has strong adsorption capacity for methane, with the average maximum on-site desorption gas volume reaching 5.09 m³/t. In the meantime, it also has high gas content, with the average on-site desorption gas content reaching 1.35 m³/t. Based on characterization of effective thickness, organic matter abundance and brittle mineral content, this study compares the hydrocarbon accumulation conditions of the Dalong Formation shale with those of the other shales in the study area with recently achieved breakthroughs. It is demonstrated that the Dalong Formation shale in the Northeastern Chongqing Area bears strong shale gas generation potential, high reservoir capacity and large gas content, which provide critical geological conditions for shale gas enrichment.

ACKNOWLEDGEMENTS

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COMPARISON OF SAMPLE PREPARATION METHODS FOR DETERMINATION OF HEAVY METALS IN CATTLE HAIR BY ICP-OES

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ABSTRACT

The purpose of this study is to compare the effectiveness of wet acid, surfactant and alkaline digestion procedures for determination of heavy metals in cattle hair samples. Cattle hair samples were collected from the tails of Holstein cattle and washed sequentially with acetone, distilled water, and acetonitrile for removal of external contaminations. Analyses were carried out by ICP-OES after applying to acid digestion (HNO₃:H₂O₂), acid digestion under addition of surfactant (Triton X-100), and alkaline digestion using Tetramethylammonium hydroxide. The accuracies of methods for Cu, Cr, Fe, Mn, Ni and Zn metals were evaluated by using NCS ZC 8100 2b hair certified reference material. The results showed that Cu, Fe results obtained by application of Triton X-100 and Cr, Fe, Ni and Zn results obtained by application of Tetramethylammonium hydroxide were not compatible with certified values, whereas wet acid digestion method provided satisfactory recoveries for all examined metals in cattle hair samples. Relative standard deviations were found below 5% in wet acid digestion method. The concentrations of Zn, Cr, Ni, Cu, Fe and Mn in cattle hair samples were in the range of 88-157, 0.94-3.22, 0.00-2.96, 14.4-33.0, 36-101, 0.20-3.00 μg g⁻¹ respectively. Our results indicated that wet digestion method was the most efficient for the analysis of Cu, Cr, Fe, Mn, Ni, and Zn in the cattle hair sample with excellent precision and recovery.

KEYWORDS:
Wet acid digestion, TMAH, Triton X-100, cattle hair, ICP-OES.

INTRODUCTION

Essential metals such as Cr, Fe, Cu, Mn and Zn are required by many organisms to keep their body working effectively. An optimal concentration of metals is needed during normal development and growth of the living body, as they are constituents of numerous vital enzymes. In cases of shortage or overabundance of these elements, the metabolism of living cells can alter erratically. On the other hand, some metals like Ni are toxic even at low concentrations and they could adversely affect a living organism just by being present in the lowest of volumes [1]. Metals that are released to the atmosphere as a result of industrial activities disperse into soil, air and water sources. These elements can enter organisms living nearby such areas via different routes; they can accumulate in skin, hair and tissues [2] and cause serious adverse effects on their metabolism over time [3].

Monitoring of metal levels in the biological material could be used for the assessment of environmental pollution. During past few decades, bone, blood, plasma, urine, nail and hair samples taken from human and animals in industrial areas have been used as indicators of toxic and essential elements [4-8]. Determination of metals in human and animal hair to monitor the deficiency of essential metals and exposure to toxic metals is a method that is getting an increasing amount of attention on the environmental sciences. Flesch [9] and Hammeret al. [10] suggested the use of hair samples for trace element analysis. In some cases hair analysis is better than blood analysis, because several trace elements accumulate in the hair. Hair samples have some advantages. Hair samples is carried out easily and painlessly, and nospecific equipment is needed. Samples can be stored at room temperature in polyethylene bags for a long time, and their composition does not change measurably. Hair provides information on the trace element concentrations of the intracellular space (blood provides information on the extracellular space) [11]. In addition, trace elements present in the body are incorporated into the hair during its growth; the exogenous trace elements are fixed on its surface. On one hand, this property is useful because environmental exposures are studied easily, but, on the other hand, it is a disadvantage, because special washing procedures are needed to remove the exogenous trace elements when endogenous trace element concentrations are to be determined. An additional advantage is that with the knowledge of the growth rate of the hair, it is possible to select the examined previous period (usually 2-3 months) [12].
The metal analyses in biological samples require utilization of selective and sensitive techniques. Element analysis of solid samples like hair with instruments requires full dissolution of samples prior to analysis. It is difficult to make direct analysis of the solid samples for many devices. The accuracy of the analyses is mainly depended on how well the samples were decomposed. Therefore, many different digestion procedures have been used for the digestion of solid samples. Common methods for preparing of solid samples are dry ashing [13], ultrasonic acid leaching [14], wet digestion in open [15] or closed vessels with microwave-assisted acid digestion [16]. Additionally, some sample preparation methods are used chemical reagents like Tetramethylammonium hydroxide [17, 18] and Triton X-100 [19] for digestion of solid samples. These are added to the solid samples to obtain enough energy to break crystalline structure and molecular bonds of solid material to ease digestion. The applications of Triton X-100 and Tetramethylammonium hydroxide (TMAH) digestion methods, especially TMAH (samples are readily prepared in a single solution) can be performed more easily compared to the wet acid digestion method [20].

The analysis of metals in solid samples requires utilization of selective and sensitive techniques. Many techniques have been reported for determination of metal in biological samples, including FAAS [21, 22], ETAAS [23, 24], ICP-OES [25] and ICP-MS [26-29]. Compared to other analytical techniques, ICP methods have higher advantages due to its ability to analyze multi elements at the same time, being fast, having lower limits of detection and having a wider linear working range. The analyses of trace metals with a high accuracy are a major advantage of these techniques.

In this study, the efficiency of three pre-treatment methods, i.e. acid digestion (HNO₃:H₂O₂), acid digestion under addition of surfactant (Triton X-100), and alkaline digestion using Tetramethylammonium hydroxide (TMAH) for determination of Cr, Cu, Fe, Mn, Ni, and Zn metals in tail hair of cattle by inductively coupled plasma-optical emission spectroscopy (ICP-OES) were evaluated.

### MATERIALS AND METHODS

**Apparatus.** Metal analyses were carried out by ICP-OES. The ICP-OES measurements were performed using a, Thermo-Fisher Scientific iCAP 6500 inductively coupled plasma atomic emission spectrometer (Thermo-Fisher Scientific, Dartford, UK). A Thermo Scientific iCAP 6500 ICP-OES (Duo) was used in conjunction with an ASX-520 auto sampler. Internal standard (5ppm Yttrium) was added on-line using a Y-connector. The optimum instrumental settings and operating conditions are summarized in Table 1. Also digestions of samples were carried out on a digital hot plate (Wisd-Wise Therm).

<table>
<thead>
<tr>
<th></th>
<th>ICP-OES operating conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF power</td>
<td>750 W</td>
</tr>
<tr>
<td>Nebulization flow rate</td>
<td>0.55 L min⁻¹</td>
</tr>
<tr>
<td>Auxiliary argon flow rate</td>
<td>0.5L min⁻¹</td>
</tr>
<tr>
<td>Pump Tubing</td>
<td>Tygon Orange/White sample</td>
</tr>
<tr>
<td>Pump rate</td>
<td>50 rpm</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>Concentric</td>
</tr>
<tr>
<td>Nebulizer Argon Pressure</td>
<td>0.65 L/min / 26 MPa</td>
</tr>
<tr>
<td>Spray Chamber</td>
<td>Cyclonic</td>
</tr>
<tr>
<td>Centre tube</td>
<td>2.0 mm</td>
</tr>
<tr>
<td>Torch Orientation</td>
<td>Duo</td>
</tr>
<tr>
<td>RF Forward Power</td>
<td>1150 W</td>
</tr>
<tr>
<td>Coolant flow</td>
<td>12 L/min</td>
</tr>
<tr>
<td>Auxiliary flow</td>
<td>0.5 L/min</td>
</tr>
<tr>
<td>Integration time</td>
<td>15 seconds (UV and VIS)</td>
</tr>
</tbody>
</table>

Reagents and Solutions. All reagents were of analytical reagent grade, and high purity deionized water (Millipore, Bedford, MA, USA) was used to prepare all solutions. HNO₃ (Suprapure, 65 %), l-butanol (99.8 %), Triton X-100 and TMAH (25 % solution in water) were purchased from Merck (Darmstadt, Germany). H₂O₂ (30 %, w/w) was obtained from Sigma-Aldrich (St. Louis, MO, USA). High-purity multi element metal standard solutions in 5 % HNO₃ (Inorganic Ventures, Christiansburg, VA, USA) were used for calibration. Certified reference material (NCS ZC 8100 2b, China National Analysis Center) was used to control the accuracy of the methods. Before using, all glassware and polyethylene bottles were kept by soaking in 10 % HNO₃ and then rinsed with distilled deionized water.

**Sample Preparation.** Hair samples were collected from the tails of Holstein cattle (3-5 years old) with stainless steel scissors and washed sequentially with acetone, distilled water, and acetone for removal of external contaminations [30]. The hair was dried in an oven at 80 ± 5 °C and cut into pieces of approximately 1 cm length and stored at room temperature in plastic bags.

**Digestion Procedures. Method A (Wet acid sample digestion procedure).** The amount about 20 mg from each of dried cattle hair samples was directly weighted into glass beakers. 5 mL HNO₃ (65 %) was added to the beakers. Samples were left on electric hot plate at 60-70 °C for 1-2 hours, until they were semi-dried. Following this, 1 mL of H₂O₂ and 5 mL of HNO₃ were added to the samples and evaporated to dryness at the same temperature. At the end of this process, the color of the solution became transparent. Solutions were cooled and transferred to a 10 mL centrifuge tube with 2 M HNO₃.
Method B (Triton X-100 sample digestion procedure). 20 mg of cattle hair samples was carefully weighed into a 100 mL glass beaker. A volume of 500 μL concentrated HNO₃ (65 %) was added and watch glass was placed over the beaker. Samples were mineralized at 70 °C and evaporated to dryness for approximately an hour. 3.9 mL (0.5 %, v/v) butanol, % 0.65 (v/v) HNO₃ and % 0.01 Triton X-100 (v/v) were added to each residue with a micropipette and samples were transferred to 10 mL conical-bottom glass tubes and analyzed.

Method C (TMAH sample digestion procedure). Cattle hair samples (20 mg) were weighed into a 15 mL conical-bottom plastic centrifuge tube. 1 mL of TMAH (25 %, w/v) was added. Samples were incubated at room temperature overnight. Following day, last volumes of it were completed to 10 mL with % 1 HNO₃.

The Validity of The Three Pre-Treatment Methods. In order to find out the reliability of this pre-treatment methods used for sample preparation prior to instrumental analysis, methods were checked with using NCS ZC 8100 2b hair certified reference materials (CRM). 20 mg of four CRM samples were weighed and three different sample preparation techniques was applied to this samples as described above and determined for each metal content. The recoveries and results obtained according to the certified value are shown in Table 3.

### TABLE 2

**Analyte characteristics of the heavy metals**

<table>
<thead>
<tr>
<th>Element</th>
<th>Spectral line (nm)</th>
<th>Correlation coefficient</th>
<th>Detection limit (ppb)</th>
<th>Torch orientations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>267.716</td>
<td>0.999942</td>
<td>5</td>
<td>Radial</td>
</tr>
<tr>
<td>Cu</td>
<td>204.379</td>
<td>0.999858</td>
<td>20</td>
<td>Axial</td>
</tr>
<tr>
<td>Fe</td>
<td>233.280</td>
<td>0.999617</td>
<td>20</td>
<td>Axial</td>
</tr>
<tr>
<td>Mn</td>
<td>257.610</td>
<td>0.999894</td>
<td>5</td>
<td>Duo</td>
</tr>
<tr>
<td>Ni</td>
<td>217.467</td>
<td>0.999997</td>
<td>5</td>
<td>Axial</td>
</tr>
<tr>
<td>Zn</td>
<td>213.856</td>
<td>0.999880</td>
<td>20</td>
<td>Axial</td>
</tr>
</tbody>
</table>

### TABLE 3

**Certified and measured means using various digestion methods of trace elements in Standard certified reference materials (NCS ZC 8100 2b hair, N=4)**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Certified Values</th>
<th>Wet acid Procedure (μg/g)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Triton X-100 Digestion Procedure (μg/g)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>TMAH Digestion Procedure (μg/g)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>33.6 ± 2.3</td>
<td>32.0 ± 0.9</td>
<td>2.81</td>
<td>95</td>
<td>18.3 ± 1.7 (LR)</td>
<td>-</td>
<td>55</td>
<td>33.4 ± 1.5</td>
<td>4.4</td>
<td>99</td>
</tr>
<tr>
<td>Cr</td>
<td>8.74 ± 0.10</td>
<td>8.10 ± 0.36</td>
<td>4.50</td>
<td>93</td>
<td>8.76 ± 0.49</td>
<td>9.3</td>
<td>100</td>
<td>5.06 ± 0.7</td>
<td>(LR)</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>106 ± 16</td>
<td>164 ± 5</td>
<td>3.04</td>
<td>103</td>
<td>65 ± 7 (LR)</td>
<td>-</td>
<td>40</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>3.83 ± 0.39</td>
<td>3.93 ± 0.19</td>
<td>4.83</td>
<td>103</td>
<td>3.61 ± 0.25</td>
<td>6.9</td>
<td>95</td>
<td>3.84 ± 0.32</td>
<td>8.3</td>
<td>93</td>
</tr>
<tr>
<td>Ni</td>
<td>5.77</td>
<td>5.43 ± 0.24</td>
<td>4.42</td>
<td>94</td>
<td>5.29 ± 0.18</td>
<td>3.4</td>
<td>92</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zn</td>
<td>191 ± 16</td>
<td>201.2 ± 3.2</td>
<td>1.49</td>
<td>105</td>
<td>199 ± 7</td>
<td>3.5</td>
<td>104</td>
<td>122.9 ± 8.0</td>
<td>-</td>
<td>64</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± standard deviation, RSD: relative standard deviation, ND: not detected, LR: low result
Three sample preparation method (A, B and C) were applied to the hair CRM samples (4 different weighing was performed for each analysis) to test the result of the accuracy and validity. The concentrations of each solution for Cr, Cu, Fe, Zn, Ni and Mn were determined by ICP-OES. Each measurement was tri-replicated. The obtained results are presented in Table 3 and Figure 1. After hair CRM analysis, we detected the content of Cu, Cr, Fe, Mn, Ni and Zn metals with taking into account good CRM results in cattle hair samples. The mean metal values in cattle hair samples were presented in Table 4.

**TABLE 4**
The metal results of cattle hair analysis

<table>
<thead>
<tr>
<th>Metals</th>
<th>Mean of metal concentrations (µg g⁻¹)</th>
<th>Range values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>124*</td>
<td>118**</td>
</tr>
<tr>
<td>Cr</td>
<td>2.02</td>
<td>1.72</td>
</tr>
<tr>
<td>Ni</td>
<td>0.80</td>
<td>0.69</td>
</tr>
<tr>
<td>Cu</td>
<td>21.0</td>
<td>NC</td>
</tr>
<tr>
<td>Fe</td>
<td>56.4</td>
<td>NC</td>
</tr>
<tr>
<td>Mn</td>
<td>1.92</td>
<td>1.75</td>
</tr>
</tbody>
</table>

* x ± s (mean ± standard deviation); ** NC: Not considered.

**DISCUSSION**

Metals are redistributed naturally in the global environment by both biological and geological cycles. But, industrial activities greatly change the distribution of metals by discharge to air, water and soil and as a result cause pollutions. According to many researchers, hair samples taken from animals living in industrial areas may be used as an indicator of environmental conditions and ensure the detection of accumulation of heavy metals [31, 32]. Sample preparation in trace element analysis is the most critical and important step. The ability to obtain more accurate results for all analytical methods depends on the sample preparation step. Especially in biological samples, since heavy metals are present in a complex matrix and their concentration is not large enough to be directly determined any loss and/or contamination that may occur during the sample preparation process in trace element analysis affects the accuracy of the results. Although the wet acid digestion is largely used for matrices of different kind, different methods such as Triton X-100 and TMAH are used in some laboratories. So, to assure that accurate and reliable results, validation of digestion methods is necessary [33].

As shown from Table 3 and Figure 1, the best recoveries obtained with three different digestion methods for cattle hair samples ranged between 92-105%. Low recoveries were between 40-64%. The recoveries of Fe and Ni metals based on TMAH digestion procedure (TMDP) were founded below the detection limit. Our finding from the hair CRM results indicates that the wet acid digestion procedure (WDP) performed with HNO₃:H₂O₂ in the ratio of 10:1 is an effective digestion method for determining of Cu, Cr, Fe, Mn, Ni and Zn metals in cattle hair samples. Recovery results were founded in the range of 93-105%. The results were nearly quantitative for all examined metals (Cr, 93%, Ni, 94% and others, ≥95%). Relative standard devia-
tions (% RSD) were found below 5%. We can say that wet acid digestion method can be used as a conventional analytical method for examined metals in hair samples. Similar results have been reported previously related to the using of WDP for hair samples. Afridi et al. [14] reported that there was a good relationship between the result of wet digestion procedure with HNO₃ and H₂O₂ and certified CRM for Cr, Cu, Fe, Zn and Ni metals. Authors have reported that the validity of the WDP was confirmed by the excellent recoveries obtained for some of the elements determined by inductively coupled plasma atomic emission spectrometry for the Standard reference materials. Luna et al. [34] evaluated acid digestion sample preparation method and commonly used procedure involving dilution of samples in an alkaline solution animal serum samples to determine trace element contents. The proposed acid digestion sample preparation method produced satisfactory results for determining most toxic and essential trace element targeted in monitoring studies. The percent recoveries obtained from Triton X-100 digestion procedure (TDP) were calculated as 100, 95, 92 and 104 for Cr, Mn, Ni and Zn. The results demonstrated that the method was quite reasonable for Cr, Mn, Ni and Zn in cattle hair analysis. % RSD values were ≤ 10. But, the percent recoveries of Cu and Fe in cattle hair samples were quite low (55% and 40%, respectively). Goulle et al. [35] were determined metal and metalloids in human whole blood, plasma, urine and hair by means of TDP. The hair samples (25 mg) were digested with TDP and metal and metalloid analysis was carried out with ICP-MS. Rodrigues et al. [1] used Triton X-100 procedure in hair samples to evaluate the deficiencies of essential metals and exposures to toxic ones. According to their results, the results of Cu and Mn in CRM samples were found to be approximately 90 % for Cu and 89 % for Mn. We obtained a good recovery result for Mn (95 %) but, the recoveries were low (55 %) for Cu.

The result of TMDP indicated that TMDP were suitable only Cu and Mn (99% and 93%, respectively, RSD ≤ 10%). The recoveries of Cr and Zn were quite low (60% and 64%, respectively). In addition, the results of Fe and Ni were below the limit of detection. TMDP was studied for Fe and Zn analysis in human serum and hair samples with GFAAS by Donnicia et al. [36]. The recoveries of Fe and Zn metals in human hair were 93 (%) and 102 (%), respectively. This difference may be explained by using of different amount of samples and chemicals. 500 µL of TMAH solution was added to the 100 mg of hair samples in sample preparation in their study. However, we used 20 mg of hair samples and added 1000 µL TMAH during sample preparation. We believe that Ni and Fe metals can coprecipitate as metal hydroxides in the presence of TMAH solution. In a study, Doğan ve Dincer Kaya [37] was used alkaline digestion with TMAH in addition to conventional acidic digestion methods like dry ashing, wet ashing and microwave digestion in acid for the digestion of hair samples. They determined that the alkaline digestion in TMAH cannot be an alternative to the acidic digestion methods for determination of Zn and Pb in human hair.

CONCLUSIONS

In our study, high recovery values were obtained for Cu, Cr, Fe, Mn, Ni and Zn with WDP, for Cr, Mn, Ni and Zn with TDP and only for Cu, Mn with TMDP methods using hair CRM samples. The highest recoveries (% 93–105) were obtained using the wet digestion method. This was followed by Triton X-100 (% 40–104) and TMAH digestion (% 60–99). The percent recoveries obtained from Triton X-100 digestion procedure were calculated as 100, 95, 92 and 104 for Cr, Mn, Ni and Zn. The results demonstrated that the method was quite reasonable for Cr, Mn, Ni and Zn in hair analysis. % RSD values were ≤ 10. But, the percent recoveries of Cu and Fe in hair samples were quite low (55% and 40%, respectively). The result of TMAH digestion procedure indicated that only Cu and Mn metals were suitable (99% and 93%, respectively, RSD ≤ 10%).

The authors declare that they have no competing interests.
REFERENCES


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BIO-CONTROL CHARACTERIZATION OF TWO ENDO-MYCORRHIZAL FUNGI AGAINST VERTICILLIUM WILT OF CUCUMBER

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ABSTRACT

The effectiveness of *Glomus mosseae* and *Glomus fasciculatum* to control Verticillium wilt on cucumber was assessed under greenhouse conditions. Several parameters were considered to assess the disease reduction by the arbuscular mycorrhizal fungi (AMF) included shoot and root biomass, disease severity and nutrient contents (N, P and K). After 10 weeks from planting the height of cucumber plants inoculated with *G. fasciculatum* was significantly (138.5cm) different compared to all other treatments, but the combined treatments (*G. mosseae & Verticillium*, *G. fasciculatum & Vertici-llium*) were similar to control plants (117.5cm, 122.5cm, 121cm respectively). Leaves number were in *G. mosseae* treatment not significantly different compared with the combined inoculation of *G. mosseae* and *Verticillium*. Cucumber shoot and root biomass were significantly increased in *G. fasciculatum* treatments compared with *G. fasciculatum & V. dahliae* treatment. Disease severity was decreased significantly in the combined treatments of *G. mosseae* and *Verticillium*, *G. fasciculatum* and *Verticillium* (2.5, 3.2 respectively) as compared to *V. dahliae* infected plants (6.5). The current study illustrated that shoot and root nutrient analysis of N, P and K were increased in the combined treatments and AMF inoculated plants as compared to infected plants. Roots colonized by both species of mycorrhizal fungi were higher compared to combined treatments of the AMF and *Verticillium*. Mycorrhizal spore production was higher in plants inoculated with *G. mosseae* compared with the other treatments, *G. fasciculatum* & *V. dahliae* and *G. mosseae & V. dahliae* treated plants were more significantly tolerant to disease infection compared with Verticillium infected plants.


INTRODUCTION

Cucumber (*Cucumis sativus* L.) is the fourth most important vegetable crop worldwide [1].

Cucumber is a delicious taste vegetable and fairly good caloric value. *Verticillium dahliae* is a soil-borne fungus that causes *Verticillium* wilt of cucumber and many other crops [2-3]. The fungus survives in the soil as small microsclerotia. The fungus form actively growing mycelium and conidial spores that infect root surface cells of many plant species or invade directly through root wounds [4-5]. The pathogen occurs worldwide attacking more than 300 plant species, including fruit trees, vegetables, flowers, and field crops as well as weeds [6].

Several strategies were followed to manage the disease worldwide, including disease resistance breeding and organic amendment application [7]. Agricultural practices such as long rotations 4 to 5 years with non-host crops, avoid moving soil or debris from infested areas to unknown infested one were highly effective in controlling the pathogen [8]. In general using fungicides is not effective and not economical for the control of Verticillium wilt diseases [9]. Biological controls of pathogens have gained importance in sustainable agricultural management strategies. This is due to the fact that biological control exploits natural resources such as mycorrhizosphere and rhizosphere organisms, which can improve and develop antagonistic effects against soil-borne pathogens [10]. The beneficial fungi *Trichoderma harzianum* and *T. viride* were used against Verticillium wilt in strawberry; this mixture was able to reduce the number of infected plants by 60% [11].

Arbuscular mycorrhizas (AM) represent the most widespread and oldest symbiosis with commercial plants [12]. They are known as a key component of the soil microorganisms and they form symbiotic association relationships with the fibrous root plants, enhancing the nutritional uptake status of host plants and protecting them against numerous soil-borne diseases [12-14]. AMF that form symbiotic relationships with the roots of most terrestrial plants are well known to enhance the mineral status of host plants to protect them against
several soil-borne plant pathogens [15-16].

Several reports are available on bio-control influences of endomycorrhizal fungi against Verticillium wilt. In eggplant, it was proposed that G. mosseae can reduce the pathogenic effect of V. dahliae by increasing the uptake of P and N [17]. The effectiveness of AMF against Verticillium wilt in pepper (Capsicum annuum) were varied among different Glomus species [18]. G. mosseae was more effective in plant growth stimulation and more able to protect the plant from wilt disease. Thus, the aim of this research was to assess the effectiveness of G. mosseae and G. fasciculatum to control Verticillium wilt disease of cucumber.

MATERIALS AND METHODS

Soil preparation. Sterilized soil (121°C for 1h) was mixed with sandy soil at 3:1 v/v with pH 6.8. Commercially, sensitive and certified cucumber seeds (C.V. Alpha Beta) were used. The seeds were surface sterilized with ethyl alcohol (70%) for 10 seconds and then washed with sterile distilled water. Three seeds were planted directly in each plastic pot (20x20cm diameter). Two weeks later, the seedlings were thinned to one seedling/pot. Plants were grown for 10 weeks under glasshouse conditions (28°C±5).

AMF spores preparation. G. mosseae and G. fasciculatum spores were taken originally from the Laboratory of Plant Pathology/School of Agriculture/University of Jordan. Inoculum of G. mosseae and G. fasciculatum was consisted of 50g soil (about 10 spores per gram dry soil) from a mother culture in pots planted with chickpea (Cicer arietnum) inoculated with G. mosseae and G. fasciculatum (separately). The soil inoculum from each species of AMF was mixed very well with the soil before plantation [19].

Pathogen isolation. Numerous twigs were taken randomly from Verticillium infected olive trees. The branches were cut into 1-2 cm. The infected branches were soaked in sodium hypochlorite (0.3%) for 1 minute, then rinsed in sterile distilled water for 1 minute and dried on sterilized filter paper before transferring them into a Petri dish containing V. dahliae selective medium [20]. The conidial suspension of V. dahliae was prepared (10^7 CFU/ml) and 5 ml was added directly to the soil surface of each pot 5 weeks after planting cucumber seeds.

Assessment of Disease severity and AMF colonization rate. The first disease symptoms were shown 2 weeks after inoculation. Wilt severity was visually estimated depending on the degree of infected shoots following the scale of Horsfall, 1945 [21]. Cucumber roots were cleared with KOH (10%) in a hot water bath and the roots were stained with trypan blue (0.05%) [22]. Percentage of adventitious and lateral roots colonized by AMF was evaluated microscopically [23]. AMF spores were isolated from the soil by wet sieving and decanting technique [19]. The healthy and mature spores were selected and counted visually under a binocular microscope (Table 3).

Growth parameters and N, P, and K determination. The number of cucumber leaves and flowers were recorded. Fresh and dry weights of shoot and plant roots were determined after drying (75°C for 24h) in the oven. At the end of the experiment cucumber leaves were collected and dried (75°C for 24h). Dried cucumber leaves (0.3g) were digested using concentrated sulfuric acid (H_2SO_4) to determine N concentration by the Kjeldahl method [13]. Cucumber dried leaves (0.3 g) was digested using H_2SO_4 and Chloric acid (HClO_3). The mixture was added to 8ml of molybdate solution, the volume was completed to 1L with distilled water. The blue color formed after 10 minutes was measured at 720 nm using a spectrophotometer to estimate P content. A standard curve was prepared using monobasic potassium phosphate [13]. Ten grams of cucumber leaves were burned in a muffle furnace at 450°C. Two grams of dried leaves were diluted with acid for dehydration and removal of silica. Potassium content was determined using a flame photometer (780nm) [13].

Data analysis. The experiment was arranged in a complete randomized design (CRD) with 6 treatments and 4 replications. The data were subjected to the analysis of variance (ANOVA) using SPSS software [24]. The Tukey Post Hoc Test (P = 0.05) was used for means separation for all parameters except for the N, P and K concentrations, where the means were separated using the least significant differences (LSD) (P ≤ 0.01).

RESULTS

Biomass measurements. The statistical analysis indicated that mycorrhizal fungi increased plant height at the end of the experiment. Plants inoculated with G. fasciculatum were significantly the highest compared to other treatments at the end of the 8th, 9th and 10th week from planting. Plant height of Verticillium treated plants was the shortest at the end of the 10th weeks of plant age and was significantly different compared with other treatments. At the end of this experiment, plant height was found to be in G. mosseae + Verticillium, G. fasciculatum + Verticillium and the control were not different statistically (P = 0.05) (Table 1).
TABLE 1
Plant height (cm/plant) of cucumber inoculated or not with V. dahliae and or G. fasciculatum and G. mosseae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 8</th>
<th>Week 9</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM</td>
<td>90.2 a</td>
<td>117.7 a</td>
<td>130.2 b</td>
</tr>
<tr>
<td>GF</td>
<td>93.5 a</td>
<td>125.5 a</td>
<td>138.5 a</td>
</tr>
<tr>
<td>VD</td>
<td>77.2 b</td>
<td>97.5 b</td>
<td>106.0 d</td>
</tr>
<tr>
<td>GM +VD</td>
<td>80.7 bc</td>
<td>105.2 b</td>
<td>117.5 c</td>
</tr>
<tr>
<td>GF +VD</td>
<td>80.5 bc</td>
<td>103.6 b</td>
<td>122.5 bc</td>
</tr>
<tr>
<td>Control</td>
<td>82.5 bc</td>
<td>106. b</td>
<td>121.0 c</td>
</tr>
</tbody>
</table>

Means followed by the same letter within columns are not significantly different as determined by Tukey Post Hoc Test (P=0.05). Whereas Glomus mosseae (GM), Glomus fasciculatum (GF), Verticillium dahliae (VD) and Control.

TABLE 2
Number of cucumber leaves inoculated or not with V.dahliae and/or G. fasciculatum and G. mosseae at different weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 8</th>
<th>Week 9</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM</td>
<td>22.0 ab</td>
<td>24.5 b</td>
<td>28.0 b</td>
</tr>
<tr>
<td>GF</td>
<td>23.5 a</td>
<td>29.4 a</td>
<td>33.5 a</td>
</tr>
<tr>
<td>VD</td>
<td>12.5 d</td>
<td>16.5 c</td>
<td>17.5 d</td>
</tr>
<tr>
<td>GM +VD</td>
<td>19.5 bc</td>
<td>21.2 b</td>
<td>28.2 b</td>
</tr>
<tr>
<td>GF +VD</td>
<td>20.5 ab</td>
<td>24 b</td>
<td>24 c</td>
</tr>
<tr>
<td>Control</td>
<td>18.0 c</td>
<td>21.5 b</td>
<td>24.5c</td>
</tr>
</tbody>
</table>

Means followed by the same letter within columns are not significantly different as determined by Tukey Post Hoc Test (P=0.05). Whereas Glomus mosseae (GM), Glomus fasciculatum (GF), Verticillium dahliae (VD) and Control.

FIGURE 1
Leaves drop of cucumber inoculated or not with V. dahliae and/or G. fasciculatum and G. mosseae.

Leaves number. G. fasciculatum was able to produce significantly the highest number of leaves compared with the rest treatments (Table 2). After 10 weeks from plantation, the number of cucumber leaves was in G. fasciculatum and G. mosseae significantly increased compared with the control treatment. Verticillium and AMF treatments were found to be different in the last two weeks of plant growth (Table 2). Cucumber plants treated with G. mosseae were not significantly different from G. mosseae + V. dahliae treatment. At the end of the experiment both treated plants with AMF + V. dahliae were statistically different (Table 2). Also both Glomus species treatments were significantly different from each other.

Leaves drop. Verticillium inoculated plants lost a significant number of leaves when compared to the other treated plants. The combined treatment of G. mosseae and V. dahliae was not varied statistically when comparing to the G. mosseae + V. dahliae. The G. fasciculatum + V. dahliae treatment differed from the control treatment significantly (Figure 1). The significance between treatments was recorded at the 10th week of plant growth.
FIGURE 2
Flowers number of cucumber inoculated or not with *V. dahliae* and/or *G. fasciculatum* and *G. mosseae.*
(Means followed by the same letter are not significantly different from each other as determined by Tukey Post Hoc Test (P=0.05). Whereas *Glomus mosseae* (GM), *Glomus fasciculatum* (GF), *Verticillium dahliae* (VD) and Control (CO).

TABLE 3
Disease severity of cucumber inoculated or not with *V. dahliae* and/or *G. fasciculatum* and *G. mosseae*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease severity/ Weeks</th>
<th>Week 8</th>
<th>Week 9</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>VD</td>
<td>6.0 a</td>
<td>6.0 a</td>
<td></td>
<td>6.5 a</td>
</tr>
<tr>
<td>GM + VD</td>
<td>2.7 b</td>
<td>2.7 b</td>
<td></td>
<td>2.5 b</td>
</tr>
<tr>
<td>GF + VD</td>
<td>2.5 b</td>
<td>3.0 b</td>
<td></td>
<td>3.2 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within columns are not significantly different as determined by Tukey Post Hoc Test (P=0.05). Whereas *Glomus mosseae* (GM), *Glomus fasciculatum* (GF), *Verticillium dahliae* (VD) and Control (CO).

TABLE 4
Concentration of N, P and K of cucumber leaves inoculated or not with *V. dahliae* and/or *G. fasciculatum* and *G. mosseae*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrogen (%)</th>
<th>Potassium (%)</th>
<th>Phosphorus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM</td>
<td>2.39 a</td>
<td>1.47 b</td>
<td>0.37 a</td>
</tr>
<tr>
<td>GF</td>
<td>1.69 b</td>
<td>1.79 a</td>
<td>0.37 a</td>
</tr>
<tr>
<td>VD</td>
<td>0.17 d</td>
<td>0.76 c</td>
<td>0.16 c</td>
</tr>
<tr>
<td>GM + VD</td>
<td>1.11 c</td>
<td>1.80 a</td>
<td>0.24 b</td>
</tr>
<tr>
<td>GF + VD</td>
<td>1.39 bc</td>
<td>1.51 b</td>
<td>0.25 b</td>
</tr>
<tr>
<td>Control</td>
<td>1.02 c</td>
<td>1.53 b</td>
<td>0.27 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within columns are not significantly different as determined by the least significant difference test (LSD) (P≤0.01). Whereas *Glomus mosseae* (GM), *Glomus fasciculatum* (GF), *Verticillium dahliae* (VD) and Control.

**Flowers number.** The flowers number of cucumber treated with *G. mosseae* was significantly the maximum compared to all other treatments. The combined treated plants were not different statistically when compared with the control and *G. fasciculatum* cucumber plants alone (Figure 2). The

**N, P and K contents.** Plants inoculated with *G. mosseae* had the highest N contents (P=0.05) when compared to all other treatments (Table 4). The lowest content was significantly detected in *Verticillium* infected plants. However, the treatments *G. mosseae + V. dahliae, G. fasciculatum + V. dahliae* and the control were not significantly different from each other (Table 4). The highest amount of K was detected in *G. mosseae + V. dahliae* combination of *G. mosseae + V. dahliae* and *G. fasciculatum + V. dahliae* did not show the typical symptoms of the Verticillium wilt disease. The symptoms were typical in the *V. dahliae* plants and it was significantly different from the combined treated plants with AMF + the pathogen (Table 3). *V. dahliae* treatment followed by *G. fasciculatum.* The lowest percentage of K was found in *V. dahliae* treatment. Shoots of *G. fasciculatum + Verticillium,* control and *G. mosseae* cucumber plants were not different from each other in their K contents. The highest amount of P was recorded in plants inoculated with both *G. mosseae* and *G. fasciculatum.* The lowest concentration of P was found in plants infected with Verticillium. The treatments *G. mosseae + V. dahliae, G. fasciculatum + V. dahliae*
and control were not statistically different concerning their P content (Table 4).

**AMF colonization rate and spores number.**
The root colonization was 85% in *G. fasciculatum* treatment, followed by *G. mosseae* (70%) and both were significantly different from each other. The combined treatments with *Glomus* and *Verticillium* was not varied statistically (Figure 3). The spores number counted in the *G. fasciculatum* was the highest among the mycorrhizal treatments followed by *G. mosseae*. Significant reduction for the spore number was found in the double treatment (*G. fasciculatum* + *V. dahliae*) (Figure 4).

**Root and shoot biomass.** Both mycorrhizal species were able to increase the root and shoot dry and fresh matter significantly in the single treatment of *G. mosseae* as well as *G. fasciculatum* (Table 5). Plants inoculated with *G. fasciculatum* + *V. dahliae* and *G. mosseae* + *V. dahliae* were not different significantly in root fresh weight and dry weight compared with the control. Control treatment recorded the significant difference in root fresh weight and root dry weight compared with both *G. fasciculatum* and *G. mosseae* dry weights (Table 5). Verticillium infected treatment showed the lowest root and shoot weight (fresh, dry) compared with all other plants. Shoot fresh and dry weights were the highest in *G. fasciculatum* treated plants compared with all other treatments. Shoot fresh weight was higher in *G. fasciculatum* + *V. dahliae* treatment compared with *G. mosseae* + *V. dahliae* and the control, but there were no significant differences between them (P=0.05). On the other hand, the pathogen treatment showed the lowest dry root weight. Combined treatments and control were not varied statistically in shoot dry and fresh weights (Table 5).

**FIGURE 3**
*Percentage of root colonization for tow mycorrhizal fungi combined with or without V. dahliae.*
(Means followed by the same letter are not significantly different as determined by Tukey Post Hoc Test (P=0.05). Whereas *Glomus mosseae* (GM), *Glomus fasciculatum* (GF), *Verticillium dahliae* (VD) and Control (CO).

**FIGURE 4**
*Spores number per 10g dry soil of Glomus mosseae, Glomus fasciculatum with or without V. dahliae inoculation.*
Means followed by the same letter are not significantly different as determined by Tukey Post Hoc Test (P=0.05). Whereas *Glomus mosseae* (GM), *Glomus fasciculatum* (GF), *Verticillium dahliae* (VD) and Control (CO).
The current study results were in agreement with those obtained by others who found that plants inoculated with Glomus spp., became taller than the control and the pathogen treatments [33-34].

Leaves number was increased in G. fasciculatum treatment as a result of plant height increase. The current study results were in agreement with the results reported earlier, they illustrated that G. mosseae can increase the number of tomato leaves significantly in a different percentages [30].

The present study demonstrated that disease severity of Verticillium wilt was reduced as a result of using mycorrhizal fungi during the experiment. G. mosseae + V. dahliae, G. fasciculatum + V. dahliae and control treatments had the lowest disease severity in the 8th, 9th and 10th weeks and the highest disease severity was recorded in V. dahliae infected plants. The possible reason behind the reduction of disease severity in the combined inoculated plants because AMF caused an increase in the phenolic, flavonoids and iso flavonoids production in the host plant. The present results were similar to the results reported by other investigators [35], where they found that G. fistulosum prevailed over the Phytophthora fragariae in wild strawberries, also tomato and wild strawberry colonized by endomycorrhiza were offset the negative influence of P. fragariae and P. incotinae respectively. In a previous study, they found that AMF can significantly suppressed the Verticillium wilt in strawberry in highly susceptible strawberry cultivar ‘Elsanta’ and moderately in ‘Senga Senga’ and Somaclone K40 [36]. The results found in this research concurred with earlier results where they found that the mycorrhizal fungi and T. harzianum were effectively reduced the disease severity of cucumber by 61.4 %, 56.1 % and 66.7 %, respectively [37].

Leaves drop of V. dahliae infected plants was increased significantly as results of xylem infection. The root hair formation was decreased because the infection with V. dahliae spread in the root system. The opposite happened with plants inoculated with mycorrhizal fungi and pathogen. The results documented in this research were in agreement with others, they reported that inoculation with G. mosseae, G. manihotis and Gigaspora gigantea increased the number of leaves and flowers in tested trees [38]. In the current study the flowers number in both combined treatments were not different significantly compared to the control plants, but it was significantly more in the G. mosseae inoculated plants. These results matches with other studies, where different species of mycorrhizal fungi can increase plant height, early flowering and increased flower number of Crossandra infundibuliformis at
different levels [39].

Mycorrhizal plants had significantly higher N, P and K concentrations compared to non-mycorrhizal plants. The presence of mycorrhizal colonization resulted in a high concentration of P contents in leaves. The current findings were in agreement with others who concluded that the beneficial effectiveness of the AMF reduced the pathogenic effect of V. dahliae and the P and N uptake were higher in mycorrhizal treatments than in the control [17, 47]. Root necrosis caused by the pathogen reduced the ability of plant roots to absorb more N, P and K nutrients from the soil. Plants treated with both mycorrhizal fungi and the pathogen was intermediated in phosphorous contents because the mycorrhizal fungi protect cucumber plant from wilt disease. The ability of increased nutrient uptake by the AMF association may allow host root to be more vigorous and more tolerant to pathogen damage [40]. The research conducted by Orak, and Demir was matched with the present results; they found that P content in mycorrhizal plant leaves was increased compared with the untreated plants [41]. Also, they reported that disease severity was related to the nutrient concentration. Other researchers suggested that the highest growth rate of onion was observed for N and P treated plants implying that N and P fertilizer has a more positive effect on growth of onion plants than AMF [42]. Also it was found that mycorrhizal symbiosis and sporulation were affected by plant phenology, physiology and different environmental conditions [18, 43]. The current research results approved that the pathogen was the main factor for the reduction of spore production in the combined treatments (G. mosseae + V. dahliae and G. fasciculatum + V. dahliae), this may be related to the production of some substrates in the mycorrhizosphere from the infected roots which can inhibit and suppress spore germination and hyphal production. These results were not in agreement with results reported earlier where they found that the Ralstonia solanacearum was inhibited by spore germination of G. mosseae due to the volatiles produced by spore germination [44-45].

The root colonization percentage was significantly different between the treatments. In Verticillium treatment, the pathogen was able to reduce the ability of AMF to colonize the roots compared with the non-pathogenic treatments. The current results were similar to other studies where they found that root colonization of Ash gourd (Benincasa hispida) by AMF as well as mycorrhizal spore density in soil were also higher in AMF inoculated treatment than non-mycorrhizal. Also they documented that inoculation with Fusarium oxysporum significantly reduced the root colonization and spore density of mycorrhizal plants [46, 47].

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NOTICE

EFFECT OF THERMAL PROPERTY OF POWDER ON THE GAS EXPLOSION SUPPRESSION BASED ON RESPONSE SURFACE METHOD

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ABSTRACT

Gas explosion is the leading accident in underground coal mining in China. Using the self-improved 20 L spherical experimental system, the impacts of Al(OH)₃, Mg(OH)₂ and Urea on the suppression of mine gas explosion were investigated. The thermal characteristics of Al(OH)₃, Mg(OH)₂ and Urea powders were studied. Suppression mechanisms of gas explosion were analyzed and compared. The results showed the performance of Urea was better than that of the other two materials because of Urea's faster decomposition rate. When 0.25 g/L Urea powder was added, the explosion limits were reduced by about 50%. Urea plays a key part in the whole explosion processes, and it can effectively suppress the forward reaction between gas and oxygen. Besides, atoms as N, P are capable of participating in chain reaction and reacting with active groups, significantly suppressing the gas explosion.

KEYWORDS:
Gas explosion, Explosion suppression, powder, Al(OH)₃, Mg(OH)₂

INTRODUCTION

The gas explosion is the serious accident in coalmine, which has the strong destructiveness and the burstiness, often causes the personnel casualty and the property damage. In order to realize the safety of low concentration gas transport, the materials and equipments may be designed to prevent the explosion and its dissemination in the conduit. At present, many scientific researches about gas explosion are done. Nie Baisheng carried out the experiments on the obstruction explosion by the foamed ceramics, and the damping of maximum overpressure achieves 5% [1,2]. Lin Ying has Studies the feasibility of suppression with the super water mist. The speed of the explosion's flame has reduced in the mist area, and its propagation time has lengthened. The phenomenon of flame lie has appeared [3]. Wen Hu has investigated the suppressing gas explosion by Al(OH)₃ with a best density scope [4]. Luo Zhenmin concludes that the nanometer level powder used to suppress explosion in the 20L pot has better effect than the micron level [5]. At present the powder is one of auxiliary techniques. The gas explosion and its destructive power are limited in the smallest degree. The harms brought by the detonation is reduced. The experiment research is used as the main method of the gas explosion suppression by powder. The feasibility of explosion suppression by powder has been verified by the study on maximum pressure, rate of pressure rising and the other parameters. The essential characteristics of the powder suppression aren’t discussed. The thermal analysis method is used in this article. The different analysis of gas explosion suppressions are based on the thermal characteristics of powders. There are the theoretic and the practical significance in protecting the gas’s transmission and enhancing the security.

In China, 95% coal mines are underground ones, for which gas explosion is always the greatest threat because of the complicated geological conditions and some other objective factors. Recently, gas explosion happened frequently and brought about great casualty and economic loss. Inert gas, water mist, powder, porous materials and aerosol are common gas explosion suppressants. Qiu et al. and He [7,8] had studied the influences of CO₂ and N₂ on the gas explosion limits and critical oxygen concentration under different conditions. According to Di Benedettoa, Di Sarlia, Salzana, Cammarotaa, and Russob [9] the maximum pressure and its rising rate increase with decreasing CO₂ content or increasing O₂ concentration. Xing and Fan [10] had conducted explosion proof experiments with different kind of powder at various concentrations. They found that larger concentration and smaller particle size got better effect. In the gas explosion proof experiment with NH₄H₂PO₄, Cai and Zhang [11] concluded that smaller particle size got better ex-
explosion proof effect and a smaller powder concentration could lead to a good suppression effect. Chen et al. and Krasnyansky [12, 13] used powder and aerosol of NH₄H₂PO₄, CaCO₃, SiO₂ and Al(OH)₃ as explosion inhibitor to carry out explosion proof experiments with large, medium and small-scale experimental tubes and spherical equipment. They concluded the influence of various powder inhibitors on explosion limits, pressure, pressure rising rate, pressure waves propagation velocity, flame propagation velocity and some other parameters of the gas, the mixture of gas and coal dust explosion.

In those studies, there are few experiments about compound inhibitors of solid. In this paper, methane, the dominant component of mine gases, was taken as experimental medium. After comparing explosion suppression effects, Al(OH)₃, Mg(OH)₂ and Urea was chosen as suppressant and their independent and combined effect on gas explosion characteristics were studied using 25 L spherical gas explosion equipment.

MATERIALS AND METHODS

According to the characteristic of explosion suppressing process, this system was autonomous refitted based on the national standard dust explosion characteristics testing device. It consists of explosion reactor, gas mixing system, ignition system, powder injection system and measurement system. The STA449C thermal synchronization analyzer is used in the experiment. The initial temperature of samples is 20 °C, and it ends at 1000 °C. The rate of temperature rising is 10°C/min. N₂ nitrogen is used as the experiment ambience, and the current capacity is 20 mL/min. The experiment operates under the atmospheric pressure.

RESULTS AND DISCUSSIONS

Fig. 1 and 2 show weight loss rate of explosion. The aluminum hydroxide is an amphoteric compound. It is nonvolatile, non-toxic and chemical stability. The aluminum hydroxide as the flame-resistant material has a widespread application. During explosion testing time which is 500 ms, maximum explosion pressure and rise rate of it reduced under effect of aluminum hydroxide powder. Besides, induction period and pressure peak time expand.

The thermal transmission is reduced and slowed down because of the heat absorption which is generated by taking off the structure water of Al(OH)₃. The combustion of gas and the oxygen may be diluted by evaporation. And Al₂O₃ is the inert oxide with non-toxic, non-corrosiveness, and will not initiate secondary pollution. So that it can effectively retard the inflammation.

Mg(OH)₂ is the favourite burning inhibitor with non-toxic, smokeless and the well thermo stability. Mg(OH)₂ powder is studied by the thermal analysis experiments under the same condition. The experiment results are shown in Fig.3 and 4. After adding Mg(OH)₂ powder and 11% methane, the maximum pressure rising rate of methane became smaller. At the same time the time to reach the peak of explosion pressure was prolonged. The suppression mechanism of Mg(OH)₂ mainly manifests the heat absorption during its decomposition. MgO and H₂O produced by the thermal decomposition of Mg(OH)₂ are both inert. And MgO can adhere to the combustible substance surface to isolate the oxygen. And the explosion suppression can be reinforced with the dilution effect on the density of flammable gas by the steam.
FIGURE 2
Surface Plot of Weight loss rate (%) vs D, B

FIGURE 3
Surface Plot of Weight loss rate (%) vs C, B

FIGURE 4
Surface Plot of Weight loss rate (%) vs D, A
The urea is the colorless and tasteless organic compound that is composed of the carbon, the nitrogen, the oxygen and the hydrogen. It displays either the white acicular or the clavate crystal. Its Chemical formula is CO(NH$_2$)$_2$. The thermal experiment results are shown in Fig.5 and 6. The multiple pyrolysis reactions existed in process are the mechanism of the explosion suppression by urea. The greater heat can be absorbed and the greater heat transfer can be reduced. At the same time, a large amount of NH$_3$ and NCO$^-$ emerge during its thermal decomposition, which can respectively react with the active radicals H$^-$ and OH$^-$. The free radicals produced by the gas explosion may be greatly decreased by this way. Then the chain reactions are terminated to suppress the gas explosion and its dissemination.

**CONCLUSIONS**

Using the self-improved 20 L spherical experimental system, the impacts of Al(OH)$_3$, Mg(OH)$_2$, and Urea on the suppression of mine gas explosion were investigated. The thermal characteristics of Al(OH)$_3$, Mg(OH)$_2$, and Urea powders were studied. Suppression mechanisms of gas explosion were analyzed and compared. The results showed the performance of Urea was better than that of the other two materials because of Urea's faster decomposition rate. When 0.25 g/L Urea powder was added, the explosion limits were reduced by about 50%. Urea plays a key part in the whole explosion processes, and it can effectively suppress the forward reaction between gas and oxygen. The activity
of free radicals generated by the pyrolysis of Al(OH)₃ and Mg(OH)₂ is so low that it cannot trigger the free radical reactions. However there are large amounts of NH₄⁺ and NCO⁻ produced during the urea pyrolysis. OH⁻ and H⁺ can be reacted respectively with these ions. The end of the chain reactions are caused by the reducing of explosion free radicals. Besides, atoms as N, P are capable of participating in chain reaction and reacting with active groups, significantly suppressing the gas explosion.

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