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DETERMINING THE ENVIRONMENTAL RISK PERCEPTIONS OF SECONDARY EDUCATION STUDENTS

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ABSTRACT

The aim of this study was to investigate the effects of socio-demographic factors and approach towards environment on perceptions regarding environmental risk. The participants of the study are 682 high school students from seven geographical regions of Turkey. The data were collected through the Environmental Risk Perception scale, which consisted of four domains and NEP scale (NEP), which were used to determine students' approach towards environment. Demographic data were analyzed through descriptive statistics in terms of percentage and frequency. Demographic data which are the independent variables were analyzed through MANOVA to identify whether or not they influence the participants' risk perceptions. The demographic characteristics of the participants were obtained through a separate questionnaire. According to the results of the MANOVA test female students considered environmental risks significantly more important in all subscales in comparison to male students; and students from the Black Sea region had the highest average score in all subscales of the Environmental Risk Perception scale. Results of the study supported that the geographical region and gender were among the variables of risk perception. Differences between geographical regions should be investigated in terms of sustainable development. In addition, the arrangement of environmental education strategies in accordance with the environmental education needs of each geographical region may be useful for experts. Significant positive relationships were found between the NEP scale score and score for the ecological risks domain ($r=0.354$, $p<0.01$), the chemical risks domain ($r=0.304$, $p<0.01$), the resource depletion risk domain ($r=0.230$, $p<0.01$), and the global risks domain ($r=0.231$, $p<0.01$).

KEYWORDS:

Environmental education, Environmental risk perception, Secondary school students, New ecological paradigm

INTRODUCTION

While industrialism that has been our dominant world view for a long time has been regarded with growth and increased productivity, its environmental cost has only been considered as a secondary side effect [1]. In the 1960s, scientists began to uncover changes in nature and the resulting consequences [2]. Rachel Carson's 'Silent Spring' that is widely accepted to have a significant role in triggering environmentalist social movements especially in the West and that changes perspective on environmental issues was published during these years [3, 4, 5]. The public interest in environmental issues created by this and other similar publications led to the prohibition of DDT (dichlorodiphenyltrichloroethane) in the US and to the beginning of a new era [4]. Since then, environmental awareness has been integrated into the basic paradigms of many and varied social disciplines [6]. This change also led to the occurrence of formal environmental education [2]. At the same time, this effort is the response to the increasing environmentalist approach and to the global environmental crisis that is finally being widely perceived [7]. In evaluating environmental and technological threats that started within the 1970s, during which research on risk perception was a part of the same trend, there are studies in which differences among the views of the public and experts are observed [8]. The history of research on environmental perception within the Western literature has reached half a century [9, 10].

Researches on environmental risk perception are based on two main approaches. One of these is known as the psychometric paradigm that focuses on revealing the cognitive structure of risk perception and attempts to determine the domains that may be common for all communities [10]

Especially the study carried out by Fischhoff, Slovic, Lichtenstein, Read, and Combs [11] provides essential information on how lay people and experts evaluate risks and how the general public perceives technological risks. However, such studies that adopted the psychometric paradigm were criticized since they had not taken into consideration the study participants' socio-demographic characteristics such as age, gender, educational background, income and occupation [12, 13]. Another movement in researching risk perception is related to studies explaining the differences between sociological groups in risk evaluation and these are gathered under the "cultural theory of risk perception" [13]. Risks caused by environmental problems being manageable require obligatory social participation [14]. Social participation is possible through the public perceiving and understanding the risks because their reaction to risks or dangers shapes compatibly with their awareness, perception, and identification [15, 16, 17, 18]. Those with the necessary awareness can play a role in supporting or opposing environmental policies [19]. Similarly, sensitivity towards environmental issues is also reported by several studies to be one of the significant indicators of positive environmental behavior [20, 21, 22]. Therefore, knowing the environmental risk perception as an indicator of environmental sensitivity and how it is shaped is going to help researchers to convey a positive attitude towards the environment as the main purpose of environmental education.

Environmental concern has been associated commonly with socio-demographic variables such as age, gender, income, education, residential statement, or geographical region [2, 23, 24, 25, 26]. There isn't any consensus that these socio-demographic variables have positive or negative effects on environmental opinion, concern, or support of environment protection policies. Ingelhart [27] suggested that wealthy and advanced industrialized nations are more concerned about the environment. His post-materialist theory points out that high income and education level provide value shift from materialist to post-materialist. There are supporting research results for this explanation [26, 28, 29]. The socio-demographic variables in correlation with environmental concern reported most frequently in the literature have included middle to upper class status, ethnicity, and higher level of education, younger people and urbanites [30]. Arcury and Christianson [31] have indicated that living in urban or rural area have no influence on being more knowledgeable about environment, having more pro-environmental attitudes and acting more environment friendly, but education and

income have influence on these factors. In the social class context, researchers have conducted a study of Korean third-grade elementary school students and determined that girls and students whose parents have higher educational background displayed higher environmental literacy [32]. The results of this study show that cognitive and affective components about environmental issues can be constructed through intra-familial interaction even early in life. In this context according to Taskin [33], when environmental awareness is addressed as part of post-materialist values, it is expected for well-educated parents to have a positive effect on their children in terms of having an environmentalist attitude. However, it should be investigated whether education and income status, which influence environmentalism, have the same influence on environmental risk evaluation. As a matter of fact, Taskin [33] found a difference in favor of higher classes in regard to awareness of environmental issues, but determined that there was no socio-economic difference in terms of the recognition of ecological paradigm as an indicator of positive attitude towards environment. Thus, environmental issues should be examined through studies on different socio-economic classes where environmental matters are investigated concretely rather than based on general expressions. In this way, the importance of environmental issues for classes as well as the real difference between classes may be determined.

Although men tend to have more positive attitudes toward science [34], women have been found to be more knowledgeable in some scientific areas, such as the environment [35], and differences between men and women regarding environmental concern are evident [36]. Women are more concerned about the environment and have a more pro-environmental world view [24]. In addition to the high environmental concern of women, women tend to evaluate risk (environmental or other risks) as being more problematic than men do [8].

Guagnano and Markee [23] stated that lack of agreed measurement tools and employment of geographically limited small samples were the restrictive aspects of the studies addressing environmental concerns from a socio-economic perspective, and emphasized that attitudes, values, and beliefs of people were affected by the history and culture of different geographical areas they lived in. Thus, the present study was based on the idea that people living in different geographical areas of Turkey would be affected by the historical and traditional structure of the region as well as its economic condition in the process of evaluating environmental issues. As a matter of fact, when

Turkey is addressed from a geographical perspective, it is observed to contain a great diversity on many subjects.

The western part of Turkey, particularly the Marmara region, is heavily industrialized, and is commercially and culturally active. It is described as the closest region to Europe. In contrast, the Black Sea, Central Anatolia, East Anatolia, and Southeast Anatolia are economically dependent on agriculture and have a weak industrial infrastructure [37]. The Mediterranean and Aegean regions are both industrialized and agricultural; these parts of Turkey are also popular among tourists.

Özaslan, Dinçer, and Özgür [38] have analyzed the social (demographic, employment, education, health, infrastructure, other welfare) and economic (manufacturing, construction, agriculture, financial) components of Turkey's provinces. The rank of the social and economic development of Turkey's regions is as follows: Marmara, Aegean, Central Anatolia, Mediterranean, Black Sea, Southeast Anatolia and East Anatolia. Tikka, Kuitunen, and Tynys [39] have stated that people coming from the most densely crowded regions seem to be the most worried about the environment.

In addition to demographic variables, views on nature-human relationship are also effective in forming environmental risk perception. Environmental problems are socio-scientific issues. Therefore, people's evaluation of risk-related environments is based not only on knowledge but on their world view as well. Research suggests that personal evaluations of environmental problems and their approach towards nature are closely related. The theory of value-belief-norm states that general beliefs about environmental sensitivity to humans' intervention in nature function as a filter to evaluate the correctness or the reasonability of the new information about the environment [40]. The NEP, which has often been used in such studies and whose consistency has been proved, is a worldview that consists of general beliefs that the intervention of people has hazardous effects on nature, the natural resources are limited, and human beings are part of nature [14, 42, 43].

The paradigm, which is based on the assumption of "the interdependency of human beings and ecosystems" and carries interaction between the environment and general public to a different dimension, completely rejects the human-centered perspective that is based on arguments that development and growth are unlimited, the environment will cover the ever increasing demand, and environmental hazards can be prevented through technologic development

The aim of this study is to investigate the effects of socio-demographic factors on perceptions regarding environmental risk. To achieve this aim, the cognitive structure of environmental risk perception was determined.

This study focuses on the following research questions:

What is the level of environmental risk perception of secondary school students?

Is there any difference between female and male students' perceptions of environmental risk?

What is the effect of socioeconomic factors (family income, mothers' and fathers' education) on students' perceptions of environmental risk?

What is the effect of the geographical region on students' perceptions of environmental risk?

Is there any relationship between students' environmental risk perception and their attitudes towards environment?

METHOD

Study group: In order to provide a fair representation of the subsamples in the population the stratified sampling technique was employed in the study. Different geographical regions were used for the homogeneity of the participants. Therefore, the population was stratified along with seven geographical regions. The study group consists of 682 students who are 10th, 11th, or 12th graders who reside in 16 randomly selected cities across the country. The demographic characteristics of the sample are showed in Table 1.

TABLE 1
Demographic Characteristics of the Sample

| | Categories | (%) |
|-----------------------------------|------------------|------|
| Gender | Female | 56 |
| | Male | 44 |
| Geographical region | Marmara | 26.0 |
| | Central Anatolia | 17.2 |
| | Mediterranean | 15.2 |
| | Aegean | 11.7 |
| | Southeast | 10.8 |
| | Black Sea | 9.9 |
| | East Anatolia | 9.2 |
| Educational background of mothers | Primary school | 40 |
| | Secondary school | 14 |
| | High school | 26 |
| | University | 20 |
| Educational background of fathers | Primary school | 21 |
| | Secondary school | 14 |
| | High school | 28 |
| | University | 37 |
| Monthly income | Below 230\$ | 6 |
| | 230 \$ – 360 \$ | 16 |
| | 361 \$ – 500 \$ | 21 |
| | 501 \$ – 640 \$ | 16 |
| | 641 \$ – 770 \$ | 10 |
| | 771\$ – 910 \$ | 12 |
| | Above 910 \$ | 19 |

Research instruments: The data of the study were collected through the use of two different tools. In order to identify the students' environmental risk perception level, the Environmental Risk Perception Scale was used. The scale was developed by Slimak and Dietz [40] to determine risk perceptions related to environmental issues through 24 environment related questions and it was adapted into Turkish by [44]. 23 Likert-type items are under the four sub-domains of 'chemical risks', 'source-related risks', 'ecological risks' and 'global environmental risks'. The Cronbach's α internal consistency coefficient of the domains varies between 0.82 and 0.69 [44].

The Environmental Risk Perception Scale includes a list of 23 environmental problems accompanied by short explanations (Table 2). Participants were asked 'How important are the human activities or environmental changes listed

below to you?' To rank each risk item, a Likert-style rating (1–7) was used with 1 meaning 'not important' and 7 meaning 'very important'.

The approach of the participants towards the environment was analyzed through the use of the NEP Scale, which was first developed by Dunlap and Van Liere [41] and included 12 items was later revised by Dunlap et al. [42] in 2000 as the NEP Scale that includes 15 Likert-type items. It was adapted into Turkish by authors and three language specialists. In the end, in accordance with the consensus of the authors and language specialists, the scale items were finalized and it was used in a pilot study with 320 secondary school students. Its Cronbach's α was found to be 0.71. In addition, the demographic characteristics of the participants were analyzed through a separate questionnaire.

TABLE 2
Items of Environmental Risk Perception Scale

| 1=not important | 4= mildly important | 7=very important |
|---|---------------------|------------------|
| <i>Risk Items</i> | | |
| 1. <i>Acid rain</i> caused by the deposition of acid-producing sulfur dioxide into streams and on forests, usually from the burning of coal | | |
| 2. Global warming caused by excessive amounts of <i>greenhouse gases</i> like carbon dioxide and methane that may lead to weather extremes, such as temperature increases, flooding, sea level rise, extreme storms | | |
| 3. The ozone hole caused by ozone-depleting substances like refrigerants that reduces the protective <i>ozone layer</i> and leads to an increase in ultraviolet radiation from the sun. Also known as stratospheric ozone depletion | | |
| 4. Drilling for <i>oil extraction</i> from sea platforms and on lands and the transportation of oil and petroleum products (e.g., pipelines, tank trucks, and supertankers) that may result in spills | | |
| 5. <i>Hazardous waste areas</i> which may release toxic chemicals into streams and estuaries and landscape | | |
| 6. <i>Radiation</i> : Release of radioactive materials associated with nuclear power generation | | |
| 7. <i>Persistent and toxic organic pollutants</i> (e.g., PCB's, DDT, dioxin, benzene) that are discharged into surface streams or into the air from chemical manufacturing plants. These chemicals are long-lived in the environment and can be transported great distances | | |
| 8. <i>Heavy metals</i> like lead, zinc, and cadmium released into surface waters from mining operations and mercury released from the burning of coal | | |
| 9. <i>Pesticides</i> : Insecticides used to treat insect pests; herbicides used to treat weeds; and rodenticides used to kill animal pests | | |
| 10. <i>Eutrophication</i> : the over enrichment of waters due to nitrogen fertilizer runoff and nitrogen oxide deposition in watersheds. This may lead to algal blooms and depletion of dissolved oxygen in waters | | |
| 11. <i>Sewage</i> : Untreated household waste water discharged into streams. | | |
| 12. The growing of genetically modified agricultural products (e.g., corn); also known as genetically modified organisms or <i>GMAPs</i> | | |
| 13. <i>Invasive species</i> : Non-native species which were transported via human and threat to the existence of native species. | | |
| 14. <i>Clear-cut logging of large tracts of forests</i> for pulp, paper, and wood products. | | |
| 15. Destruction and <i>fragmentation of natural areas</i> (habitats) due to urbanization | | |
| 16. The <i>damming of rivers</i> for electric power generation, flood prevention | | |
| 17. Destruction and <i>loss of wetlands</i> by residential, commercial, industrial, agricultural, or recreational development | | |
| 18. <i>Pollution of inland water</i> (also known as nonpoint pollution) contaminated with agricultural chemicals and sediment | | |
| 19. <i>Open mining</i> | | |
| 20. <i>Overgrazing</i> of range and pasture lands due to excessive numbers of livestock for a specific area (<i>extreme pasturage</i>) | | |
| 21. <i>Sport fishing</i> (e.g., fishing for bass, trout, catfish, deep sea and coastal fish) and <i>sport hunting</i> (e.g., hunting for deer, squirrels, waterfowl, and other wildlife) | | |
| 22. <i>Commercial fishing</i> (e.g., fishing for tuna, lobsters, or crabs for human consumption) | | |
| 23. Worldwide human <i>population growth</i> | | |

Procedure and data analysis: Based on the research support protocol of the body granted in 2009, the data collection tools were duplicated by the Ministry of National Education's Research and Development Directory (EARGED) and were sent to secondary schools of randomly selected cities within the seven geographical regions and were collected.

Demographic data were analyzed through descriptive statistics in terms of percentage and frequency. MANOVA employed the measures of environmental risk perception as dependent variables, the categorical demographic data as a fixed-factor independent variable. According to

Result of Box's M test assumption of homogeneity of variance-covariance matrices are violated. Therefore, Pillai's criterion was used instead of Wilk's lambda to evaluate multivariate significance, as Tabachnick and Fidell [45] suggested.

In evaluating the explained variance, eta square value (η^2) was used. Classification of the η^2 values resulted in the following categories: $\eta^2 = 0.01$ "small effect", $\eta^2 = 0.06$ "medium effect" and $\eta^2 = 0.14$ or higher "large effect" [46].

In addition, by calculating the Pearson correlation coefficient between the NEP scale values used to determine the students' approach

towards the environment and the scores from the subdomains of the environment risk perception scale, the magnitude and direction of the relation was identified

RESULTS

In Table 3, the mean scores and standard deviations based on the 7-Likert style rating and

evaluation of the environmental risk perception scale of 23 environment related questions answered by the participants were listed from greater to smaller and the standard deviation values were provided.

The mean values of the risk items in the environmental risk scale were found to vary between 4.57 and 6.18 (Table 3).

TABLE 3
Mean Scores and Standard deviations on the Environmental Risk Scale

| Risk Items | M | SD | Risk Items | M | SD |
|---------------------------------------|------|------|------------------------|------|------|
| 2. greenhouse effect | 6.18 | 1.39 | 13. invasive species | 5.60 | 1.54 |
| 6. radiation | 6.16 | 1.38 | 23. population growth | 5.57 | 1.63 |
| 5. hazardous waste areas | 6.11 | 1.39 | 12. GMAP* | 5.49 | 1.64 |
| 7. persistent toxic organic compounds | 6.10 | 1.37 | 16. dam constructions | 5.26 | 1.76 |
| 11. sewage | 6.06 | 1.42 | 4. oil extraction | 5.24 | 1.63 |
| 1. acid rains | 6.01 | 1.36 | 10. eutrophication | 5.23 | 1.64 |
| 15. damage to natural areas | 5.97 | 1.35 | 9. pesticides | 5.18 | 1.53 |
| 3. ozone layer | 5.95 | 1.43 | 21. sportive hunting | 5.09 | 1.81 |
| 14. cutting in forests | 5.92 | 1.47 | 20. extreme pasturage | 4.71 | 1.74 |
| 17. wetland loss | 5.74 | 1.48 | 19. open mining | 4.57 | 1.82 |
| 18. pollution of inland water | 5.66 | 1.53 | 22. commercial fishing | 4.57 | 1.96 |
| 8. heavy metals | 5.63 | 1.55 | Total | 5.57 | 1.04 |

*GMAP: Genetically modified agricultural products

The factors these items are collected under are called 'chemical risks' (Cronbach's $\alpha=0.89$), 'risks about consumption of natural sources' (Cronbach's $\alpha=0.82$), 'ecological risks' (Cronbach's $\alpha=0.82$) and 'global environmental risks' (Cronbach's $\alpha=0.78$). The mean values from the Environmental Risk Perception Scale and their standard deviations are given in Table 4 along with the demographic variables.

Table 4 shows that the participants regard global environmental problems as the most significant concern with a mean of 6.05 and that the risks about source loss as the least significant problem (M = 4.96). Regarding gender, female

participants are found to have higher mean scores in all domains of the scale associated with environmental risks than male participants. As for geographical regions, those from the Black Sea region are found to have the highest mean scores in all domains of the scale, whereas those from Central Anatolia have the lowest mean scores in all domains of the scale. The educational background of the parents and the family income are found to have no significant difference among the student's environmental risk perception scale's sub-domain means

TABLE 4
Mean Values from the Environmental Risk Perception Scale and their Standard Deviations Along with Demographical Variables

| Demographical characteristics | | Factors | | | | | | | |
|-----------------------------------|------------------|----------------|------|----------------------|------|------------------|------|--------------|------|
| | | Chemical risks | | Source-related risks | | Ecological risks | | Global risks | |
| | | M | SD | M | SD | M | SD | M | SD |
| Gender | Female | 6.14 | 1.03 | 5.10 | 1.27 | 5.93 | 1.02 | 6.21 | 1.00 |
| | Male | 5.72 | 1.25 | 4.79 | 1.34 | 5.51 | 1.24 | 5.84 | 1.31 |
| Geographical region | Black Sea | 6.29 | 0.88 | 5.40 | 1.08 | 6.05 | 0.81 | 6.26 | 0.92 |
| | Mediterranean | 6.17 | 1.00 | 5.27 | 1.23 | 6.04 | 0.94 | 6.28 | 1.07 |
| | East Anatolia | 6.06 | 0.96 | 4.88 | 1.37 | 5.59 | 1.24 | 6.07 | 1.21 |
| | Aegean | 6.03 | 0.90 | 5.14 | 1.24 | 5.97 | 0.91 | 6.07 | 1.04 |
| | South-eastern | 5.91 | 0.99 | 4.75 | 1.30 | 5.60 | 1.24 | 6.08 | 1.10 |
| | Marmara | 5.86 | 1.15 | 4.88 | 1.32 | 5.63 | 1.13 | 5.91 | 1.25 |
| | Central Anatolia | 5.62 | 1.59 | 4.63 | 1.36 | 5.50 | 1.39 | 5.88 | 1.28 |
| Educational background of mothers | Primary school | 5.95 | 1.24 | 4.94 | 1.33 | 5.72 | 1.27 | 6.08 | 1.19 |
| | Secondary school | 6.03 | 1.04 | 5.17 | 1.19 | 5.93 | 0.96 | 6.03 | 1.26 |
| | High school | 5.83 | 1.18 | 4.82 | 1.30 | 5.61 | 1.16 | 5.93 | 1.18 |
| | University | 6.07 | 0.96 | 5.03 | 1.33 | 5.85 | 0.93 | 6.13 | 0.97 |
| Educational background of fathers | Primary school | 5.92 | 1.30 | 4.99 | 1.27 | 5.79 | 1.16 | 6.16 | 1.18 |
| | Secondary school | 5.93 | 1.10 | 5.18 | 1.28 | 5.67 | 1.32 | 5.88 | 1.31 |
| | High school | 5.94 | 1.21 | 4.97 | 1.30 | 5.74 | 1.14 | 5.95 | 1.20 |
| | University | 5.99 | 1.03 | 4.86 | 1.34 | 5.75 | 1.07 | 6.12 | 1.05 |
| Income | Below 230\$ | 5.87 | 1.33 | 4.78 | 1.21 | 5.65 | 1.21 | 5.94 | 5.94 |
| | 230 \$ – 360 \$ | 6.02 | 1.17 | 5.08 | 1.31 | 5.75 | 1.19 | 6.10 | 6.10 |
| | 361 \$ – 500 \$ | 5.94 | 1.16 | 5.06 | 1.26 | 5.76 | 1.20 | 6.11 | 6.11 |
| | 501 \$ – 640 \$ | 5.90 | 1.21 | 4.93 | 1.24 | 5.85 | 1.12 | 6.08 | 6.08 |
| | 641 \$ – 770 \$ | 5.96 | 1.15 | 4.95 | 1.31 | 5.66 | 1.30 | 5.89 | 5.89 |
| | 771 \$ – 910 \$ | 6.11 | 0.94 | 5.00 | 1.41 | 5.82 | 1.00 | 6.16 | 6.16 |
| | Above 910 \$ | 5.87 | 1.15 | 4.83 | 1.36 | 5.64 | 1.06 | 5.96 | 5.96 |
| | Total | 5.95 | 1.15 | 4.96 | 1.31 | 5.74 | 1.14 | 6.05 | 1.16 |

In order to see the effects of demographic characteristics on the participants' risk perceptions on the four subdomains, MANOVA was used. The results of MANOVA showed that geographical regions have significant effects on the residents' perceptions of risk. Pillai's criterion= 0.065, $F(24, 2336) = 1.619$, $p < 0.05$; $\eta^2 = 0.016$. Gender is also found to have significant effects on risk perception. Pillai's criterion= 0.018, $F(4, 581) = 2.607$, $p < 0.05$; $\eta^2 = 0.018$. However, other demographic features including income and educational background of parents are found to have no

statistically significant effect on the risk perceptions of the participants.

According to Cohen's [46] classification of η^2 values, multiple variances in dependent variables accounts for a small portion of the geographical region-based effects and gender-based effects and they have small effect sizes. Table 5 shows the results of the follow test (ANOVA) related to the difference between the sub-domains of the Environmental Risk Perception Scale.

TABLE 5
ANOVA Test Results on the Environmental Risk Perception Scale

| Source | Dependent variables | <i>df</i> | MS | <i>F</i> | <i>p</i> | Partial η^2 |
|----------------------------|--------------------------------|-----------|---------------|--------------|---------------|------------------|
| Geographical region | Chemical risks | 6 | 2.712 | 2.104 | 0.051 | 0.000 |
| | Risks about source loss | 6 | 4.589 | 2.749 | 0.012* | 0.027* |
| | Ecological risks | 6 | 2.446 | 1.940 | 0.072 | 0.000 |
| | Global risks | 6 | 2.207 | 1.709 | 0.117 | 0.000 |
| Gender | Chemical risks | 1 | 5.863 | 4.549 | 0.033* | 0.008* |
| | Risks about source loss | 1 | 6.909 | 4.139 | 0.042* | 0.007* |
| | Ecological risks | 1 | 6.982 | 5.539 | 0.019* | 0.009* |
| | Global risks | 1 | 11.966 | 9.263 | 0.002* | 0.016* |

*Analysis was performed at 0.05 significance level

The ANOVA results show that geographical regions led to a significant effect only in the domain of risks about source exhaustion and its η^2 value states that it can account for only 2.7% of the variance, indicating that it has a small-size effect. Gender, on the other hand, led to significant effects in all domains and its η^2 value indicates that it accounts for 1.6% of the variance for the domain of global environmental risks, and that it has exploratory power of lower than 1% for the remaining domains.

To identify the relation between the students' environmental risk perception and their tendency towards accepting the new environmental paradigm, the Pearson correlation coefficients between the mean scores in the NEP Scales and the mean scores in the Environmental Risk Perception Scale were calculated. A positive significance of 0.01 was identified between the scores of the NEP the following subdomains: Ecological Risks ($r=0.354$; $p<0.01$), Chemical Risks ($r=0.304$; $p<0.01$), Source Exhaustion Risks ($r=0.230$; $p<0.01$), and Global Risks ($r=0.231$; $p<0.01$).

DISCUSSION

In this study, the relationship among various socio-demographic factors and the students' environmental risk perception levels and risk perception were examined. The mean score of the participants in the Environmental Risk Perception Scale is found to be 5.57, which is higher than the average value. Therefore, it can be suggested that the students perceive environmental issues at a higher level and that their awareness of such issues is high as well. The environmental topics regarded

as significant by the participants are respectively the greenhouse effect, radiation, hazardous waste areas, persistent toxic organic compounds, sewage, acid rains, damage to natural areas, damage to the ozone layer, cutting of trees in forests and wetland loss. Similar listings have also been gathered from various studies [10, 40, 44, 47, 48, 49, 50]. Within this study, the environmental issues that are at the top of the list such as radiation and chemical waste are of those with dreading consequences. However, environmental issues that are not observed immediately and directly but are possibly hazardous such as damage to natural areas, forests, and wetland areas are also considered as of high risk by the students. These environmental issues are also among the risky environmental issues studied by experts. Lazo, Kinnell, and Fisher [51] and McDaniels, Axelrod, and Slovic [18] also identified that habitats being damaged, wetland areas being ruined, and the extinction of species are considered as important environmental issues. Thus, in the light of results it can be argued that students do not have only a human-centered approach towards environmental issues.

Considering the mean scores of the domains of the environmental risk scale indicate that ranking of the domains were shaped by their popularity. Global risks such as global warming or ozone depletion are the themes which are most debated by media and naturally their consequences are influential in global scale. Chemical risks are the secondly most important risk domain evaluated risk domain within the importance list. These risks have low probability of occurrence but high consequences; therefore, the public is more concerned about these risks.

As for demographic characteristics, although the geographical region and gender have small

sized effects, they have significant effects on the participants' perceptions about environmental risks. However, others demographic factors including monthly income and the educational background of parents are found to have no significant effects on the participants' perceptions of environmental risks. The students from the Black Sea and Mediterranean regions are found to have higher levels of risk perception than those from the Marmara and Central Anatolia regions. However, when the ANOVA results are considered in regards of the geographical regions, a significant difference is found only in the domain of source exhaustion-related risks. This may be suggested as a result of the economic status differences among the Black Sea, Marmara, and Mediterranean regions. Due to the topography change in the Black Sea Region caused by the recent launch of a hydropower plant the extension of the mining activities, the habitat that has become dependent on the natural sources offered by the region over a long period of time can be considered to be at a higher level of risk perception when compared to other geographical regions. Therefore, these developments may result in increased levels of environmental concerns in the region. In addition, given that the regional economy is partly based on seafood, the existence of a dilemma between earning a living and the source exhaustion is known [52]. Teksoz, Tekkaya and Erbaş [53] stated similar concerns for the Aegean, Mediterranean and Black Sea regions. However, they also found higher levels of environmental sensitivity in the Marmara region when compared to other geographical regions. They have attributed this difference to this region being developed in terms of commerce, industry, tourism, and transportation as well as its proximity to Europe. We, however, believe that the socio-economic disparities have a different influence on environmental risk perception in contrast to its influence on awareness. The influence of socio-economic status on environmental awareness can be different than its influence on environmental risk perception. While a high socio-economic status can lead to high environmental awareness [53], our view is that those with a much lower socio-economic status have a much higher risk perception because the perceived vulnerability, which is one of the indicators of risk perception, can be inversely correlated with the socio-economic status. The socio-economic disparities among the geographical regions support the region being studied as a variable.

Lima, Barnett, and Vala [54] studied the data from 25 countries and concluded that people from developed countries with advanced technological infrastructure have lower levels of environmental

sensitivity than those from less developed countries with underdeveloped technological infrastructure. They have also stressed that the society requires more time to get used to the technological developments. Also, in the current study, the students from the Marmara region, which has a developed industrial infrastructure, are found to have lower levels of sensitivity towards environmental risks and this might be because of adapting to the present dangers brought with the technological and industrial development and for this reason it is likely that they are less sensitive.

Kılınç, Boyes, and Stainisstreet [55] have indicated that although the students from Central Anatolia Region have awareness of the benefits of nuclear energy generation, the students from the Black Sea and Mediterranean Regions have negative evaluation about nuclear power plant. The vulnerability perception of regions differ each other. The Sinop province of the Black Sea Region and the Mersin province of the Mediterranean Region being chosen for nuclear plant generation has started a new argument related to where they are going to be generated. In addition to differences in environmental issues, people of the East Anatolia and Southeast Anatolia Regions face terrorist incidents more intensively than the other parts of Turkey. This event can influence the risk perception of people from these two regions. Despite the source of risk, facing high risk can cause high level of risk perception.

According to the findings of the research, a significant difference was identified in favor of the female students under the gender subdomain of the environmental risk perception scale. However, it should be stated that influence degree of the variability depending on gender is quite low in this study. Similar results were identified in other studies [24, 50, 56, 57]. In an extensive research of Davidson and Freudenburg [58] focusing on gender and environmental risk perception, it was concluded that women have higher levels of perceptions regarding health-related risks, security risks and thus environmental risks. However, in studies focusing on concerns of environmental issues, while women display a higher level of concern if the issues are related to health threats such as nuclear energy, radioactive waste, hazardous chemicals, there is not a significant difference between genders against general environmental issues [59]. In parallel with these results, Prokop and Kubiato [60] stated that as women make more investment for reproduction in comparison to men, in regard to an evolutionary perspective suggesting that they are more sensitive towards issues associated with disease such as environmental pollution, in explaining gender

related differences in environmental concerns, the disease-threat model was effective. However, the present study determined that women are not only sensitive towards chemical risks that contain health risk, but they are also more sensitive towards risks concerning the globe, ecology, and source exhaustion when compared to men. It can be assumed that due to their fertility, against any kind of risk, women are more sensitive in comparison to men and therefore they have a higher risk perception.

The fact that family income and the educational statuses of parents do not have any influence on environmental risk perception in Turkey proves that our education system is not successful in creating environmental awareness. Income and educational status do not have any effect on environmental risk evaluation in the Turkish community; vulnerability to environmental issues is observed to be a more dominant factor. As a matter of fact, the Black Sea Region, which is a region that can be affected economically by environmental issues the most, being the region with the highest environmental risk perception supports this fact. Similarly, Abramson and Inglehart [61] associate environmental concerns in the developing countries with the conditions experienced, that is, the status of being affected or not being affected by environmental issues rather than environmentalism. This perspective is supported by the findings of the present study.

With this study, a weak but positive correlation between the sub-domains of the Environmental Risk Perception Scale and the NEP Scale were identified. For this reason, it can be suggested that having an environmentalist world view or not is a stronger determinant in environmental risk perception. These findings are also supported by those of Teksoz, Tekkaya and Erbaş [53], who found that the variance between the levels of responsibility, awareness, and concern towards the environment and resources according to the geographical region is of only 0.9%. Similarly, Slimak and Dietz [40] concluded that the NEP is a much stronger indicator of environmental risk perceptions than demographical characteristics. It is further determined that the NEP is mostly related to the domain of ecological risks and then to the domain of chemical risks. Its relation to the domains of source exhaustion and global environmental risks is found to be lower. Also, in other studies related to environmental risk perception, a correlation between the NEP and levels of environmental risk perceptions was found [10, 40, 48, 62]. Furthermore, Stern, et. al. [63] argued that adopting the NEP is related to environmental awareness, or in other words to risk

perception, and that awareness has effects on assuming responsibility in environmental issues.

CONCLUSIONS

Results of the study supported that the geographical region and gender were among the variables of risk perception. The fact that regions which have more at stake in terms of environmental opportunities had higher risk perception levels in comparison to others points to the socio-economic aspect of the difference resulting from geographical regions. In this regard, differences between geographical regions should be investigated in terms of sustainable development. This data can be beneficial in explaining differences of risk perception. In addition, the arrangement of environmental education strategies in accordance with the environmental education needs of each geographical region may be useful for experts. Although the present study is limited for an evidence-based discussion on gender differences, gender socialization may explain high risk perception level within the context of the Turkish social structure. Future studies may test gender differences at the levels of environmental risk and concern by using hypotheses accounting for the difference. In addition to having identified a significant difference in terms of geographical region and gender, when its relation with environmental risk perception is taken into consideration, the acceptance level of the NEP is a more significant variable when compared to demographic variables.

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ANTIGENOTOXIC AND THE ANTIOXIDANT CAPACITY OF TOTAL EXTRACT OF TWO LICHENS

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ABSTRACT

In this study, we aimed to determine the antigenotoxic and antioxidant capacity of total extract of two lichens against to Aflatoxin B₁ (AFB₁). For this aim, we obtained the total extracts from *Letharia vulpina* (LTE) and *Vulpicida pinastris* (VTE) lichens species. AFB₁ induced oxidative stress and genotoxicity in human lymphocytes in vitro. Our results showed that 5 μM concentration of AFB₁ increased the frequency micronucleus (MN) and malondialdehyde (MDA) levels, decreased the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) level. On the other hand, cotreatment of 5, 10 and 20 μg/ml concentrations of lichen extracts with AFB₁ decreased the frequency of MN and MDA level and activities of SOD, GPx and GSH level increased. The results of the experiments have clearly shown that LTE and VTE have strong antioxidative and antigenotoxic effect in human lymphocytes in vitro.

KEYWORDS:

Letharia vulpina, *Vulpicida pinastris*, Antigenotoxicity, Antioxidant

INTRODUCTION

Lichens are symbiotic organisms in which fungi and algae or cyanobacteria form an intimate biological union [1]. Lichens live from arctic zone to equator. They produce very important secondary metabolites including usnic acid, cetraric acid, vulpinic acid, gallic acid, and quercetin. These secondary metabolites are used in different area from medicine to cosmetic industry [2-4].

Especially in the folk medicine of different countries of the World, lichens metabolites are used. Lichen secondary metabolites have lots of biological activities, such as antimicrobial [5-7], antimutagenic [8-13], antiviral [14], anti-inflammatory [15-18], antioxidant [8-13, 19-20]

and anticancer [21]. Turkez et al (2010) found that methanol, acetone, and n-hexane extracts obtained from *Pseudovernia furfuracea* have anti-genotoxic effect and antioxidant capacity in human lymphocytes in vitro [22].

So far approximately 800 lichen secondary metabolites are identified. The most common lichen compounds are aromatic polyketides, in particular depsides, depsidones, diphenyl ethers and dibenzofurans, thus making lichens a rich source of phenolic compounds [23].

Especially these metabolites are not found in higher plants. Lichens secondary metabolites existing in the thallus over 1–5% of the dry weight, but sometimes comprising up to 20% from the fungal symbiont [24].

In the folk medicine, *Letharia vulpina* (L.) Hue is used for relieving digestive problems as a highly diluted infusion. Withal, *L. vulpina* and *V. pinastris* were also used to hunt foxes and wolves with a mixture of meat, lichens and broken glass because of their toxic effects [25-26]. In many places, these types of lichen used for dye as raw material. In addition to that, *L. vulpina* is used to treat cutaneous eruption and eczema in North Europe and other some European countries, *V. pinastris* is used for poison, likewise *L. vulpina* [27].

L. vulpina has atranorin, norstictic acid, vulpinic acid, arabitol, erythritol, galactoside, glucose, lichenin, mannitol, sucrose; citric acid, glycolic acid, malic acid; alanine, aspartic acid, glycine, serine, tyrosine and valine. In addition that it contains highly toxic lichen acids, likewise (-) usnic acid, pinastric acid and vulpinic. However *V. pinastris* has richly fatty acids, depsides and depsidones [7, 28]. So far no report has shown antigenotoxic and antioxidative effects of these lichens.

In this study, we investigated the antigenotoxic and antioxidative effect of total extracts of *L. vulpina* and *V. pinastris* against AFB₁ in the human blood culture by using MN test and biochemical analysis including superoxide dismutase (SOD) and glutathione peroxidase (GPx)

activities, glutathione (GSH) and malondialdehyde (MDA) level.

MATERIALS AND METHODS

Plant material. Lichen samples (*Letharia vulpine* (L.) Hue, *Vulpicida pinastri* (Scop.) J.-E.Mattsson&M.J.Lai) were collected from Erzurum, Artvin and Giresun provinces between June-August 2010, Turkey. Air-dried samples were observed and studied with a Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i light microscope with standard identification methods for lichens. The samples were identified using various flora books and papers [29-32]. Vouchers are stored in the herbarium of Kazım Karabekir Education Faculty (ATA-KKEF-1857, ATA-KKEF-1863), Ataturk University, Erzurum, Turkey.

Preparation of total extracts. Air-dried and powdered lichens (20 g) were extracted with 400 mL of n-Hexane, Diethylether, Acetone and Methanol respectively using the Soxhlet extractor (Isopad, Heidelberg, Germany) for 24h at a temperature not exceeding the boiling point of the solvents [33]. The extracts were filtered using Whatman filter paper (no. 1). Then concentrated in vacuum at 40 °C using a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland). After then, all extracts collected in a cap and yielding a waxy material. The extracts were lyophilized and kept in the dark at +4 °C until tested.

Microscopic Evaluation. Peripheral heparinized human blood lymphocytes were taken from four (age: 24-28) nonsmoking healthy individuals. Lymphocyte cultures were set up by adding 0.5 mL of heparinized whole blood to RPMI-1640 chromosome medium supplemented with 15% heat-inactivated fetal calf serum, 100 IU/mL streptomycin, 100 IU/mL penicillin, and 1% L-glutamine. Lymphocytes were stimulated to divide by 1% phytohemagglutinin. AFB₁ (in concentration of 5 µM), *L. vulpina* total extract (LTE) and *V. pinastri* total extract (VTE) (in concentrations of 5, 10 and 20 µg/ml) were added to the cultures just before incubation. The experiments were performed on control group, AFB₁ treated group (5 µM), LTE (10 µg/ml), VTE (10 µg/ml), and AFB₁ with different concentrations of LTE (5, 10 and 20 µg/ml), and VTE (5, 10 and 20 µg/ml).

For MN analysis, cytochalasin B was added 44h after PHA stimulation to a final concentration of 6 µg/ml. Twenty-eight hours later (after 72h of culture) the cells were harvested by centrifugation (1200g x 10min). The supernatant was removed,

the cells were mixed thoroughly and 6 ml of cold hypotonic solution (0.05M KCl) was added. The cells were subsequently incubated at 37 °C for 7 min and centrifuged again (1200gx10min). The pellet was mixed thoroughly and 5 ml fresh fixative (1:3 acetic acid:methanol) was added dropwise. This fixation procedure was repeated three times and the tube was centrifuged again. The cell pellet was then resuspended in 1 ml of fresh fixative, dropped on to a clean microscope slide, incubated at 37 °C or at room temperature overnight, and stained with Giemsa dye. Coded slides were scored blind by two independent individuals. Only binucleated cells were scored for MN analysis. For each subject, at least 1000 binucleated cells were analyzed for the presence of MN. For the MN scoring, the micronucleus criteria described by Countryman and Heddle were used: a diameter less than 1/3 of the main nucleus, non-refractility, not touching, and with the same color as the nucleus or lighter [34].

Biochemical Analysis. The cell homogenates were prepared at a 1:10 (w:v) dilution in 10 mM potassium phosphate buffer, pH 7.4. Samples were centrifuged at 3000 rpm for 10 min at 4°C, and the supernatants were collected and immediately assayed for enzyme activities. Cu, Zn-SOD, GSH and GPx activity and MDA levels in the cell culture supernatant was measured by the method of Paglia and Valentina Sun et al., and Ohkawa [35-37], respectively [11,38-39]. All samples were measured in sixfold.

SOD assay. Cu, Zn-SOD activity in the cell culture supernatant was detected by the method of Sun et al. [37]. 2.45 mL of assay reagent [0.3mM xanthine, 0.6mM Na₂EDTA, 0.15mM nitroblue tetrazolium (NBT), 0.4 M Na₂CO₃, 1 g/L bovine serum albumin] was combined with 100 µL of the sample. Xanthine oxidase (50 µL, 167 U/L) was added to initiate the reaction, and the reduction of NBT by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system, was determined by measuring the absorbance at 560nm. Cu, Zn-SOD activity was expressed in units of SOD per mg protein, where 1 U is defined as that amount of enzyme causing half-maximal inhibition of NBT reduction.

GSH assay. GSH levels in the cell culture supernatant were assessed according to the method of Tietze and Anderson [38-39]. Briefly, 100 µL of sample was placed to a 3 mL cuvette and then 750 µL of 10mM 5-5'-dithio-bis-2-nitrobenzoic acid (DTNB) solution (100 mM KH₂PO₄ plus 5 mM Na₂EDTA, pH 7.5 and GSH-RD, 625 U/l) was

added and incubated for 3 min at room temperature. Then, 150 μL of 1.47mM β -NADPH was added, mixed rapidly by inversion, and the rate of 5-thio-2-nitrobenzoic acid formation (proportional to the sum of reduced and oxidized GSH) was measured spectrophotometrically for 2 min at 412 nm. The reference cuvette contained equal concentrations of DTNB and NADPH but no sample, and values were presented as μmol per gram protein.

GPx assay. GPx activity in the cell culture supernatant was measured by the method of Paglia and Valentine [35]. Briefly, 50 μL of sample was combined with 100 μL of 8mM NADPH, 100 μL of 150 mM reduced GSH, 20 μL of glutathione reductase (30 units/mL), 20 μL of 0.12 M sodium azide solution, and 2.65 mL of 50 mM potassium phosphate buffer (pH 7.0, 5 mM EDTA) and the tubes incubated for 30 min at 37 °C. The reaction was initiated with the addition of 100 μL of 2 mM H_2O_2 solution, mixed rapidly by inversion, and the conversion of NADPH to NADP was measured spectrophotometrically for 5 min at 340 nm. The enzyme activity was expressed as units per g protein using an extinction coefficient for NADPH at 340 nm of 6.22×10^{-6} .

MDA assay. MDA levels in the cell culture supernatant were determined spectrophotometrically according to the method described by Ohkawa et al. [36]. A mixture of 8.1% sodium dodecyl sulphate, 20% acetic acid, 0.9% thiobarbituric acid was added to 0.2 mL of sample, and distilled water was added to the mixture to bring the total volume up to 4 mL. This mixture was incubated at 95 °C for 1h. After incubation, the tubes were left to cool under cold water and 1 mL distilled water with 5 mL n-butanol/pyridine (15:1, v/v) was added, followed by mixing up. The samples were centrifuged at $4000 \times g$ for 10 min. The supernatants were removed, and absorbances were measured with respect to a blank at 532 nm.

1,1,3,3-Tetraethoxypropane was used as the standard. Lipid peroxide levels were expressed as $\mu\text{mol/l}$ MDA. Protein concentrations in the cell culture supernatant were determined by Bradford method [40]. All photometrical measurements were performed with a DU 530 spectrophotometer (Beckman Instruments, Fullerton, California, USA) in a quartz cuvette.

Statistical analysis. The statistical analysis of MN frequencies was performed by use of the χ^2 test. For statistical analysis of biochemical parameters Mann–Whitney U-test was used. A p value of ≤ 0.05 was regarded as indicative of statistical significance for all tests used. Results were expressed as mean \pm SD. For these procedures, SPSS 16.0 version for Windows (SPSS Inc, Chicago, Illinois, USA) was used.

RESULTS AND DISCUSSIONS

AFB₁ increased MN frequencies in peripheral lymphocytes as seen Table 1,2. This increase is found to be statistically significant ($p < 0.05$). When peripheral lymphocytes treated with lichen total extracts, the MN frequencies were reduced. Biochemical results showed that AFB₁ significantly increased MDA level and caused a significant reduction on SOD, GPx activities and GSH level. ($p < 0.05$). Cotreatment of AFB₁ with lichen total extracts reduced the level MDA and increased the activities of SOD, GPx and GSH level. Statistical analysis showed that differences of SOD, GSH and GPx activities and MDA level are important.

TABLE 1
The effects of AFB₁ (5 $\mu\text{g/ml}$), LTE (10 $\mu\text{g/ml}$) and different concentrations of LTE (5, 10 and 20 $\mu\text{g/ml}$) with together AFB₁ on the number of MN in human peripheral lymphocytes.

| | Counted of Cell | Number of MN | MN/Cell |
|--|-----------------|--------------|-------------------------------|
| Control | 1012 | 16 | 4.74 \pm 0.19 ^a |
| AFB ₁ 5 μM | 1011 | 26 | 7.22 \pm 0.52 ^{ef} |
| LTE (10 $\mu\text{g/ml}$) | 1013 | 18 | 4.83 \pm 0.19 ^{ab} |
| AFB ₁ (5 μM)+LTE (5 $\mu\text{g/ml}$) | 1015 | 22 | 6.40 \pm 0.19 ^{de} |
| AFB ₁ (5 μM)+LTE (10 $\mu\text{g/ml}$) | 1014 | 19 | 5.42 \pm 0.19 ^{cd} |
| AFB ₁ (5 μM)+LTE (20 $\mu\text{g/ml}$) | 1014 | 17 | 4.83 \pm 0.19 ^{bc} |

TABLE 2
The effects of AFB₁ (5 µg/ml), VTE (10 µg/ml) and different concentrations of VTE (5, 10 and 20 µg/ml) with together AFB₁ on the number of MN in human peripheral lymphocytes.

| | Counted of Cell | Number of MN | MN/Cell |
|--|-----------------|--------------|-------------------------|
| Control | 1009 | 14 | 4.16±0.12 ^a |
| AFB ₁ 5 µM | 1010 | 23 | 6.43±0.42 ^{ef} |
| VTE (10 µg/ml) | 1011 | 16 | 4.35±0.20 ^{ab} |
| AFB ₁ (5µM)+ VTE (5 µg/ml) | 1013 | 21 | 5.82±0.20 ^{de} |
| AFB ₁ (5µM)+ VTE (10 µg/ml) | 1014 | 17 | 4.93±0.20 ^{cd} |
| AFB ₁ (5µM)+ VTE (20 µg/ml) | 1014 | 15 | 4.33±0.20 ^{bc} |

Our result showed that AFB₁ increased the MN frequency and MDA level, decreased the activity of SOD, GPx and GSH level. AFB₁ is a highly toxic substance. AFB₁ mechanisms are not known clearly. In previous studies found that AFB₁ could stimulate the release of free radicals, including reactive oxygen species (ROS), which leads to chromosomal aberrations [41-42]. Furthermore, previous studies showed that AFB₁ is mutagenic in different model systems and produces MN, sister chromatid exchange (SCE), unscheduled DNA synthesis, and chromosomal strand breaks and forms adducts in rodent and human cells [11-12,43-44].

In this study, the protective role of two lichen total extracts against AFB₁ was assessed. In our present results, total extracts of lichens have been shown to possess anti-mutagenic/anti-oxidant effect. In previous lichen studies, extracts (especially methanol) of some lichens have been shown to possess anti-mutagenic effects with comet assay, tunnel assay, SCE and MN in human lymphocytes and *Salmonella typhimurium* (TA100

and TA98; TA1535 and TA1537) assay [6,8-9,45-47].

Biochemical analysis of this study show that AFB₁ increased MDA level decreased the activity of SOD, GPx and GSH level. However observed effect of AFB₁ at higher concentration decreased after treatment with different concentrations of LTE and VTE. This is the first report on the antigenotoxic and antioxidant capacity of LTE and VTE. Many scientist researchers were studied methanol or other solvent but very few researchers studied total extracts.

Oxidative damage and lipid peroxidation have been considered to be main mechanisms in the toxicity of AFB₁ although the mechanism of cellular damage caused by AFB₁ has not been fully elucidated [48-49,55]. Odabasoglu *et al.*, [18] determined that gastroprotective and antioxidant effects of usnic acid. Researchers [10-11] were reported that different lichen species, which are including (-)usnic acid, atranorin, cetraric acid and different lichen major components, showed antigenotoxic and antioxidant properities against AFB₁.

TABLE 3
Effects of AFB₁ and different concentrations of LTE on SOD, GPx activities and GSH, MDA levels. ^ap<0,05 compared with control group, ^bp<0,05 compared with AFB₁ (5µM) group, ^cp<0,05 compared with LTE, ^{de}p<0,05 compared with LTE different concentrations.

| | SOD (U/mL) | GPx (U/mL) | GSH (µmol/L) | MDA (nmol/mL) |
|----------------------------------|--------------------------|----------------------------|---------------------------|---------------------------|
| Control | 1.09±0.04 | 1.15±2.03 | 3.84±1.66 | 0.73±0.92 |
| AFB ₁ (5mM) | 0.57±0.22 ^a | 0.73±0.92 ^a | 1.28±0.55 ^a | 1.15±2.30 ^a |
| LTE (10 µg/ml) | 0.88±0.03 ^{ab} | 0.97±2.06 ^{ab} | 3.20±0.55 ^{ab} | 0.70±2.60 ^b |
| AFB ₁ +LTE (5 µg/ml) | 0.94±0.22 ^{abc} | 0.86±2.30 ^{abc} | 2.24±0.54 ^{abc} | 0.98±0.70 ^{abc} |
| AFB ₁ +LTE (10 µg/ml) | 0.97±0.29 ^{abc} | 1.02±2.38 ^{abcd} | 3.20±0.56 ^{abd} | 0.85±0.35 ^{abcd} |
| AFB ₁ +LTE (20 µg/ml) | 1.04±0.15 ^{bcd} | 1.10±1.18 ^{abcde} | 3.80±0.54 ^{bcde} | 0.79±0.34 ^{bc} |

TABLE 4

Effects of AFB₁ and different concentrations of VTE on SOD, GPx activities and GSH, MDA levels. ^ap<0,05 compared with control group, ^bp<0,05 compared with AFB₁ (5µM) group, ^cp<0,05 compared with LTE, ^dp<0,05 compared with VTE different concentrations.

| | SOD (U/mL) | GPx (U/mL) | GSH (µmol/L) | MDA (nmol/mL) |
|----------------------------------|--------------------------|----------------------------|--------------------------|--------------------------|
| Control | 1.20±0.12 | 1.28±0.01 | 2.84±0.05 | 2.13±1.84 |
| AFB ₁ (5µM) | 0.62±0.22 ^a | 0.43±1.50 ^a | 0.83±0.14 ^a | 2.99±3.69 ^a |
| VTE (10 µg/ml) | 0.92±0.24 ^b | 1.08±0.01 ^{ab} | 2.84±0.05 ^b | 2.13±1.84 ^b |
| AFB ₁ +VTE (5 µg/ml) | 1.01±0.21 ^b | 0.92±1.50 ^{abc} | 2.62±0.05 ^{abc} | 2.56±1.85 ^{ac} |
| AFB ₁ +VTE (10 µg/ml) | 1.12±0.18 ^{bc} | 1.37±0.01 ^{abcd} | 2.72±0.19 ^b | 2.21±3.20 ^{bc} |
| AFB ₁ +VTE (20 µg/ml) | 1.26±0.05 ^{bcd} | 1.48±0.01 ^{abcde} | 2.84±0.11 ^{bd} | 2.02±0.10 ^{bcd} |

Antimutagenic effects might be due to antioxidant system. AFB₁ might be reduced by antioxidant enzymes activities [1]. When the levels of antioxidants are declined, oxidative stress develops. In order to understand this situation, we measured the level of MDA and antioxidant enzymes such as SOD, GSH and GPx. AFB₁ decreased antioxidant capacity and increased genetic damage. At the end of this study results indicate that antigenotoxic effect of LTE and VTE could be related to its antioxidant potential or blocked AFB₁ efficient mechanism.

Previous studies, the antigenotoxic effect and antioxidant capacity of lichen extract have been linked to usnic acid, vulpinic acid, pinastric acid, cetraic acid, atranorin, depsides, depsidons and phenolic compounds, which did not react covalently with AFB₁ [10-11, 49-50].

L. vulpina and *V. pinastris*'s major components are atranorin, norstictic acid, vulpinic acid, (-)usnic acid, pinastric acid, richly fatty acids, depsides and depsidones. Lauterwein et al., [50] showed that vulpinic acid, (-)usnic acid have antimicrobial, antimutagenic, antioxidant and insecticidal activities. Furthermore, vulpinic acid isolated from *V. pinastris* has same activity like usnic acid [51-52]. In addition to, vulpinic acid has insecticide activities and showed effect on the some plant pathogens [53-55]. *Usnea longissima* and their inhibitor activities on the Epstein-Barr virus were shown by Yamamoto. Halici et al., reported that the water extract of *U. longissima* had a protective effect in indomethacin induced ulcers, and this protective effect was linked to the antioxidant effect of the lichen extract [17].

Therefore, our study results indicate that the antigenotoxic effect of LTE and VTE could be related to its antioxidant potential. The fractionation and isolation the crude extract of lichen samples containing the active components responsible for antigenotoxic and antioxidant activities are being conducted to test this hypothesis.

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MODELING ULTRASOUND-ASSISTED DECOLORIZATION EFFICIENCY OF REACTIVE BLUE 19 AND REACTIVE YELLOW 145 FROM AQUEOUS SOLUTIONS BY COTTONSEED CAKE

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ABSTRACT

Cottonseed cake as a low-cost and abundant by-product in Turkey was utilized as an adsorbent for the decolorization of Reactive Blue 19 (RB19) and Reactive Yellow 145 (RY145) from aqueous solution based on adsorption and ultrasound-assisted adsorption. This study modeled effects of the following six factors on decolorization efficiency: the two process types, six initial pH values, three adsorbent concentrations, four temperatures, eleven reaction times, and five and eight initial dye concentrations of RY145 and RB19, respectively. The maximum decolorization efficiency was obtained consistently with ultrasound-assisted adsorption at the lowest initial dye concentration (300 mg/L of RB19 and 200 mg/L of RY145), the highest cottonseed cake concentration (1.5 g/L), and the highest temperature (55°C). The best-fit multiple non-linear regression models were cross-validated accounting for 90.8% to 97.2% of variation in decolorization efficiency. Maximum adsorption capacities of cottonseed cake were estimated at 330.7 mg/g and 231.5 mg/g for RY145 and RB19, respectively. The adsorption kinetics of RB19 and RY145 on cottonseed cake was of endothermic and spontaneous nature.

KEYWORDS:

Color removal, empirical modeling, isotherms, kinetics, low-cost adsorbent

INTRODUCTION

Dyes as colored compounds with complex structures have been commonly used especially in textile industry [1]. Colored wastewaters, particularly, associated with reactive azo dyes used for dyeing cellulose fibers are a consequence of batch processes in both the dye-manufacturing and -consuming industries [2]. The discharge of such wastewaters into the recipient streams adversely affects not only their water quality but also their

aesthetic nature [3]. The intensive uses of azo dyes and their reaction products such as aromatic amines have been shown to be of highly carcinogenic nature [4, 5]. For these reasons, the removal of dyes before their disposal as wastewaters is of great significance in terms of ecological and public health. Physical and/or chemical methods such as chemical coagulation, ozonation, and electrochemical and biological treatments have been conventionally used to achieve decolorization of wastewater. However, these conventional decolorization processes may not be economically feasible due to excessive chemical usage, associated toxic sludge, and low biodegradability of dyes [6-8]. Adsorption of pollutants from aqueous solution plays an important role in wastewater treatment since it eliminates the need for huge sludge-handling processes [9]. Although activated carbon is the most commonly used adsorbent for the treatment of dye-related wastewaters, this process has been proved to be very costly [10].

The potential uses of different low-cost adsorbents have been explored for the removal process of various dyes from aqueous solutions. These adsorbents include sepiolite [11], cross-linked chitosan beads [12], vermiculite [13], sawdust [8], activated clay [14], rice husk [2], dolomitic sorbents [15], bagasse fly ash [2,16], perlite [17], apple pomace and wheat straw [18], algae [10,19], and powdered peanut hull [20]. The applications of advanced oxidation processes (AOPs), especially, ultrasound to adsorption processes have recently received much attention as an environmentally benign remediation [21-32]. The objective of the present study was to model decolorization efficiency of adsorption and ultrasound-assisted adsorption onto cottonseed cake of Reactive Yellow (RY145) and Reactive Blue 19 (RB19) as a function of process types, initial pH, initial dyestuff concentrations, adsorbent concentrations, temperature, and reaction time.

MATERIALS AND METHODS

Adsorbent. Cottonseed cake was used as the adsorbent and was supplied from a local textile factory in Adana, Turkey. After cottonseed cake was washed with deionized water to remove the soluble impurities, it was dried at 80°C for 24 h. Finally, cottonseed cake was sieved to different sizes using American Society for Testing and Materials (ASTM) standard sieves after being grinded using a ball mill (Mertest LB 220). In all the experiments, 50-100 mesh size cottonseed cakes were used without any other treatment and stored in a CaCl₂ desiccator during the experiment.

Reactive dyestuff solution. The commercial azo dyes with the Color Index generic name of

RY145 (C₂₈H₂₀ClN₉O₁₆S₅Na₄; molecular weight = 1026.26 g/mol; λ_{max} = 420) and RB19 (molecular weight = 626.54 g/mol; λ_{max} = 592) were obtained from a local textile firm in Çorlu (Turkey) and used without further purification. The stock solutions of the reactive dyestuff were prepared in a constant concentration of 1.0 g/L and then diluted to appropriate concentrations. Working solutions of the desired concentrations were obtained using successive dilutions. The initial pH of each solution was adjusted to the required value with concentrated and diluted H₂SO₄ and NaOH solutions before mixing with cottonseed cake.

Experimental procedure. Batch sorption experiments were carried out following the experimental set-up shown in Fig. 1.

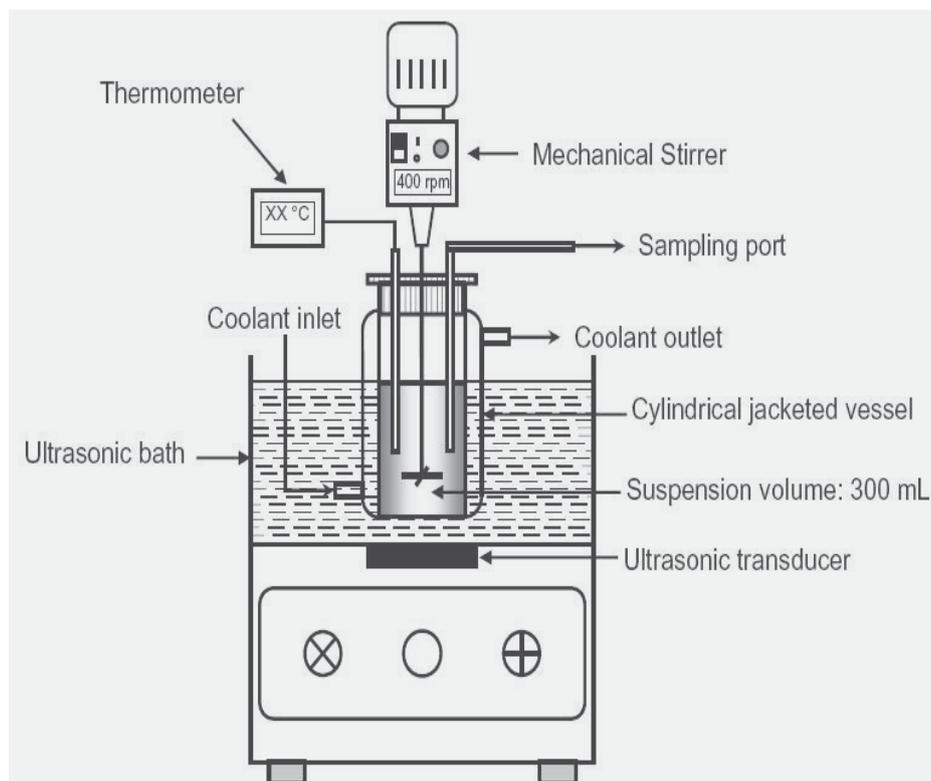


FIGURE 1
A schematic view of the experimental set-up used in the present study.

The experiments were effectuated in a 1000-ml cylindrical jacketed vessel put on an overhead mechanical stirrer (IKA RW 20, Kutay Group, Turkey) which stirred the mixture at an agitation speed of 400 rpm. The agitator was used to have well-mixed suspension characteristics for solid particles. The vessel was set in a digital ultrasonic

cleaner (WiseClean WUC D10H, Wisd Laboratory, Germany) operating at a frequency of 40 kHz and an ultrasonic power of 344 W. Pre-determined concentration of cottonseed cake was contacted with reactive dyestuff solutions of 300 mL at different temperatures for the decolorization of aqueous dye solutions. The experiments were

repeated for the two process types of adsorption and ultrasound-assisted adsorption, the six initial pH values of 1, 1.5, 2, 2.5, 3 and 4, the eight initial dye concentrations of 200, 225, 250, 275, 300, 325, 350 and 375 mg/L for RY145 and five initial dyestuff concentrations of 300, 350, 400, 450 and 500 mg/L for RB19, the three cottonseed cake concentrations of 0.5, 1.0 and 1.5 g/L, the four temperatures of 25, 35, 45 and 55°C, and the 11 reaction times of 0, 5, 10, 15, 20, 30, 45, 60, 75, 90 and 120 min.

Dye analysis. The solutions were analyzed at predetermined time intervals for the final concentrations of RY145 and RB19 using a UV/vis spectrophotometer (SHIMADZU UV-2100, Biomerieux, France) for the maximum absorbance value at wavelengths of 420 nm and 592 nm, respectively. Dye concentration was calculated from a calibration curve. Decolorization efficiency (DE, %) at any time was estimated as follows [1, 23, 32-34]:

$$DE = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

where C_0 and C_t are the initial and measured concentration values of the samples at a specified interval during a four-hour reaction, respectively. Dyestuff concentrations (C_{RY145} and C_{RB19} , mg/L) after each experiment were calculated using the best-fit calibration plot (absorbance expressed in IU = $0.0189 \cdot C_{RY145}$; $R^2 = 0.99$; $p < 0.001$; $A = 0.0161 \cdot C_{RB19}$; $R^2 = 0.99$; $p < 0.001$). Zero point charge (pH_{zpc}) of cottonseed cake and isoelectric points (pH_{iep}) of RY145 and RB19 were measured using a zeta potential meter (Zetasizer, 3000HSA, Malvern, UK) and a titration method, respectively.

Kinetic, isotherm and thermodynamic studies. Kinetic parameters of chemical reaction of the two decolorization processes were derived from the experimental data and were used to estimate activation energy of reaction for the processes. Langmuir and Freundlich isotherm studies were performed under pH 2, the initial dye concentration range of 200 to 500 mg/L, the two-hour reaction time, and the temperature range of 25 to 55°C. Finally, Gibbs free energy change, entropy, and enthalpy of chemical reactions were determined using isotherm constants.

Statistical analyses. Decolorization efficiency for RB19 was modeled in response to changes in the following experimental conditions: (1) adsorbent concentration, process type, and reaction time under the constant initial dye concentration (225 mg/L) and temperature (25°C);

(2) initial dye concentration, process type, and reaction time under the constant adsorbent concentration (1.0 g/L) and temperature (25°C); and (3) temperature, process type, and reaction time under the constant initial dye (225 mg/L) and adsorbent (1.0 g/L) concentrations. Decolorization efficiency for RY145 was modeled in response to changes in the following experimental conditions: (1) initial dye concentration, process type, and reaction time under the constant initial dye concentration (300 mg/L) and temperature (25°C); (2) initial dye concentration, process type, and reaction time under the constant adsorbent concentration (0.5 g/L) and temperature (25°C); and (3) temperature, process type, and reaction time under the constant initial dye (300 mg/L) and adsorbent (0.5 g/L) concentrations. Anderson-Darling test, and the plots of residuals versus fitted values and residuals versus the order of the data were performed to check the assumptions of normality, constant variance, and autocorrelation, respectively, during one-way analysis of variance (ANOVA) and multiple non-linear regression (MNL) models.

Tukey's pairwise comparisons following one-way ANOVA was used to detect significant differences in mean decolorization efficiency among the types of process, the reaction times, the initial dye concentrations, the adsorbent concentrations, and the temperatures. Best-fit MNL models were used to account for the efficacy of explanatory factors and their interactions in the prediction of decolorization efficiency. The best subsets procedure was used to choose the best-fit MNL models with the highest goodness-of-fit measured by adjusted coefficient of determination (R^2_{adj}), and the highest predictive power measured by coefficient of determination based on leave-one-out cross-validation (R^2_{CV}). The process type of adsorption versus ultrasound-assisted adsorption was incorporated in the MNL models as a dummy variable with adsorption being held as the baseline. Variance inflation factor (VIF) for multicollinearity and Durbin-Watson (D-W) statistics for autocorrelation were reported for the best-fit MNL models. All the statistical analyses were performed using Minitab 17 (Minitab, Inc., State Collage, PA).

RESULTS AND DISCUSSION

Decolorization efficiency as a function of initial pH. Descriptive statistics of decolorization efficiency for the six datasets of RY145 and RB19 were presented in Table 1.

TABLE 1
Descriptive statistics of decolorization efficiency (DE, %) data under three experimental conditions for each of RB19 and RY145.

| Dataset | RB19 under the constant conditions of | | | RY145 under the constant conditions of | | | |
|--------------|---|----------------------|---|--|---|--|--|
| | 20°C and IDC of 225 mg/L | 20°C and AC of 1 g/L | AC of 1 g/L and IDC of 225 mg/L | 20°C and IDC of 300 mg/L | 20°C and AC of 0.5 g·L ⁻¹ | AC of 0.5 g/L and IDC of 300 mg/L | |
| <i>n</i> | 66 | 110 | 88 | 66 | 176 | 88 | |
| Mean | 68.8 | 67.7 | 77.7 | 73.6 | 68.4 | 68.9 | |
| SD | 24.0 | 18.9 | 13.2 | 20.6 | 19.2 | 11.0 | |
| Median | 75.1 | 67.3 | 79.2 | 78.3 | 68.1 | 70.8 | |
| Min | 12.7 | 20.3 | 37.4 | 33.3 | 19.0 | 31.5 | |
| Max | 100.0 | 100.0 | 99.9 | 99.2 | 102.9 | 86.0 | |
| Skewness | -0.50 | -0.19 | -0.64 | -0.34 | -0.18 | -0.74 | |
| Kurtosis | -0.87 | -0.67 | 0.11 | -1.29 | -0.70 | 0.43 | |
| Distribution | Normal with JT | Normal | Normal with JT | Normal with JT | Normal with JT | Normal with JT | |
| AD | 0.15 | 0.41 | 0.11 | 0.38 | 0.25 | 0.14 | |
| <i>p</i> | 0.95 | 0.32 | 0.99 | 0.38 | 0.73 | 0.96 | |
| JT | $-0.544+0.619 \times \ln((DE-11.275)/(101.366-DE))$ | | $-1.011+1.164 \times \ln((DE-23.289)/(102.964-DE))$ | $-0.397+0.489 \times \ln((DE-32.866)/(99.709-DE))$ | $-0.188+1.175 \times \ln((DE-13.612)/(116.079-DE))$ | $-1.081+1.183 \times \ln((DE-22.440)/(89.742-DE))$ | |
| Location | -0.030 | | 0.022 | -0.004 | -0.006 | 0.008 | |
| Scale | 1.010 | | 1.012 | 1.030 | 1.020 | 1.009 | |

AD: Anderson-Darling normality test; JT: Johnson transformation; IDC: initial dye concentration; AC: adsorbent concentration.

All the datasets of decolorization efficiency exhibited Gaussian distribution with or without Johnson transformation. Out of the six initial pH values, the minimum decolorization efficiency was obtained at the initial pH 4 for both RY145 and RB19. Decolorization efficiency increased with a decreasing pH ($p < 0.05$; $n = 132$) and reached its maximum at the lowest pH 1 for RY145 (Fig. 2) and RB19 (Fig. 3) as the reaction time progressed.

pH_{zpc} of cottonseed cake, and pH_{IEP} of RY145 and RB19 were measured at 1.4, 2.4 and 3.0, respectively. Effect of initial pH on decolorization efficiency appears to depend on zero point charge (pH_{zpc}) of cottonseed cake and isoelectric points (pH_{IEP}) of RY145 and RB19. The dyes get negatively charged with pH values above 3.0, thus serving as a driving force between the dye and the negatively charged adsorbent [28]. For example, decolorization efficiency was reported to

increase when the initial pH of reactive dye solution was set in a range of 1.5 to 2.5 which was attributed to the strongly electrostatic attraction force between adsorbent and dyestuff [29]. Therefore, pH 2.0 was assumed to be the optimum pH value for the decolorization experiments conducted in the present study.

Decolorization efficiency as a function of process type, reaction time, and adsorbent concentration under constant initial dye concentration and temperature. According to Tukey's pairwise comparisons, the ultrasound-assisted adsorption resulted in the highest decolorization efficiency of *ca.* 99% with the highest adsorbent concentration (1.5 g/L) both for RB19 and for RY145 under the constant initial dye concentrations of 225 mg/L and 300 mg/L and temperature of 25 °C ($p < 0.001$) (Table 2).

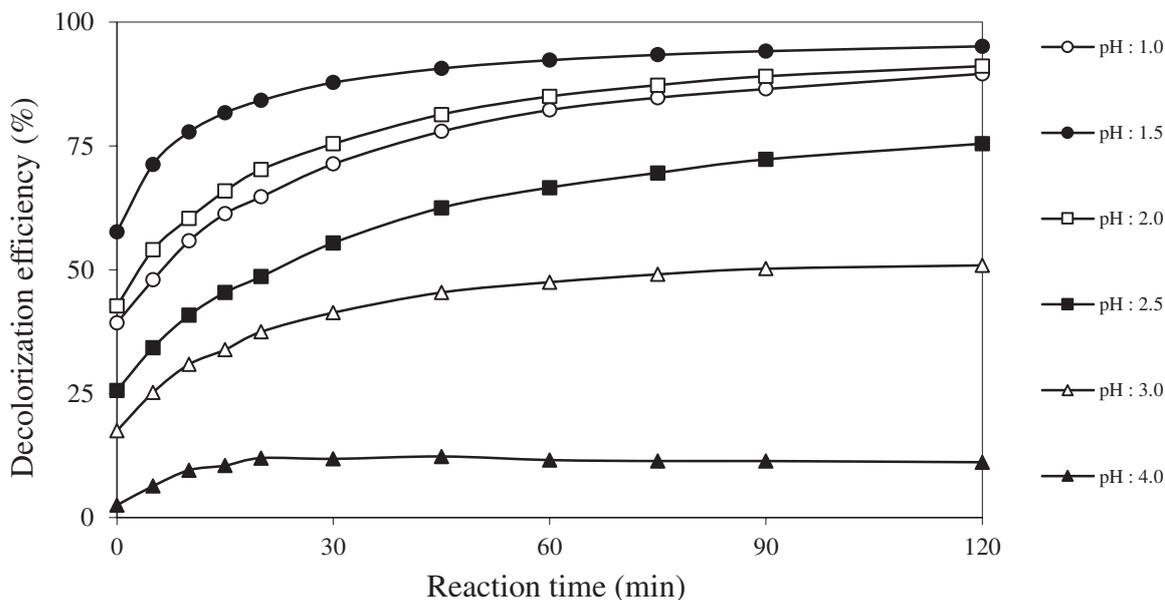


FIGURE 2
 Effect of initial pH on decolorization efficiency (300 mg·L⁻¹ initial RY145 concentration, 0.5 g·L⁻¹ cottonseed concentration, 25 °C and two-hour reaction time).

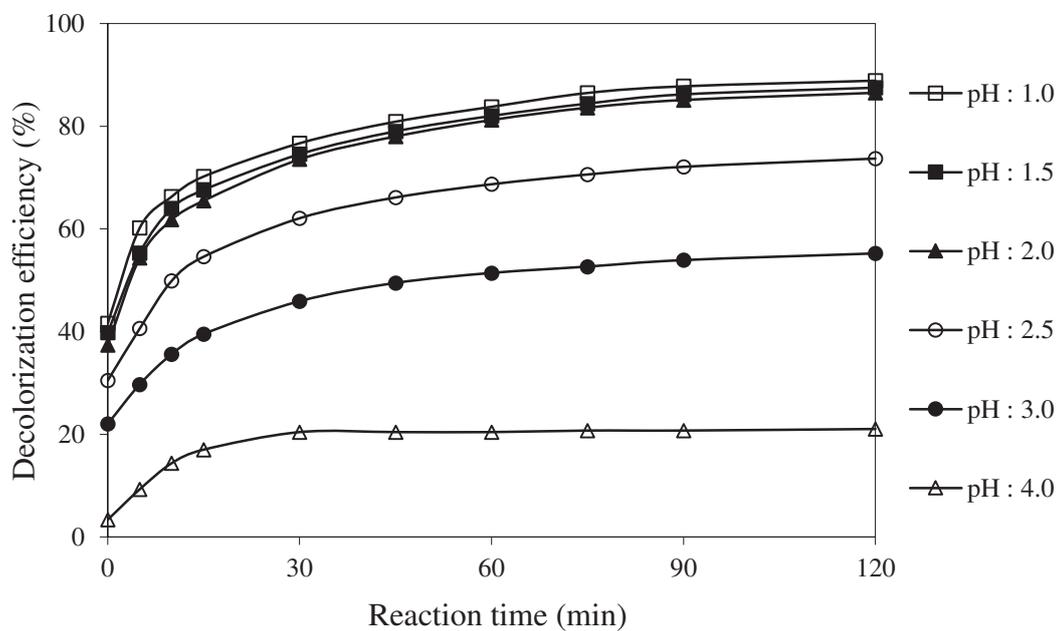


FIGURE 3
 Effect of initial pH on decolorization efficiency (225 mg·L⁻¹ initial RB19 concentration, 1.0 g·L⁻¹ cottonseed concentration, 25 °C and two-hour reaction time).

TABLE 2
Tukey pairwise comparisons of decolorization efficiency (DE, %) for RB19 and RY145 in terms of process type, adsorbent concentration (AC, g/L), initial dye concentration (IDC, mg/L), reaction time (RT, min), and temperature (T, °C) under three experimental conditions.

| Explanatory variable | RB19 under the constant conditions of | | | RY145 under the constant conditions of | | |
|----------------------------------|---------------------------------------|------------------------------------|-----------------------------------|--|------------------------------------|-----------------------------------|
| | 20°C and IDC of 225 mg/L | 20°C and AC of 1 g/L | AC of 1 g/L and IDC of 225 mg/L | 20°C and IDC of 300 mg/L | 20°C and AC of 0.5 g/L | AC of 0.5 g/L and IDC of 300 mg/L |
| Process type | (<i>n</i> = 33; <i>p</i> = 0.04) | (<i>n</i> = 55; <i>p</i> = 0.001) | (<i>n</i> = 44; <i>p</i> > 0.05) | (<i>n</i> = 33; <i>p</i> > 0.05) | (<i>n</i> = 88; <i>p</i> > 0.05) | (<i>n</i> = 44; <i>p</i> > 0.05) |
| Adsorption | 62.8 ± 25.7 ^a | 60.4 ± 17.7 ^a | 76.7 ± 14.4 ^a | 69.00 ± 22.1 ^a | 67.5 ± 18.0 ^a | 68.1 ± 11.9 ^a |
| UAA | 74.7 ± 20.8 ^b | 75.0 ± 17.3 ^b | 78.6 ± 11.9 ^a | 78.26 ± 18.2 ^a | 69.3 ± 20.3 ^a | 69.7 ± 9.9 ^a |
| Reaction time (min) | (<i>n</i> = 6; <i>p</i> > 0.05) | (<i>n</i> = 10; <i>p</i> = 0.001) | (<i>n</i> = 8; <i>p</i> = 0.001) | (<i>n</i> = 6; <i>p</i> > 0.05) | (<i>n</i> = 16; <i>p</i> = 0.001) | (<i>n</i> = 8; <i>p</i> = 0.001) |
| 0 | 43.1 ± 22.2 ^a | 42.2 ± 14.3 ^a | 52.5 ± 8.8 ^a | 54.4 ± 20.2 ^a | 43.4 ± 15.0 ^a | 48.7 ± 9.3 ^a |
| 5 | 54.2 ± 22.9 ^a | 50.7 ± 13.8 ^{ab} | 62.9 ± 5.5 ^b | 62.9 ± 21.2 ^a | 55.1 ± 13.9 ^{ab} | 56.1 ± 4.0 ^b |
| 10 | 61.5 ± 23.2 ^a | 56.6 ± 14.3 ^{abc} | 69.0 ± 4.6 ^{bc} | 72.2 ± 22.0 ^a | 60.0 ± 14.9 ^{abc} | 61.5 ± 3.9 ^{bc} |
| 15 | 65.9 ± 23.6 ^a | 61.7 ± 14.8 ^{abcd} | 71.5 ± 4.4 ^{cd} | 70.7 ± 19.2 ^a | 62.9 ± 15.8 ^{bcd} | 63.6 ± 3.7 ^{cd} |
| 20 | 69.4 ± 23.4 ^a | 67.7 ± 15.6 ^{bcd} | 75.6 ± 4.3 ^{cde} | 72.6 ± 19.5 ^a | 66.8 ± 17.0 ^{bcd} | 66.5 ± 3.4 ^{cd} |
| 30 | 70.0 ± 20.6 ^a | 70.5 ± 15.2 ^{bcd} | 78.7 ± 4.5 ^{def} | 76.1 ± 19.3 ^a | 71.0 ± 17.2 ^{bcd} | 69.9 ± 3.0 ^{de} |
| 45 | 75.1 ± 21.9 ^a | 74.8 ± 14.6 ^{cd} | 83.2 ± 4.3 ^{efg} | 78.2 ± 20.1 ^a | 74.6 ± 16.5 ^{cde} | 73.7 ± 2.5 ^{ef} |
| 60 | 77.6 ± 23.3 ^a | 77.7 ± 14.6 ^{cd} | 86.5 ± 4.1 ^{fgh} | 79.4 ± 20.5 ^a | 76.9 ± 16.1 ^{cde} | 76.3 ± 2.5 ^{efg} |
| 75 | 79.0 ± 23.7 ^a | 79.6 ± 14.4 ^d | 89.5 ± 3.9 ^{gh} | 80.2 ± 21.3 ^a | 78.9 ± 15.6 ^{de} | 78.7 ± 2.5 ^{fgh} |
| 90 | 79.8 ± 23.6 ^a | 80.7 ± 14.5 ^d | 91.4 ± 4.1 ^{gh} | 80.7 ± 21.4 ^a | 80.3 ± 15.4 ^{de} | 80.1 ± 2.4 ^{fgh} |
| 120 | 80.8 ± 23.3 ^a | 82.3 ± 14.2 ^d | 93.5 ± 4.3 ^h | 82.0 ± 21.3 ^a | 82.4 ± 14.9 ^e | 82.6 ± 3.0 ^{gh} |
| Adsorption concentration (g/L) | (<i>n</i> = 22; <i>p</i> = 0.001) | | | (<i>n</i> = 22; <i>p</i> = 0.001) | | |
| 0.5 | 41.5 ± 13.6 ^a | | | 50.4 ± 9.4 ^a | | |
| 1.0 | 77.2 ± 14.7 ^b | | | 77.2 ± 13.8 ^b | | |
| 1.5 | 87.5 ± 12.5 ^c | | | 93.2 ± 7.4 ^c | | |
| Initial dye concentration (mg/L) | | (<i>n</i> = 22; <i>p</i> = 0.001) | | | (<i>n</i> = 22; <i>p</i> = 0.001) | |
| 300 (200) | | 82.4 ± 15.0 ^a | | | 89.4 ± 12.7 ^a | |
| 350 (225) | | 78.2 ± 15.9 ^{ab} | | | 84.4 ± 12.8 ^a | |
| 400 (250) | | 66.8 ± 16.8 ^{bc} | | | 79.8 ± 13.2 ^{ab} | |
| 450 (275) | | 60.1 ± 15.2 ^{cd} | | | 72.0 ± 13.5 ^{bc} | |
| 500 (300) | | 50.9 ± 12.6 ^d | | | 65.1 ± 12.4 ^{cd} | |
| (325) | | | | | 58.8 ± 11.6 ^{de} | |
| (350) | | | | | 51.6 ± 11.6 ^{ef} | |
| (375) | | | | | 46.2 ± 12.2 ^f | |
| Temperature (°C) | | | (<i>n</i> = 22; <i>p</i> > 0.05) | | | (<i>n</i> = 22; <i>p</i> > 0.05) |
| 25 | | | 72.7 ± 13.4 ^a | | | 65.1 ± 12.4 ^a |
| 35 | | | 76.2 ± 12.6 ^{ab} | | | 67.9 ± 10.4 ^a |
| 45 | | | 78.5 ± 12.8 ^{ab} | | | 70.6 ± 9.9 ^a |
| 55 | | | 83.3 ± 12.5 ^b | | | 71.9 ± 10.4 ^a |

UAA: ultrasound-assisted adsorption

The best-fit MNL models explained 96% for RB19 and 93.2% for RY145 of variations in decolorization efficiency. The predictive power (R^2_{cv}) of the best-fit MNL models was high

(95.4% for RB19; 92.2% for RY145) as a function of process type, adsorption concentration, reaction time, and the quadratic and cubic terms of adsorbent concentration (Table 3).

TABLE 3
Best-fit multiple non-linear regression models of decolorization efficiency (DE, %) based on stepwise selection using the indicator variable of process type with adsorption as the reference level (alpha-to-enter and -to-remove values = 0.05).

| Dye type | Under constant experimental condition | Explanatory variable | Coefficient | SE of coefficient | T value | VIF | D-W | SE of model (%) | R ² _{adj} (%) | R ² _{cv} (%) |
|-----------------|--|----------------------|-------------|-------------------|---------|------|-----|-----------------|-----------------------------------|----------------------------------|
| RB19 | 20°C and IDC of 225 mg/L (n = 66) | Intercept | -46.8 | 4.6 | -10.0 | | 0.8 | 4.7 | 96.0 | 95.4 |
| | | RT (min) | 1.3 | 0.1 | 10.0 | > 10 | | | | |
| | | AC (g/L) | 147.5 | 10.1 | 14.6 | > 10 | | | | |
| | | UAA | 11.8 | 1.1 | 10.0 | 1 | | | | |
| | | RT ² | -0.017 | 0.002 | -6.5 | > 10 | | | | |
| | | AC ² | -50.7 | 4.9 | -10.1 | > 10 | | | | |
| | 20°C and AC of 1 g/L (n = 110) | RT ³ | 0.00007 | 0.00001 | 5.1 | > 10 | | | | |
| | | Intercept | -108.4 | 82.5 | -1.3 | | 0.9 | 3.0 | 97.4 | 97.2 |
| | | RT (min) | 1.3 | 0.06 | 21.2 | > 10 | | | | |
| | | IDC (mg/L) | 1.4 | 0.6 | 2.2 | > 10 | | | | |
| | | UAA | 14.5 | 0.5 | 25.3 | 1 | | | | |
| | | RT ² | -0.017 | 0.001 | -13.0 | > 10 | | | | |
| | IDC ² | -0.003 | 0.001 | -2.3 | > 10 | | | | | |
| | AC of 1 g/L and IDC of 225 mg/L (n = 88) | RT ³ | 0.00007 | 0.000007 | 9.9 | > 10 | | | | |
| | | IDC ³ | 0.000003 | 0.000001 | 2.2 | > 10 | | | | |
| Intercept | | 41.5 | 1.5 | 26.4 | | 1.0 | 3.3 | 93.7 | 93.0 | |
| RT (min) | | 1.15 | 0.07 | 14.6 | > 10 | | | | | |
| T (°C) | | 0.34 | 0.03 | 10.8 | 1 | | | | | |
| UAA | | 1.8 | 0.7 | 2.6 | 1 | | | | | |
| RT ² | -0.013 | 0.001 | -8.5 | > 10 | | | | | | |
| RT ³ | 0.00005 | 0.000009 | 6.4 | > 10 | | | | | | |
| RY145 | 20°C and IDC of 300 mg/L (n = 66) | Intercept | -1.7 | 4.0 | -0.4 | | 1.0 | 5.3 | 93.2 | 92.2 |
| | | RT (min) | 0.9 | 0.1 | 6.7 | > 10 | | | | |
| | | AC (g/L) | 66.0 | 6.2 | 10.5 | > 10 | | | | |
| | | UAA | 9.2 | 1.3 | 7.0 | 1 | | | | |
| | | RT ² | -0.014 | 0.003 | -4.6 | > 10 | | | | |
| | | RT ³ | 0.00006 | 0.00001 | 3.7 | > 10 | | | | |
| | 20°C and AC of 0.5 g/L (n = 176) | AC ³ | -7.1 | 1.8 | -3.8 | > 10 | | | | |
| | | Intercept | 119.7 | 1.9 | 61.1 | | 0.7 | 4.5 | 94.3 | 94.0 |
| | | RT (min) | 1.23 | 0.07 | 15.9 | > 10 | | | | |
| | | IDC (mg/L) | -0.255 | 0.006 | -42.3 | 1 | | | | |
| | | UAA | 1.7 | 0.6 | 2.4 | 1 | | | | |
| | | RT ² | -0.015 | 0.001 | -10.0 | > 10 | | | | |
| | RT ³ | 0.00006 | 0.000009 | 7.8 | > 10 | | | | | |
| | AC of 0.5 g/L and IDC of 300 mg/L (n = 88) | Intercept | 41.0 | 1.4 | 27.5 | | 0.8 | 3.1 | 91.8 | 90.8 |
| | | RT (min) | 0.94 | 0.07 | 12.6 | > 10 | | | | |
| T (°C) | | 0.23 | 0.03 | 7.7 | 1 | | | | | |
| UAA | | 1.55 | 0.67 | 2.3 | 1 | | | | | |
| RT ² | | -0.011 | 0.001 | -7.3 | > 10 | | | | | |
| RT ³ | | 0.00004 | 0.000008 | 5.5 | > 10 | | | | | |

UAA: ultrasound-assisted adsorption; IDC: initial dye concentration; AC: adsorbent concentration; RT: reaction time; D-W: Durbin-Watson statistic; VIF: variation inflation factor.

An increase in the adsorbent concentration or the reaction time enhanced decolorization efficiency at a rate of 147.5% per one g/L and 1.3% per one min for RB19 and at a rate of 66% per one g/L and 0.9% per one min for RY145, respectively. Ultrasound-assisted adsorption yielded decolorization efficiency better than adsorption by 11.8% for RB19 and by 9.2% for RY145. Ultrasound-assisted adsorption was the fastest in paving the way for the highest decolorization efficiency with an increase in adsorbent concentration and reaction time. In agreement with the present study, the same positive relationship

between reaction time and decolorization efficiency was found [31, 32, 35].

Decolorization efficiency as a function of process type, reaction time, and initial dye concentration under the constant adsorbent concentration and temperature. The process types, and the initial dye concentrations differed significantly from one another in terms of mean decolorization efficiency ($p < 0.001$) (Table 2). The highest decolorization efficiency of 91.8% for RB19 and 99.9% for RY145 was obtained with ultrasound-assisted adsorption at the lowest initial dye concentrations of 200 mg/L and 300 mg/L

under the constant adsorbent concentrations (1.0 g/L and 0.5 g/L) and temperature (25 °C), respectively. Similarly, decolorization efficiency of methyl orange with ultrasonic irradiation-assisted ozonation was found to decrease with the increasing initial dye concentration [23]. The best-fit MNLR models for RB19 and RY145 accounted for 97.4% and 94.3% of variations in decolorization efficiency and had high R^2_{CV} of 97.2% and 94%, respectively. In addition to the predictors of the process type, reaction time, and initial dye concentration, the quadratic and cubic terms of reaction time and initial dye concentration for RB19, and the quadratic and cubic terms of reaction time for RY145 were included in the best-fit MNLR models as the significant predictors ($p < 0.001$) (Table 3). The increased initial dye concentration decreased decolorization efficiency at a rate of 1.4% per one g/L, whereas the increased reaction time increased decolorization efficiency at a rate of 1.3% per one min for RB19. The increased initial dye concentration decreased decolorization efficiency at a rate of 0.255% per one g/L, whereas the increased reaction time increased decolorization efficiency at a rate of 1.23% per one min for RY145.

Consistent with our findings, an inverse relationship between initial dye concentration and decolorization efficiency was found for the decolorization of RB19 with a combined ultrasound/activated carbon system [35]. A positive relationship between adsorbent concentration and decolorization efficiency was detected for the adsorption of Reactive Black 5 by peanut hull [1]. Relative to ultrasound-assisted adsorption, adsorption led to a low decolorization efficiency by 14.5% for RB19 and 1.7% for RY145.

Decolorization efficiency as a function of process type, reaction time, and temperature under the constant initial dye and adsorbent concentrations. Decolorization efficiency was significantly higher with both the ultrasound-assisted adsorption and the increasing temperature ($p < 0.001$) (Table 2). The highest decolorization efficiency of 99.9% and 86% under the constant initial dye concentrations (225 mg/L and 300 mg/L) and adsorbent concentrations (1.0 g/L and 0.5 g/L) for RB19 and for RY145, respectively. About 94% decolorization efficiency was reported to be achieved with the adsorption of Chemazol RR195 on dehydrated beet pulp carbon with an initial dye concentration of 20 mg/L at 50 °C [36]. 98.6% decolorization efficiency for triphenylmethane dye

wastewater was obtained using ultrasonic-assisted ozone oxidation under 39.8 °C, and initial pH 5.2 [31, 32]. An optimal decolorization efficiency of Reactive Red 2 was estimated at 85% after a 120-min reaction time at pH 7 using an ultraviolet/ultrasound/TiO₂ system [12]. Supporting our findings, increased decolorization efficiencies of up to 93% and 85% were obtained with increased temperature at 40 °C and with increased reaction time in the first 10 min, respectively, as a result of ultrasound-assisted Fenton oxidation of ammunition wastewater [31, 32]. The best-fit MNLR model elucidated 85% of variation in decolorization efficiency with R^2_{CV} of 93% and 90.8% for RB19 and RY145, respectively, as a function of process type, temperature, reaction time, and the quadratic and cubic terms of reaction time (Table 3). The rate of change in decolorization efficiency was estimated at 0.34% and 0.23% per one °C and 1.15% and 0.94% per one min, for RB19 and RY145, respectively. Ultrasound-assisted adsorption performed decolorization efficiency better than adsorption by 1.8% and 1.55% for RB19 and RY145, respectively (Table 3). The same positive correlation between temperature and decolorization efficiency was reported based on the best-fit MNLR model [35].

Decolorization kinetics. Our results showed that the pseudo-second-order kinetic model described decolorization kinetics of RB19 and RY145 better than the pseudo-first-order kinetic model (Table 4) based on the following computation [28, 37, 38]:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (2)$$

The integration of Eq (2) at the boundary condition provides the following Eq (3):

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \quad (3)$$

The rearrangement of Eq (3) generates the below equation:

$$\frac{t}{q_t} = \frac{1}{k_2 \times q_e^2} + \frac{t}{q_e} \quad (4)$$

where k_2 is the rate constant of pseudo-second-order reaction (g/mg/min); q_e is the amount of dye removed at equilibrium (mg/g); and q_t is the amount of dye removed at time t (mg/g).

TABLE 4
Kinetic models and rate constants of pseudo-first-order and pseudo-second-order reactions of adsorption and ultrasound-assisted adsorption processes of RY145 and RB19 dyestuff.

| Process/ Dyes | Temperature (°C) | Pseudo-first-order model | | | | Pseudo-second-order model | | | E_A (kJ/mol) |
|---------------------|---------------------|---|-----------------|------------------------|-------|-----------------------------------|------------------------|-------|-------------------|
| | | $k_1 \times 10^2$ (min ⁻¹) | q_e (mg/g) | $q_{e,det.}$ (mg/g) | R^2 | $k_2 \times 10^4$ (g/(mg·min)) | $q_{e,det.}$ (mg/g) | R^2 | |
| Adsorption RY145 | 25 | 1.91 | 86.4 | 236.8 | 0.981 | 7.51 | 240.9 | 0.999 | 5.35 |
| | 35 | 1.52 | 78.0 | 244.7 | 0.952 | 8.01 | 248.7 | 0.999 | |
| | 45 | 1.59 | 70.3 | 254.4 | 0.953 | 8.49 | 257.0 | 0.999 | |
| | 55 | 1.66 | 68.2 | 262.8 | 0.973 | 9.18 | 265.2 | 0.999 | |
| Adsorption RB19 | 25 | 2.10 | 111.4 | 313.0 | 0.973 | 6.16 | 317.4 | 0.999 | 7.33 |
| | 35 | 2.03 | 107.2 | 321.5 | 0.974 | 6.64 | 325.7 | 0.999 | |
| | 45 | 2.01 | 100.9 | 332.3 | 0.964 | 7.10 | 336.7 | 0.999 | |
| | 55 | 1.91 | 92.0 | 339.4 | 0.969 | 8.23 | 342.4 | 0.999 | |
| UAA RY145 | 25 | 2.33 | 97.0 | 248.7 | 0.933 | 10.4 | 250.0 | 0.998 | 3.15 |
| | 35 | 2.28 | 92.9 | 253.3 | 0.988 | 10.8 | 256.4 | 0.996 | |
| | 45 | 2.21 | 86.1 | 256.6 | 0.988 | 11.2 | 261.1 | 0.997 | |
| UAA RB19 | 55 | 2.14 | 88.0 | 258.0 | 0.961 | 11.7 | 263.8 | 0.999 | 4.27 |
| | 25 | 2.40 | 122.0 | 328.4 | 0.973 | 9.21 | 328.2 | 0.999 | |
| | 35 | 2.27 | 116.8 | 333.5 | 0.974 | 9.75 | 336.3 | 0.999 | |
| | 45 | 2.39 | 109.9 | 336.1 | 0.981 | 10.10 | 345.1 | 0.999 | |
| | 55 | 2.16 | 101.5 | 339.0 | 0.952 | 10.85 | 350.8 | 0.999 | |

Our results are in agreement with findings reported by similar studies [13, 36]. Also, the increased second-order rate constant with the increasing temperature indicated that all the decolorization processes in the present study were endothermic. Activation energy (E_A) estimates of the decolorization processes decreased in the order of adsorption > ultrasound-assisted adsorption (Table 4).

Isotherms of decolorization processes.

Isotherms were used to determine the amount of adsorbent required to remove one unit mass of dyestuff and adsorption capacity. The below Langmuir (5) and Freundlich (6) isotherm models were fitted into the experimental data:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_L} + \frac{1}{q_{\max} C_e} \quad (5)$$

where q_e is the amount of adsorbed dye (mg/g); C_e is the equilibrium liquid-phase concentration (mg/L); K_L is a direct measure of sorption intensity (L/mg); and q_{\max} is a constant related to adsorbent monolayer reflecting the maximum adsorption capacity (mg/g).

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (6)$$

where q_e is the amount of dye adsorbed per unit mass of adsorbent (mg/g); C_e is the equilibrium concentration of dye in solution (mg/L); and K_f and n are Freundlich constants. Langmuir adsorption model fitted the experimental data better than the Freundlich model (Table 5), as was also found by other studies [18, 36].

R_L as a dimensionless separation constant of equilibrium was used to express the shape of Langmuir isotherm, and thus, applicability of decolorization process as follows [39]:

$$R_L = \frac{1}{1 + K_L C_0} \quad (7)$$

where K_L is the Langmuir constant; and C_0 is initial dye concentration adsorbed. According to Eq (7), process is unrealizable if $R_L > 1$; shape of isotherm is linear if $R_L = 1$; process is realizable if $0 < R_L < 1$; and process is irreversible if $R_L = 0$. In the present study for RB19 and RY145, the R_L values at all the temperatures and concentrations were found to vary between 0 and 1 which in turn showed that all the decolorization processes were realizable.

TABLE 5
Langmuir and Freundlich isotherm constants of adsorption and ultrasound-assisted adsorption.

| Process/Dyes | Temperature (°C) | Langmuir isotherm | | | Freundlich isotherm | | |
|---------------------|------------------|-------------------|--------------|-------|---------------------|------|-------|
| | | q_{\max} (mg/g) | K_L (L/mg) | R^2 | K_f | n | R^2 |
| Adsorption RY145 | 25 | 270.3 | 0.091 | 0.999 | 169.4 | 12.9 | 0.978 |
| | 35 | 277.8 | 0.114 | 0.999 | 185.1 | 14.7 | 0.963 |
| | 45 | 285.7 | 0.150 | 0.999 | 200.7 | 16.5 | 0.981 |
| | 55 | 290.7 | 0.226 | 0.999 | 213.8 | 18.0 | 0.981 |
| Adsorption RB19 | 25 | 197.1 | 0.162 | 0.999 | 36.9 | 5.2 | 0.893 |
| | 35 | 204.0 | 0.243 | 0.999 | 42.1 | 6.0 | 0.902 |
| | 45 | 216.7 | 0.385 | 0.996 | 44.8 | 4.8 | 0.875 |
| | 55 | 221.4 | 0.464 | 0.997 | 46.4 | 5.0 | 0.971 |
| UAA RY145 | 25 | 310.3 | 0.094 | 0.991 | 174.1 | 13.6 | 0.978 |
| | 35 | 317.8 | 0.123 | 0.999 | 194.4 | 15.4 | 0.965 |
| | 45 | 325.7 | 0.157 | 0.991 | 204.3 | 16.5 | 0.982 |
| | 55 | 330.7 | 0.235 | 0.991 | 238.8 | 18.0 | 0.978 |
| UAA RB19 | 25 | 209.2 | 0.171 | 0.998 | 42.1 | 6.0 | 0.893 |
| | 35 | 217.3 | 0.258 | 0.999 | 46.2 | 6.5 | 0.882 |
| | 45 | 228.7 | 0.404 | 0.999 | 53.2 | 3.8 | 0.885 |
| | 55 | 231.5 | 0.503 | 0.997 | 56.2 | 5.5 | 0.971 |

Thermodynamic parameters. Gibbs free energy as a function of entropy and temperature provides information about spontaneity of a chemical reaction [40]. Gibbs free energy and entropy can be calculated as follows [9, 17]:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K \quad (8)$$

$$\ln K = \frac{\Delta S}{R} - \frac{\Delta H}{R T} \quad (9)$$

where ΔG is Gibbs free energy (kJ/mol); ΔH is enthalpy of reaction (kJ/mol); K is chemical equilibrium constant (L/mol); and T is temperature (K). When $\ln K$ versus $1/T$ graph is plotted, the slope and intercept of the plot can be used to determine enthalpy and entropy of the reaction, respectively. Negative Gibbs free energy changes for all the temperatures indicated that the process in

the present study was endothermic and spontaneous (Table 6).

The increase in entropy depending on the process type indicates increased structural heterogeneity of the dye, and positive change in entropy can be attributed to high affinity of the adsorbent to the dyes as was also indicated by related literature [32, 37]. Although no study exists about decolorization of reactive dyes using cottonseed cake, the high adsorption capacity of cottonseed cake was revealed in this study compared to adsorption, biosorption and photocatalytic degradation capacities of different adsorbents for different dyes (Table 7).

The use of cottonseed cake or its various forms such as cottonseed cake-based activated carbon, and dehydrated or strong acid-modified cottonseed cakes appears to be a promising adsorbent in decolorization process.

TABLE 6
Thermodynamic constants of RY145 and RB19 for adsorption and ultrasound-assisted adsorption processes.

| Dyes | Temperature (°C) | K_L (L/mol) | ΔG (kJ/mol) | ΔS (kJ/mol) | ΔH (kJ/mol) |
|---------------------|------------------|---------------|---------------------|---------------------|---------------------|
| Adsorption RY145 | 25 | 11580.6 | -23.18 | 0.158 | 24.13 |
| | 35 | 14438.9 | -24.52 | 0.158 | |
| | 45 | 19023.2 | -26.05 | 0.157 | |
| | 55 | 28555.8 | -27.97 | 0.158 | |
| UAA RY145 | 20 | 16019.1 | -23.98 | 0.172 | 27.51 |
| | 30 | 19440.1 | -25.28 | 0.171 | |
| | 40 | 25144.2 | -26.78 | 0.170 | |
| Adsorption RB19 | 50 | 45875.3 | -29.27 | 0.173 | 18.02 |
| | 20 | 10150.3 | -27.74 | 0.157 | |
| | 30 | 15413.7 | -29.70 | 0.156 | |
| | 40 | 24681.2 | -31.84 | 0.157 | |
| UAA RB19 | 50 | 38946.5 | -33.26 | 0.158 | 20.54 |
| | 20 | 14458.3 | -28.02 | 0.177 | |
| | 30 | 20343.2 | -30.67 | 0.176 | |
| RB19 | 40 | 30329.4 | -32.24 | 0.178 | 20.54 |
| | 50 | 44676.2 | -34.89 | 0.179 | |

CONCLUSIONS

Mean decolorization efficiency of ultrasound-assisted adsorption was consistently higher than that of adsorption across all the six experimental datasets for RB19 and RY145 which is most likely to be attributed to its lower activation energy. However, no significant difference was detected in decolorization efficiency between the process types for all the RY145 datasets, and for the RB19 data under the constant initial dye and adsorption concentrations. The lowest initial dye concentration, and the highest adsorbent concentration for RB19 and RY145, and the highest temperature for RB19 yielded significantly higher decolorization efficiency. Except for the RB19 and RY145 datasets under the constant temperature and initial dye concentration, the highest reaction times led significantly to the highest decolorization efficiency. All the predictors common in the best-fit

MNLR models of both RB19 and RY145 showed the maximum rate of change in decolorization efficiency for RB19 than for RY145. Except for initial dye concentration, the direction of the relationship between a given predictor and decolorization efficiency was consistently positive for the common predictors except for the quadratic term of reaction time (negative) across all the best-fit MNLR models of RB19 and RY145. The most and least influential linear predictors for decolorization efficiency were found to be adsorbent concentration, and reaction time for both RB19 and RY145, respectively. Future studies about pilot plant-scale AOPs such as photocatalytic degradation of real versus synthetic effluents, and their economic feasibility remain to be explored incorporating various designs of experiment, artificial neural networks, and adaptive-network based fuzzy inference systems.

TABLE 7
A comparison of adsorbent capacities (q_{\max}) for different reactive dyes.

| Adsorbent | Dye | Decolorization process | q_{\max} (mg/g) | References |
|------------------------------------|---------------------|--------------------------------|----------------------|------------|
| Bagasse fly ash | Orange G | Adsorption | 18.8 | [2] |
| | Methyl Violet | | 26.2 | |
| Coir pith carbon | Congo Red | Adsorption | 6.72 | [7] |
| | Reactive Blue 2 | | 2498 | |
| Cross-linked chitosan beads | Reactive Red 2 | Adsorption | 2422 | [12] |
| | Reactive Yellow 89 | | 1911 | |
| | Amaranth | | 14.9 | |
| Powdered peanut hull | Sunset Yellow | Adsorption | 13.9 | [20] |
| | Fast Green FCF | | 15.6 | |
| | Ractive Blue 19 | | 159 | |
| <i>P. oxalicum</i> pellets | Reactive Blue 241 | Adsorption | 122 | [41] |
| | Reactive Blue 145 | | 137 | |
| <i>C. lipolytica</i> | Remazol Blue | Biosorption | 250 | [42] |
| Spent brewery | Acid Orange 7 | Adsorption | 30.5 | [43] |
| Rice husk | Methylene Blue | Adsorption | 40.6 | [44] |
| TiO ₂ +SiO ₂ | Reactive Yellow 145 | Photocatalytic degradation | 5.13 | [45] |
| | Reactive Blue 19 | | 117.6 | |
| Wheat bran | Reactive Red 195 | Adsorption | 119.1 | [46] |
| | Reactive Yellow 145 | | 196.1 | |
| | Reactive Blue 19 | | 231.5 | |
| Cottonseed cake | Reactive Yellow 145 | Ultrasound-assisted adsorption | 330.7 | This study |
| | Reactive Blue 19 | Adsorption | 221.4 | |
| | Reactive Yellow 145 | | 290.7 | |

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TOXICITY OF SIX ETHANOL PLANT EXTRACTS AGAINST THE GREEN PEACH APHID *MYZUS PERSICAE* SULZER (HOMOPTERA: APHIDIDAE)

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ABSTRACT

A study was conducted to test the aphicidal activity of the ethanol leaf extract of six plant species of the most widely spread plants in Jordan. LC50 and LC90 were calculated using SPSS program. Completely Randomized Design (CRD) evaluation experiment was conducted to test the six plant species against the green peach aphid (GPA) *Myzus persicae* which was maintained in two cultures, in the Lab and in the green house for ten generations to get a susceptible strain before conducting the bioassay. Ethanol leaf extracts were obtained by soaking and then concentrated and dried. Dipping method was used in the bioassay. Results were significantly different in the toxicity assessment and in the CRD evaluation. The LC50 for the leaf extract of *Ricinus communis* L was the lowest (553 ppm) and for *Robinia pseudoacacia* L was the second most toxic plant extract (1150 ppm) after 24 hours, while *Lantana camara* L ethanol leaf extract was the least toxic (6660 ppm). CRD evaluation showed that *Robinia pseudoacacia* had 76.46%, followed by *Ricinus communis* which had 58.6% mortality rates, respectively. On the other hand *Nerium oleander* Mill caused the least mortality rate 25.2%, compared to the negative control and the positive control (Cypermethrin) which caused 3.24% and 90.82% mortality rates after 24 hours, respectively.

KEYWORDS:

Toxicity, Plant extract, Ethanol, Green peach aphid.

INTRODUCTION

Environmental pollution has been considered the outcome of the intensive use of chemical pesticides, which adversely affect the human

health, disturb biological control agents, and cause pesticides resistance. Food and agricultural products quality and safety have been considered the aim of both the consumer and the producer who focused on the pesticide free products and organic farming [1]. Pest management should consider the environment and should be applied in the integrated crop management (ICM) and integrated pest management (IPM) concept, by applying economically and friendly techniques. Therefore, the use of bio pesticides has gained a lot of interest specially chemicals from plant origin which negatively affect the pests. These botanical products could be used as extracts, essential oils, or pure allelochemicals [2]. In North America and Europe, there is a public interest for long term health and the environmental effects of the synthetic pesticides and natural pesticides, both microbial and botanical. In the United States, Food Quality Protection Act of 1996 restricted the use of conventional insecticides (organophosphates and carbamates) which have been intensively used by the farmers. Therefore, alternative products favored by the Environmental Protection Agency (EPA) in the USA should be produced [3]. Moreover, insecticides resistance development in insect pests has been considered as an increasing obstacle in agriculture, forestry and public health [4].

The green peach aphid *Myzus persicae* Sulzer (Aphididae: Homoptera) is a cosmopolitan species of economic importance, extremely polyphagous, and efficient vector of plant viruses, transmitting more than 100 plant viruses. It has a wide range of genetic variations in color, life cycle, host-plant relationships and methods of resisting insecticides [5]. The green peach aphid has a striking capacity for rapid adaptation to insecticides, developing resistance to more active compounds, as it has high reproductive rate and short generation time [6]. It has been noticed that the green peach aphid has developed resistance to the commonly used

insecticides from organophosphates and carbamates in Jordan, particularly, in Jerash district and Jordan valley regions [7]. Therefore, new pest management strategies should be considered, in addition to, the biological control, pheromones, and plant derived products.

Plant extracts or essential oils which are non chemical control options have gained interest. The plant extracts are concentrated mixture preparations of plant parts obtained by suitable solvent, which is evaporated away and the residue is then adjusted to a prescribed standards [2]. Allelo chemicals are secondary metabolites which could be the components of both extracts and essential oils that affect growth, reproduction, or behavior of individuals other than the ones producing them, or structure and dynamic of populations or community of other organism species [8]. Botanical insecticides have many advantages over the current synthetic insecticides. These are non persistent in the environment as some of them are volatile composed of many constituents which lower the possibility of resistance development, and have many modes of action [3].

Pavela et al. [9], from Czech Republic, evaluated three *Impatiens* species, on *M. persicae* and found that the most effective extract was *I. parviflora* with insecticidal activity of 99.7% and 90% mortality at concentrations of 0.5% and 0.1%, respectively, followed by *I. glandulifera* and *I. nolitangere* that caused 75%, 82.5% and 70%, and 67.5% for the 0.5% and 0.1% concentrations, respectively. Extracts were then analyzed by HPLC. It was found that the presence of the caffeic acid and high flavones content of the *I. parviflora* might be the reason for mortality of aphids. Moreover, in Jordan, insecticidal activity of different extracts of *Rhamnus dispermus* against *Pterochloroides persicae*, peach trunk aphid was investigated using hexane, chloroform, acetone, and ethanol, at three concentrations. Acetone and ethanol extracts revealed higher mortality 69% and 71%, respectively at the higher concentration of 10.000 ppm compared with hexane and chloroform [10]. In Canada, 17 essential oils were screened against different pest species, patchouli oil was found to be among the most toxic to all tested species including *M. persicae* that had 50% mortality and has 8.2µl/ml LC50 and that for rosy apple aphid, *Dysaphis plantaginea* (Passerini) had 90% mortality. Lavender oil was identified as the second most toxic essential oil to *M. persicae* causing 46.7% mortality and has 2.4 µl/ml LC50.

The major constituents of citronella, lavender, patchouli and thyme oils were determined and quantified via gas chromatography-mass spectrophotometry (GC-MS) [6]. In Iraq,

insecticidal effect of *Eucalyptus camaldulensis* (Myrtaceae), *Nerium oleander* (Apocynaceae) and *Myrtus communis* (Myrtaceae) were studied on *Hyalopterus pruni* with four concentrations 4, 6, 8, and 10%. *Eucalyptus camaldulensis* caused highest mortality up to 92.6% at 10% concentration after 48 hours of treatment while *Nerium oleander* caused 40.4% after 24 hours of treatment [11]. In UK, a study was conducted to demonstrate that *Tagetes minuta* oil volatile had potential to control three aphid species including *M. persicae*. The chemical composition of the volatiles was investigated and the main constituents of the oil were identified. The purified sesquiterpene β caryophyllene produced greater effect than the monoterpenes limonene and acimene. *Tagetes minuta* oil volatiles significantly reduced aphid reproduction up to 100% and oil was fractionated by vacuum distillation [12].

Safe alternatives to insecticides could be used to manage the green peach aphid population in the IPM context or organic agriculture. These might not be harmful to natural enemies of green peach aphid. Therefore, the objectives of this study were to evaluate the toxicity of the following plant extracts against *Myzus persicae* under laboratory conditions. These plants were *Ricinus communis* L, *Solanum nigrum* L, *Nerium oleander* Mill, *Robinia pseudoacacia* (Fabaceae), *Lantana camara* L, *Inula viscosa* L., Cypermethrin insecticide as a positive control and the extract solvent as negative control were included, by using ethanol as the solvent. The results will be useful in the aphid control without environmental contamination and to avoid aphid resistance.

MATERIALS AND METHODS

Host plant rearing and Green peach aphids cultures. Continuous sweet pepper production of the Super Lamoy cultivar from Scantini Seed Company was provided as needed for the aphid colony establishment in the greenhouse (25-35 °C and 40-60% Relative Humidity (RH%)) where a stock culture of the green peach aphid GPA (*M. persicae*) was established. The aphid culture was maintained on sweet pepper plants during the study for ten generations before being used in the bioassay, to gain susceptible strain of GRP [7]. When the plant became at 7-8 plant leaves, then the transplants were planted in plastic pots (15-20) cm in diameter, with 50% soil and 50% Peatmose. The plants were irrigated regularly, and fertilized weekly using 20:20:20 NPK water soluble fertilizer. Topsin fungicide was used after planting to prevent wilt diseases. The plant pots were placed in twelve aluminum cages with dimensions of

60x60x60 cm, with fine mesh screen, to keep them free from insect infestation. From 8-10 pepper pots were used per cage. The doors of the greenhouse were tightly closed by organza.

In March 2014, the GPA infested leaves of the sweet pepper brought from Ghor Al Safi region, were used to infest pepper plants under greenhouse conditions in Rabba Center for Agricultural Research and Extension. The GPA was confirmed by Prof. Tawiq Al Antary. Slides were prepared according to Blackman and Eastop [5]. When the plants became heavily infested with the GPA colonies, they were used to infest few other sweet pepper plants in the aluminum cages by using small paint brush. The used greenhouse was with 25-35 Co and 40-60% relative humidity (RH %).

Another GPA culture was established and maintained under laboratory conditions by using six wooden cages (60x60x100) cm³ covered with fine screen mesh on all sides and with a sliding movable glass door. The growth laboratory conditions were 25 C^o, 35-60 RH% and 16:8 hrs (L: D) photoperiod regime to get apterous aphids. New plants were provided as needed from the greenhouse for the two cultures. Also pepper plants (transplants) were regularly irrigated and weekly fertilized with 20:20:20 NPK water soluble fertilizer. Infested plants from the greenhouse were transferred to the laboratory growth chamber and reared in the wooden cages. Fluorescent light with 4000 lux was used for 16 hours lighting period.

Collecting the plant materials. Plants which might have toxic potential to GPA were collected from the beginning of April until the end of June 2014, and their taxonomy was confirmed. The following plants, *Ricinus communis* L. (Caster) (Euphorbiaceae), *Solanum nigrum* L. Black nightshade (Solanaceae), and *Nerium oleander* Mill, *Oleander* (Apocynaceae) were collected from Gohor Al Safi region, while *Lantana camara* L. Wild sage (Verbenaceae) was collected from Potash co. garden.

Robinia pseudoacacia (Fabaceae) black locust and *Dittrichia (Inula) viscosa* L. Stinkwort (Asteraceae) were collected from Alwasiah area in the uplands. Voucher specimens from all the plants were kept in Rabba Center for Agricultural

Research and Extension. The plant leaves were shade dried on the benches at the Field Crops laboratory under room temperature until the leaves became dry to be pulverized easily. The leaves were finely ground (25 Co, 35-60 RH% and 16:8 hrs (L:D) photoperiod regime) by using Thomas Wiley laboratory mill, which was found in Rabba Center for Agricultural Research and Extension.

Plant extract preparation. The stock solutions were prepared as needed by dissolving certain weight of each extract in certain volume of acetone (solvent) until it dissolved nearly completely, the average concentration of each plant extract was estimated as shown in Table (1). Four to five concentrations were prepared by diluting the certain volume of stock solution with distilled water. Three replicates for each concentration were used. The powdered materials under investigation were weighed as (150g) and were placed in cotton bags that were tied and soaked in 375ml of ethanol in glass jars that were tightly closed to prevent evaporation of the solvent. They were kept in dark place for five days. Ethanol 99.9% (Analytical grade AG from ACI Labscan limited) was used as organic solvent. After five days of soaking, the cotton bags were squeezed to get the rest of the extract. The extracts were then filtered and evaporated under reduced pressure. After drying, the extracts were weighed and sealed with foil paper and preserved in the refrigerator until used in the experiments.

Bioassay procedures. Plant extracts toxicity assessment. Modified leaf dipping was used. At least 20 apterous aphids were dipped, by dipping GPA infested sweet pepper leaf disks in the prepared solutions for 10 seconds, the volume of the solution should be at least 20 ml [10]. The plant extract stock solutions were prepared as needed by dissolving certain weight of each extract in certain volume of acetone (solvent) until it is dissolved nearly completely. Table (1) shows the average weight of each plant extract used per solvent (acetone). Four to five concentrations were prepared by dilutions with distilled water. Three replicates for each concentration were used, in order to conduct toxicity tests to LC₅₀ and LC₉₀ for each plant extract.

Table 1
Concentrations of the plant extracts that were used in the toxicity assessment.

| Plant | Ethanol extract used mg/ml |
|-----------------|----------------------------|
| R. communis | 3.2 |
| R. pseudoacacia | 5.33 |
| vescosa | 13.0 |
| S. nigrum | 4.24 |
| N. oleander | 13.75 |
| L. camara | 20.0 |

Modified leaf dipping was used and at least 20 apterous aphids were used by dipping sweet pepper leaf disks in the prepared solution for 10 seconds [10]. The excess solution was taken off by placing it on a tissue paper, then the leaf disks were placed in 5.5 cm Petri dishes with moist cotton, and were left to dry. The Petri dishes were covered with lid's in which four holes were made for ventilation. These placed under lab conditions, 16:8 L:D period, 25±3 Co, and 35-65 relative humidity (RH%). Number of dead aphids was counted after 24 hours of treatment. Percentage mortality was then calculated. The highest concentration gave less than 100% mortality down to 80%, while the lowest concentration gave more than 20% mortality. The control gave 5% or less mortality rate. Assessment was carried out under the dissecting binocular microscope based on the lack of the antennal and leg movement by touch with fine brush. Third and fourth nymphal instars and adult apterous aphids stages were counted. Two counters were used, a silver one was used to count the alive GPA, and a black one was used to count the dead GPA. The data were reported in special tables.

Evaluation of the plant extracts toxicities.

Eight treatments were evaluated. The same six plant species were evaluated, in addition to the positive control (cypermethrin insecticide from, the pyrethroids) and the negative control which was acetone diluted as the used concentration of the stock solution. For each treatment, there were five replicates with CRD design for each experiment. There were at least 20 apterous aphids (excluding the first, second nymphal and allate stages). Preliminary work was conducted to determine the proper concentrations that were used.

The concentrations used were obtained by 35% dilution of the stock solution. Sweet pepper leaf disks were dipped for 10 seconds in the prepared solutions. The excess solution was taken off by placing the leaf disks on a piece of tissue paper, and then was placed in a 5.5cm Petri dish with a moistened cotton. These were then left to dry and then covered by the Petri dish lid, with four

small holes that were made by using hot needle. The dishes were placed under 16:8 L:D, 25Co, and 35-50 RH% conditions. Assessment was carried out under the binocular microscope. Those aphids were with lack of antennal and leg movement were considered dead. The mortality percentages were determined after 24, 48, and 72 hours after treatments and were reported in special tables. Two counters were used for assessment. MSTAT C version 2.1 (Michigan State University) was used for statistical analysis. The positive control was cypermethrin insecticide that was used as recommended by the manufacturer. The negative control was acetone that was used in 35% concentration.

Statistical analysis. SPSS program (version 20) was used to obtain the LC50, LC90, 95% confidence limits (CL), L.E.P.R (line estimated by probit regression) and Slope ± SE, and chi square was used to separate between means.

RESULTS

Toxicity of the ethanol plant extracts.

Abbott's formula [13] and the SPSS software version 20 were used to estimate the parameters of toxicity, such as the slope, its standard error and 95% confidence limits (CL) for the concentrations used. Pearson's Chi-square test was used to determine the fit of the statistical model [14], for all the plant extracts. The calculated chi-square (X²) value was less than the X² tabulated at the corresponding degree of freedom (df) and at 0.05 probabilities which means there are goodness of fit at that probability level. Toxicity of the ethanol plant extracts is shown in Table (2) in ppm against the GPA. R. communis had the lowest LC50 concentration which was 553ppm, while Robinia pseudoacacia had 1150ppm, Solanum nigrum had 1371ppm, Inuala viscosa had 3792, Neruim oleander had 5615ppm and Lantana camara had the highest LC50 value of 6650ppm. All the LC50

values were significantly different (95% CL. Didn't overlap).

The slopes of the regression line for the tested ethanol plant extract were 4.9 for the *R. communis*, 4.27 for *R. Pseudoacacia*, 7.2 for *S.nigrum*, 6.44 for *I. viscosa*, 5.4 for *N. oleander* and 4.15 for *L. camara*. *R. communis* may be considered as the most toxic followed by *Ro. Pseudoacacia*. The LC90 values for the ethanol extracts are shown in

Table(3), *R. communis* had also the lowest LC90 which was 1010ppm, followed by *S. nigrum* of 2067 ppm, and *R.pseudoacacia* 2293 ppm, and *I. viscosa* 5996 ppm, *N. oleander* had 9695 ppm and *L. camara* which had also the highest LC90 value of 13571ppm. The LC90 values for *S. nigrum* and *R. pseudoacacia* were not significantly different (95% CL overlap).

TABLE 2
Comparative toxicity of ethanol extracts LC50 against *Myzus persicae* using dipping method under laboratory conditions after 24 hours.

| Plant (Treatment) | LC50* ppm | 95% CL1 | L.E.P.R2 | Slope ± SE | df. | Chi-Square |
|-----------------------------|-----------|-----------|--------------|-------------|-----|------------|
| <i>Ricinus communis</i> | 553 a | 506-595 | -13.45+4.9X | 4.9 ± 0.46 | 10 | 7.577 |
| <i>Solanum nigrum</i> | 1371 c | 1302-1437 | -22.53+7.2X | 7.2 ± 0.76 | 9 | 3.302 |
| <i>Nerium oleander</i> | 5615 e | 5312-5920 | -20.3+5.4X | 5.4 ± 0.05 | 10 | 14.385 |
| <i>Robinia pseudoacacia</i> | 1150 b | 1075-1223 | -13.08+4.27X | 4.27 ± 0.37 | 12 | 11.349 |
| <i>Lantana camara</i> | 6660 f | 6206-7118 | -15.85+4.15X | 4.15 ± 0.47 | 10 | 7.543 |
| <i>Inula viscosa</i> | 3792 d | 3613-3978 | -23.05+6.44X | 6.44 ± 0.61 | 10 | 13.203 |

1: 95% confidence limits for the LC50 or LC90 in ppm

2: L.E.P.R. line estimated by probit regression

*: LC50 Or LC90 values having different letters are significantly different (95% CL didn't overlap)

-SPSS program for probit analysis was used and chi square for means separation

TABLE 3
Comparative toxicity of ethanol extracts LC90 against *Myzus persicae* using dipping method under laboratory conditions after 24 hours.

| Plant (Treatment) | LC90* ppm | 95% CL1 | L.E.P.R2 | Slope ± SE | df. | Chi-Square |
|-----------------------------|-----------|-------------|--------------|-------------|-----|------------|
| <i>Ricinus communis</i> | 1010 a | 927-1129 | -13.45+4.9X | 4.9 ± 0.46 | 10 | 7.577 |
| <i>Solanum nigrum</i> | 2067b | 1917-2305 | -22.53+7.2X | 7.2 ± 0.76 | 9 | 3.302 |
| <i>Nerium oleander</i> | 9695d | 8844-11004 | -20.3+5.4X | 5.4 ± 0.05 | 10 | 14.385 |
| <i>Robinia pseudoacacia</i> | 2293b | 2066-2638 | -13.08+4.27X | 4.27 ± 0.37 | 12 | 11.349 |
| <i>Lantana camara</i> | 13571e | 11851-16644 | -15.85+4.15X | 4.15 ± 0.47 | 10 | 7.543 |
| <i>Inula viscosa</i> | 5996 c | 5514-6736 | -23.05+6.44X | 6.44 ± 0.61 | 10 | 13.203 |

1: 95% confidence limits for the LC50 or LC90 in ppm

2: L.E.P.R. line estimated by probit regression

*: LC50 Or LC90 values having different letters are significantly different at (95% CL didn't overlap)

-SPSS program for probit analysis was used and chi square for means separation

The X2 values with the corresponding degrees of freedom were also shown in Tables (2 and 3). The X2 values for each extract were less than that of the tabulated X2 at the corresponding df and 5%

probability. Bioassay mortalities were plotted against the concentrations that were tested to get log dosage-probit mortality curves (Figures 1-5).

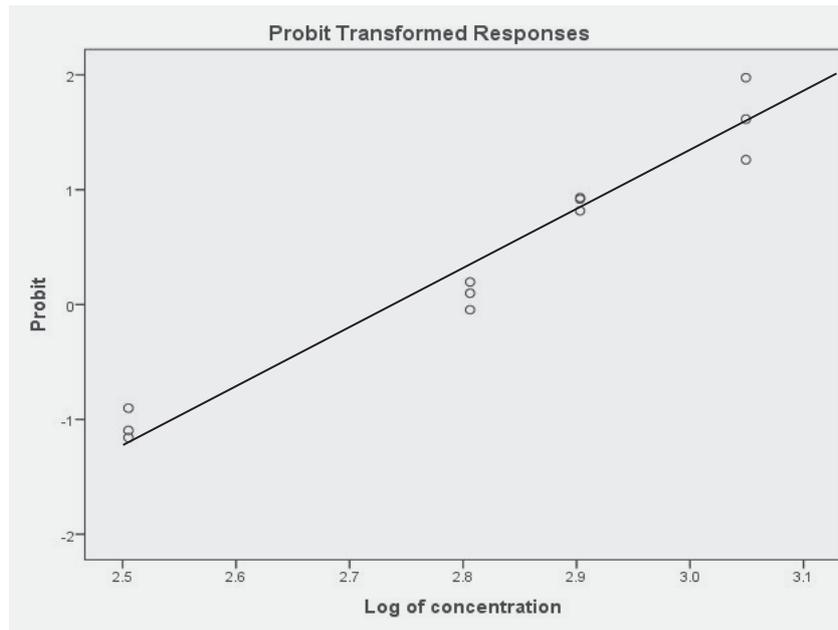


FIGURE 1
 Log dosage-probit mortality line for ethanol extract of *R. communis* against the green peach aphid by using dipping method.

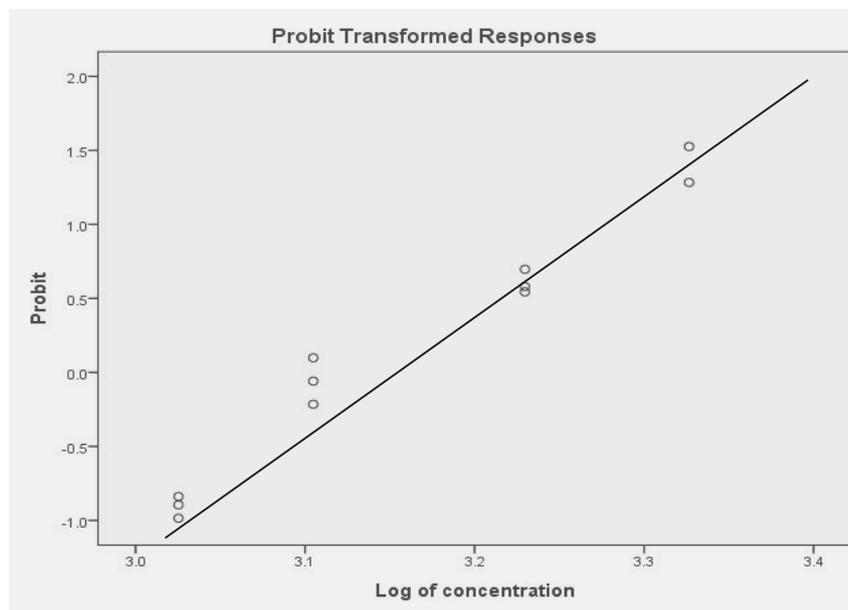


FIGURE 2
 Log dosage-probit mortality line for ethanol extract of *S. nigrum* against the green peach aphid by using dipping method.

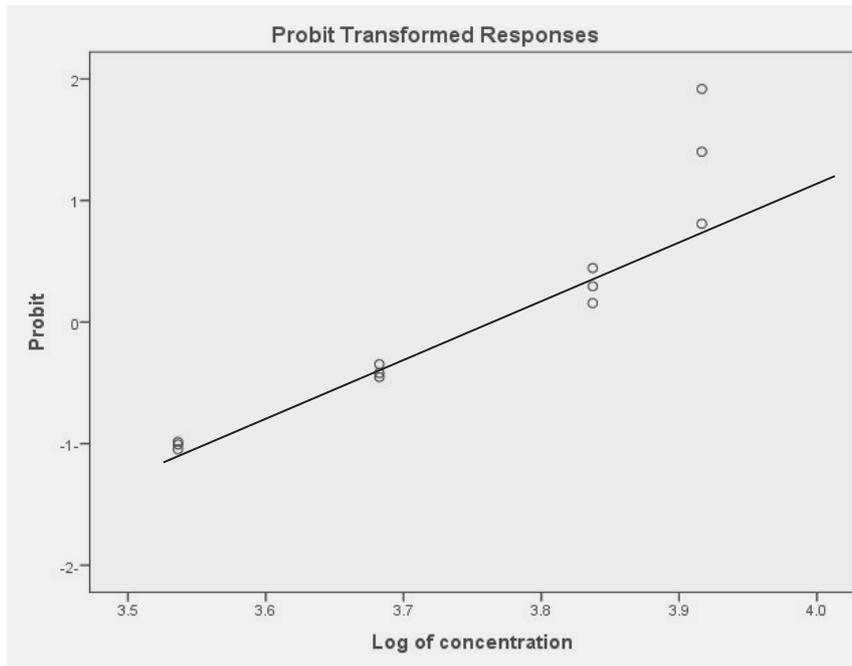


FIGURE 3
 Log dosage-probit mortality line for ethanol extract of *N. oleander* against the green peach aphid by using dipping method.

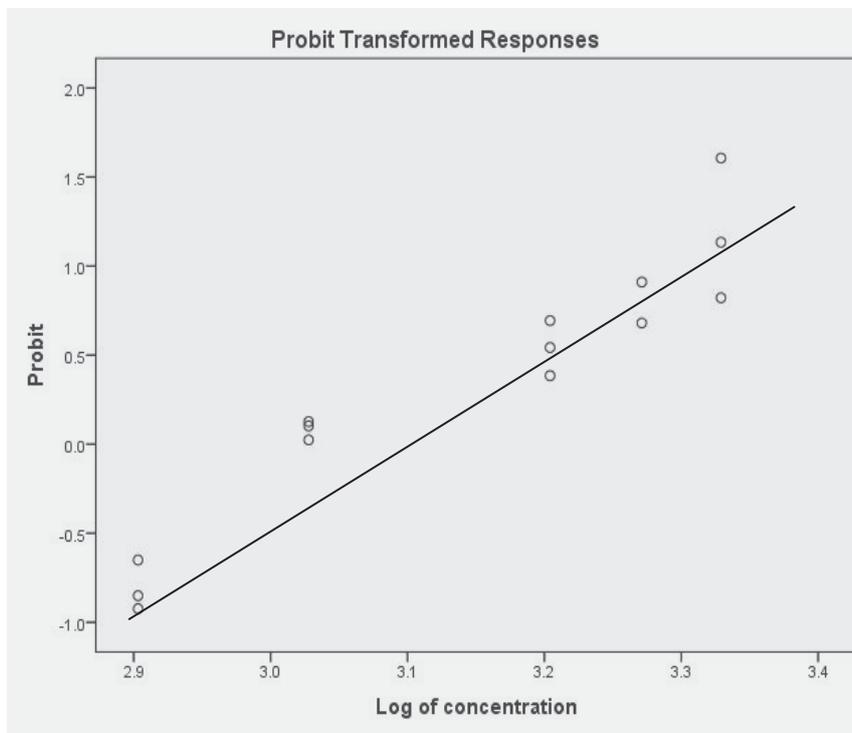


FIGURE 4
 Log dosage-probit mortality line for ethanol extract of *R. pseudoacacia* against the green peach aphid by using dipping method.

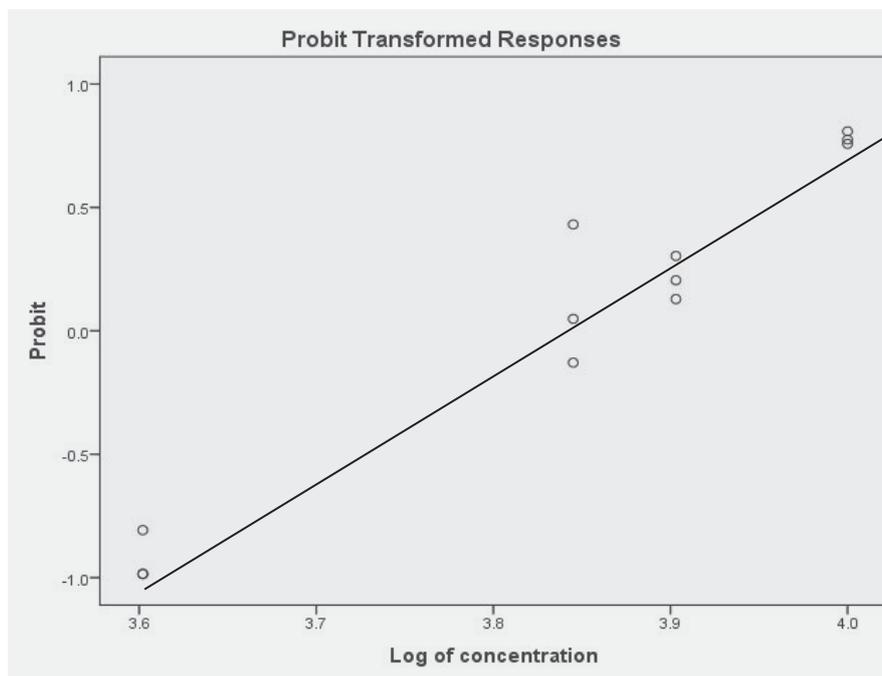


FIGURE 5

Log dosage-probit mortality line for ethanol extract of *L. camara* against the green peach aphid by using dipping method.

Plant extracts toxicity evaluation.

Evaluation of the ethanol plant extracts. Results after 24 hours are shown in Table (4). The positive control (cypermethrin insecticide) caused 90.82% mortality, followed by *R. pseudoacacia* which caused 76.46% mortality, but they were not significantly different. *R. communis* and *L. camara* caused 58.6% and 55.44% mortality rates, respectively. They were not significantly different from each other, but were significantly different from the other treatments. On the other hand, *S. nigrum*, *N. oleander*, and *I. viscosa* caused 28.54%, 25.2%, and 37.22% mortality rates, respectively, which were not significantly different from each other, but differ significantly from the other treatments. The negative control had 3.24% mortality rate and was significantly different from the other treatments.

Results after 48 hours are shown in Table (4). There was an increase in the mortality rate over 48 hours. There was no significant difference between positive control and *R. pseudoacacia* which had

93.06% and 83.3% mortality rates, respectively, followed by *R. communis* which caused 65.48% mortality rate, then *L. camara*, *S. nigrum*, *I. viscosa*, and *N. oleander*, which caused 62.32%, 56.8%, 52.44%, and 46.96% mortality rates, respectively. They were not significantly different from each other, but differ significantly from other treatments, although the negative control had 3.84% mortality. For 72 hours results, which are also shown in Table (4) little increase compared with the 48 hours results with the positive control was appeared. The positive control caused 94.76% mortality rate. The effective extract with the highest mortality was *R. pseudoacacia* that caused 85.86% mortality which was not significantly different from the control, followed by *R. communis*, *L. camara*, *I. viscosa*, and *S. nigrum*, causing 67.46, 63.78%, 60.32%, and 57.42% mortality rates. They were not significantly different from each other except *N. oleander* .. *N. oleander* caused the least mortality rate causing 48.32%. On the other hand, the negative control had 8.8% mortality rate.

TABLE 4
Mortality rates of the green peach aphid caused by the plant extracts using ethanol.

| Plant (Treatment) | Average mortality ± S E * | | |
|-----------------------------|------------------------------|----------------|----------------|
| | After 24 hours | After 48 hours | After 72 hours |
| <i>Ricinus communis</i> | 58.60± 6.5B | 65.48±7.39 B | 67.46±6.38 B |
| <i>Solanum nigrum</i> | 28.54±7.0 C | 56.8±8.29 BC | 57.42±8.28 BC |
| <i>Nerium oleander</i> | 25.20±5.43 C | 46.96±2.38 C | 49.32±2.69 C |
| <i>Robinia pseudoacacia</i> | 76.46±1.69 A | 83.3±2.37 A | 85.86±2.00A |
| <i>Lantana camara</i> | 55.44±8.34 B | 61.32±6.17 BC | 63.78±5.72 B |
| <i>Inula viscosa</i> | 37.22±4.85 C | 52.44±4.87 BC | 60.32±4.87 BC |
| Cypermethrin | 90.82±2.63 A | 93.06±3.34 A | 94.76±2.41 A |
| Control | 3.24±3.25 D | 3.84±1.10 D | 8.8±1.82 D |

*Means in each column sharing the same letters don't differ significantly using LSD test at 5% level.

DISCUSSION

This study tested the aphicidal activity using ethanol solvent system for the previous mentioned six plant species against the GPA. The efficacy of the plant extract was measured by the contact toxicity mode of action on the GPA. The GPA is a piercing sucking insect which couldn't be affected by stomach insecticides unless the insecticides are systemic [15]. Direct comparisons of the aphicidal activity of the plant extracts are not possible as no other study on these six plant species has evaluated the relationship between the extract concentrations and mortality percent of the GPA. *R. communis* could be considered the most toxic to the GPA as it has the lowest LC50 and LC90 after 24 hours as shown in Tables (2-3). *R. communis* the castor bean has been used to control insect pests in several crops (Ramos-Lopez et. al., 2010). Also castor bean *R. communis* L. has been widely studied, because of its toxic principle including the alkaloid ricinine, and the protein lectins called ricin within the seed that found useful in cancer therapy [16]. Olaiya et. al. [16] demonstrated that the GPA died 8-24 hours after feeding on the castor bean.

Extraction and thin layer chromatography (TLC) and high performance liquid chromatography analysis separated and showed the presence of ricinine in the GPA that fed on the castor bean but not on the leaves of the control which was (beans *Phaseolus vulgaris*). The ethanol extract of the *R. communis* showed the lowest

LC50 553 ppm. Ramos-Lopez et. al. (2010) found that the castor oil and ricinine were active ingredients of the *R. communis* that affected *Spodoptera frugiperda*. It was found that seed extract exhibited better insecticidal activity than leaf extracts. The half maximum larvae viability concentrations (LVC50) were 380ppm for ricinine, 269ppm for the castor oil, 483 ppm for methanol leaves extract, 507ppm for the ethyl acetate leaves extract, and 1001ppm for the hexane leaves extract. In addition to the seeds, ricinine is also present in the leaves [17]. Ramos-Lopez et. al. [18] suggested that ricinine is the compound responsible for the high activity 483 ppm (LVC50) in the MeOH Leaves extract. Therefore, the same might be applied for the ethanol extract in this study as ricinine was responsible for the aphicidal activity. Upanasi et. al. [17] isolated flavanoids and showed the potential insecticidal, ovicidal, oviposition and deterrent activities of flavonoids against *Callosobruchus chinensis* (Coleoptera: Bruchidae). Flavonoids are reported as a major class of phytochemicals constituting 5-10% of the known secondary metabolites in plants for defense mechanism exerting toxic effects on insects [17]. Methanol leaves extract caused 100% mortality rate. It was found that quercetin was the major flavonoid present in the leaf extract by using both HPTLC and HPLC [17].

In another study conducted by Ghosh et. al. [19] who found that 95% ethanol extract of *R. communis* significantly affected the mortality rate

of ticks in dose dependent manner ranging from 35±5% to 95±5% with additional effect on reproductive physiology of tick by inhibiting 36.4-63.21%. Moreover, they found that the cidal and oviposition limiting properties of the extract were separated by fractionation and detected by HPTLC and reported that quercetin, gallic acid, flavone and kaempferol which seemed to have synergistic acaricidal effect. Moreover, Taha et.al [20] stated that castor bean ethanol extract had the greatest larvicidal activity against the 3rd. larval instar of *Anopheles arabensis* (Diptera :Culicidae) with the lowest LC50 282.7ppm. This was probably due to the presence of the alkaloid ricinine and the ricie protein which are toxic substances. All these reports results might be parallel to the results obtained by this study and these results confirm the toxicity of the ethanol leaves extract of *R. communis*. The second most toxic plant extract was *R. pseudoacacia* leaves extract, there are many reports that show and confirm the results of this study. Alexenizer and Dorn [21] screened 121 medicinal and ornamental plants for the insecticidal and growth regulation activity against the milkweed bug *Oncopeltus fasciatus* (Hemeiptera: Lygaeidae) excluding *L. camara* and *N. oleander*, the other four plant species were included, and the most pronounced effect was the extract of the *Rumex crispus* which caused 50% mortality during adult molting, while the leaves extract of *R. pseudoacacia* had 35% mortality also it caused prolongation of the last fifth instar. On the other hand, *R. communis* leaves extract caused only 10% mortality rate, and *S. nigrum* caused only 5% mortality, whereas *I. viscosa* had 10% mortality. The extracts were topically applied acetone and methanol were used for the extraction in 2:1 (v/v) ratio comparing these results with toxicity evaluation Table (4) for the ethanol leaves extracts for *R. pseudoacacia* which was 76.4% mortality rates and that for *R. communis* was 58.6% % of the GPA after 24 hours ,respectively, the higher mortality rates results in this study compared to results obtained against the milkweed bug and may be due to the smaller size of the GPA than that of the milkweed bug and might be due to the thickness of the cuticle of each the milkweed bug. Korczynski and Kuzminski, [22] found that a statistically significant reduction in the size of damage that was recorded on pine twigs treated with methanol extract of leaves of 8 plants out of 24 plants screened against pine weevil *Hyllobius abietis* (Coleoptera: Curculionidae) and the strongest antifedent activity was methanolic extract of leaves of *Lupinus polyphyllus*, then *R. pseudoacacia* which had 25.2±5.2 mm² compared to the control 131.2±10.97 mm² and then

Convallaria majalis. These reports confirmed the toxicity of the *R. pseudoacacia* against the GPA.

There are several studies that might confirm the toxicity of some of these plants against insects. Obeng-Ofori and Freeman, [23] reported that water and acetone ground leaves extracts of *R. communis* which were topically applied had 100%, 96% and 100% mortality rates for both *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), while *S. nigrum* had 40%, 36%, 70% and 63% mortality, respectively, after 48 hours. These results also confirmed the toxicity of the tested plants *R. communis* and *S. nigrum* in this study. *S. nigrum* contains solanine and solasodine which might be the direct reason of killing the insects [24]. Solanine comprises 95% of the total alkaloid concentration present in the plant .However; it considered one of the major defenses as it is toxic even in small amounts. Its function and activity has been extensively studied [25].

It was also found from the present study that, the mortality caused by these plants extracts was dose dependent. The mortality increased with time after 24, 48, and 72 hours. Mamoci et. al. [26] found that *Dettrichia viscosa* (*I. viscosa*) and *Ferula communis* had biological activates against *Spodoptera lituralis* and *M. persicae*, the GPA, and aphid antifeedant action being the most important biological activity. They found that bioassay guided fractionation research for aphid antifeedant compounds resulted in the isolation of two sesquiterpene lactones; tomentosin and inuviscolide. The extracts were rich with tomentosin from *D. viscosa*. They were more active than ferulenal rich extract from *F. communis*. Katerinopoulos et. al. [27] isolated the sesquiterpene acid from *D. viscosa* as it had allelopathic activity against phytopathogenic fungi and acari.

Concerning *N. oleander* leaf extracts, which were significantly different from control. Bagari et. al. [28] reported the toxic effect of *N. oleander* leaves which was used as a stable food on the development, food intake and mortality of the fourth instar larvae of the desert locust under lab. Conditions. They found that *N. oleander* fed larvae cumulative mortality rate was over 50% and was obtained from the 4th day and the total mortality was recorded at 12th day. In addition, they found that no larvae fed with *N. oleander* molted during the experimental period, compared to the control larvae fed on *Brassica oleracea*. This effect could be due to secondary compounds contained in the leaves. Prabowo [29] reported that the use of the *N. oleander* leaf extract caused mortality of the thrips *Scolothrips rubrocinctus* (Thysanoptera:

Thripidae) 21-72% mortality at 72-144 hours after the spraying.

Roni et. al. [30] reported that *N. oleander* with different solvent leaf extracts exerted ovicidal, adulticidal, and smoke toxicity effect against the malaria vector, *Anopheles stephensi* (Diptera: Culicidae) and the leaf extract treated eggs exhibited a delayed hatchability. This might be due to the action of phytochemical present in the extract. The larvae which hatched out of the treated eggs were dead within few hours. The active substances of *N. oleander* were toxic to the malaria vector and it might have capability to interfere with the endocrine system, neuronal system and reproductive systems of *A. stephensi* [30]. El-Shazly et. al. [31] also conducted bioassay guided fractionation of an ethanolic leaf extract of *N. oleander* against the western-banded blow fly *Chrysomya albiceps* (Diptera: Calliphoridae) and isolated the cardiotoxic glycoside neriifolin from the insecticidal active fraction. They reported the insecticidal activity of the *N. oleander* for different insect species. These reports were in agreement with the *N.oleander* ethanol leaf extract results which were with significant activity against the GPA and confirmed what has been found in the present investigation.

Actually, *L. camara* had the highest LC50 and LC90 after 24 hours after the treatments. Several studies have been conducted on *L. camara* showed that it had insecticidal activity. Chandel et. al. [32] tested eight plant species under lab. and field conditions. They found that *L. camara* at 2% extract caused 79.4% mortality of the cabbage aphid *Brevicorne brassicae* (Homoptera: Aphididae) after 72 hours in the field. Deshmukhe et. al. [33] tested a crude aqueous extract from leaves of *L. camara* (*L.*) for the insecticidal activity on the fourth instar larvae of the *Spodoptera litura* (*F.*) (Lepidoptera: Noctuidae) under lab conditions. Maximum total percent mortalities of 96.66% and 66.6% were recorded at the larval and pupal intermediate stage at 40% concentration in topical treatment, which indicated the direct insecticidal action of the plant extract. LC50 and LC90 were found to be 19.95 and 35.6 and topical treatment caused deformities of 6.66% at 40% concentration which might be due to change in the ecdysteroid titre. The larval mortality might be due to feeding inhibition or gustatory repellency or impairment in the food assimilation. All these effects of the crude aqueous extract of *L. camara* leaves might be due to the active toxic group like lantadene present in the leaves. These reports might be in agreement with what has been found in the present study that *L. camara* had significant effect on the GPA.

CONCLUSIONS

In this study the results for toxicity evaluations were significantly different compared to the controls. The toxic leaf extracts for the ethanol contain active substances that have the aphicidal activity against the green peach aphid. The most toxic plant extract could be used in the control of the GPA in the green house as an alternative to the insecticides in the IPM programs or in the organic culture. The active toxic substances could be isolated and tested for further future studies.

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IMPACT OF TRAFFIC ON THE CONTENT OF TRACE ELEMENTS IN SOILS ALONG STATE ROAD 53 (POLAND)

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ABSTRACT

Development of transport causes air, soil, ground and surface water pollution which brings high risk to human life and health. Heavy metals pollution cause soil alkalization or acidification and a violation of its ionic balance. The concentrations of heavy metals in soil directly influence on plants and indirectly - animals, and humans. The aim of the study was to determine the content of lead, cadmium, chromium, nickel, manganese, iron and cobalt in soils in different distances from the State Road 53 between Olsztyn and Szczytno, northeastern Poland. The studied section of the State Road 53 in terms of trace elements in soil is not a high threat to environment. Car traffic on this route results in higher than natural accumulation of lead, zinc, nickel (in some sites), copper and cobalt in soil. The most significant downward trend of elements in soil accumulation with increasing distance from the road was recorded in the case of copper. The concentration of chromium and iron were the highest in the furthest soils from the road, which may have been caused by the agricultural use of the land or by their higher natural content in the parent rock.

KEYWORDS:

road, traffic, pollution, soil, trace elements

INTRODUCTION

Transportation is an important branch of economy, but it is harmful to the natural environment, both locally and on a global scale, causing irreversible changes in the soils. According to the Polish Central Statistical Office [1], there were 24,876,000 registered cars and tractors in Poland in 2013, of which around 75 %, i.e. 18,744,000 were passenger cars. Such a huge number of vehicles travelling over the Polish network of roads poses and also car factories, manufactures of car maintenance materials and spare parts, and the transportation infrastructure a

threat to the natural environment as an anthropogenic source of contamination of air, soils and waters [2, 3].

Beside the broadly understood transport and traffic, other sources of soil contamination are emissions from industrial plants, agriculture, municipal maintenance works and landfills, which can easily permeate into the water environment [4] or, as dust, enter the atmosphere [5-7]. Thus, they also affect crops [8-11]. The content of trace elements in soil is caused by the climate, type of soil, geomorphology of a given area [11], as well as atmospheric conditions, such as type of precipitation and wind intensity [12]. The plant cover is another contributing factor [12].

Transportation is also responsible for emission of heavy metals to the environment, which are adsorbed onto particles of solid dust (particulate matter, PM) and penetrate into soil [2]. Particulates are released from the exhaust system of an engine. They contain heavy metals, carbon, nitrogen and sulphur compounds as well as hydrocarbons. Faiz et al. [6], Liu et al. [7], Christoforidis and Stamatis [13], Johansson et al. [14], Helmreich et al. [15], Duong and Lee [16] claim that trace elements found in soil originate from the combustion of fuel or leakage of oil from cars, but they are also produced by the wear of brakes, clutch facings and tyres, from used road surfaces, rusting metal elements of a car body, from contaminations in fuels and lubricants and from metal compounds added to fuel to raise its octane number. Solid particulates have been found to comprise large quantities of elements, of which the major ones are lead, chromium, cadmium, zinc, nickel, copper, silicon, sulphur, calcium and phosphorus [2, 17]. Heavy metals emitted to the environment due to fuel combustion interfere with the chemical equilibrium of soils. Dry and wet precipitation, which contains heavy metals as well as other substances, causes excessive accumulation of elements in soil, increased acid or alkaline soil reaction, and higher solubility of heavy metals bounded with sorbents [18]. The high content of trace elements in the environment are toxic to organisms [19-27].

The above considerations have stimulated the current investigations, whose purpose was to determine the content of lead, cadmium, chromium, nickel, manganese, iron and cobalt in soils in different distances from State Road 53 between Olsztyn and Szczytno, northeastern Poland.

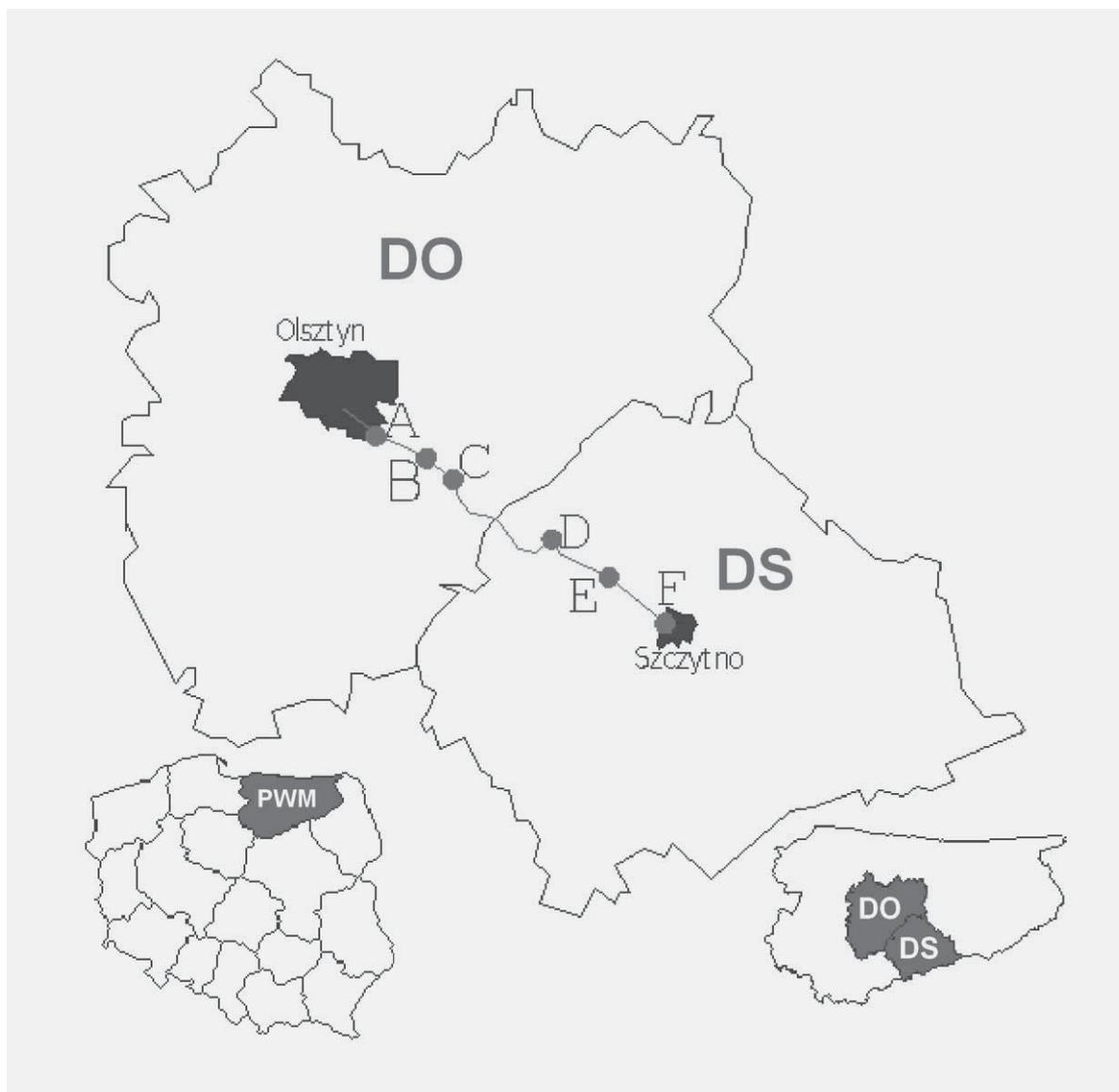
MATERIAL AND METHODS

Collection of samples. The study encompassed 6 towns and villages along State Road 53, from Olsztyn to Szczytno, northeastern Poland. Research sites were located at the exit streets from Olsztyn (53°45'02.0"N 20°32'04.9"E), Klewki (53°44'11.0"N 20°33'58.7"E), Trękusek (53°42'36.7"N 20°37'59.5"E), Pasym (53°38'15.8"N 20°48'30.1"E), Grom (53°36'49.0"N 20°52'08.3"E) and Szczytno (53°34'20.9"N 20°57'11.8"E) towns and villages which lie along the above communication route. The exact soil sampling sites are shown in Figure 1. In each of the above locations, soil was sampled from the topsoil at 4 sampling areas, situated on the edge of the road and then 25, 50 and 100 m away from it. Over a dozen single samples of soil were collected at every site from the depth of 25 cm according to ISO 10381-5 [28]. The representative sample of 1000 g weight (after mixing) for each sampling point was achieved. The study was carried out in May 2012. The average daily traffic from Olsztyn to Pasym was composed of 5 335 vehicles, including 870 lorries and 93 coaches and from Pasym to Szczytno - 4 799, 855 and 80, respectively [29]. The average daily traffic was measured using automated techniques - video recording and continuous traffic flow measurement stations, and manually - by trained observers [29]. State Road 53 is one of the three state roads connecting the Province of Warmia and Mazury with the Province of Masovia. The sampling areas in Olsztyn, Klewki and Trękusek lie in the District of Olsztyn, while Pasym, Grom, Szczytno belong to the District of Szczytno. In all the sites, the land was covered with grass growing on sandy and organic soils. Besides, the site in Klewki was located near Linowskie

Lake, and the village of Grom lies on Machniacz Lake. No larger industrial plants were found near the analyzed locations.

HNO₃ extraction of heavy metals in soil samples. The sampled soil material was dried at room temperature, crushed and passed through a 0.1 mm mesh sieve and stored at room temperature until analysis. Then, it was wet extracted in nitric acid (V) (HNO₃ analytical grade) of the concentration equal 1.40 g/cm³, in Teflon vessels HP500 placed in a MARS 5 microwave oven (Microwave Accelerated Reaction System, manufactured by CEM Corporation, USA). All analytical parameters, such as the size of a weighed portion, volume of nitric acid and temperature, were compliant with the US-EPA3051 method [30]. Each sample of 0.5 g weight was placed in a closed microwave vessel and drenched with 10 ml of concentrated HNO₃. Heating program were realized in 2 stages: 1st stage: ramp to temperature 160 °C - 10 minutes, hold - 5 minutes, 2nd stage: ramp to temperature 170 °C - 10 minutes, hold - 10 minutes. After cooling and filtering the samples were moved to measuring 50 ml volumetric flasks and made up to the mark with distilled deionized water [30].

Heavy metal analysis in soil samples. The soil samples thus prepared were submitted to determinations of the total content of trace elements: lead, cadmium, chromium, nickel, manganese, zinc, copper, iron and cobalt, with the flame atomic absorption spectrophotometric method (FAAS) in an air and acetylene flame on a SpectrAA 240FS spectrophotometer (VARIAN, Australia), using a Sample Introduction Pump System. The analysis conditions were as follows in US-EPA3051 method [30]. Certified reference material (RTC-CRM023) was used for analyses. Quality control were determined by method of analyses of blanks, duplicates, and certified reference materials made by Sigma Aldrich Chemie GmbH at the beginning and after each series of sample analyses. Recoveries of certified reference materials for all elements ranged between 89% and 111%.



PWM - Province of Warmia and Mazury; DO - District of Olsztyn; DS - District of Szczytno
 A - Olsztyn (53°45'02.0"N 20°32'04.9"E), B - Klewki (53°44'11.0"N 20°33'58.7"E),
 C - Trękuszek (53°42'36.7"N 20°37'59.5"E), D - Pasym (53°38'15.8"N 20°48'30.1"E),
 E - Grom (53°36'49.0"N 20°52'08.3"E), F - Szczytno (53°34'20.9"N 20°57'11.8"E)

FIGURE 1

Map presenting the distribution of sampling sites near State Road 53 from Olsztyn to Szczytno, northeastern Poland

Statistical analysis of the results. The results were processed statistically, using ANOVA two-factorial analysis of variance (1st factor - town/village, 2nd factor - distance from route). The Pearson's simple correlation coefficients (r) between the analyzed variables were calculated. Standard deviation errors (SD) were calculated in order to reflect the average results of chemical analysis. The Pearson's correlation coefficients were classified as follows: $r < 0.665$ - weak

correlation, $0.666 < r < 0.798$ - medium correlation and $r > 0.799$ - high correlation. The principal component analysis (PCA) was applied to evaluating the effect of traffic on the content of trace elements in soil. The contribution of variables, expressed in %, was also derived from the ANOVA two-factorial analysis of variance, using the eta-square method. All statistical calculations were aided by the software programme Statistica [31].

RESULTS

The content of trace elements in the analyzed soils depended on the distance to the road and on the locality (Tables 1, 2 and 3).

Lead. The content of lead in soils along State Road 53 from Olsztyn to Szczytno ranged from 31.4 to 82.0 mg/kg (Table 1). The highest average lead content (61.8 mg/kg) in soil was detected at the sampling sites in Grom. The highest concentration of this element was observed at the edge of the road (82 mg/kg). In Grom, Szczytno and Olsztyn, a highly significant negative correlation occurred between the content of Pb in the topsoil and the distance to the road. In Grom, the Pb accumulation at 100 m from the road was 43 % lower than at the road edge, which may have been caused the local presence of organic soils.

A lower average lead content was determined in Szczytno (54.4 mg/kg of soil), where it decreased by 29 % between the road edge and 100 m from the road. This result, apart from being impacted by the flow of traffic, might have been due to the proximity of the town Szczytno. In contrast, the lowest average content of lead (38.4 mg/kg of soil) was determined in village Klewki. Similar content were detected in Pasym, Trękusiek and Olsztyn. In Klewki and Trękusiek, the content of lead was higher in sampling sites located further from the road than at its edge. Compared with the content of lead in the topsoil given in the Regulation of the Minister for the Environment [32], which set permissible lead concentrations in the topsoil at no more than 50 mg (protected areas), 100 mg (unprotected areas, including farmland) and 600 mg/kg of soil (communication routes and adjacent areas), our results were found to be much lower than the mentioned thresholds. On the other hand, relative to the results achieved from the Monitoring of the Chemical Properties of Arable Soils in Poland [33], the soils along the Olsztyn-Szczytno road were found to contain much more lead than average for Polish soils (25.0 mg/kg of soil) and average for Province of Warmia and Mazury (12.3 mg/kg of soil). Thus, the distance to a road, average daily traffic and close proximity to a larger town had a substantial effect on the content of lead in soil.

Cadmium. The highest average content of cadmium in soil along State Road 53 from Olsztyn to Szczytno was found in Olsztyn (0.20 mg/kg of soil), with the peak concentration of 0.26 mg/kg at 100 m from the road (Table 1). Beside the emission of metals caused by moving vehicles, the cadmium

concentration may have resulted from the proximity of a large town, such as Olsztyn, and transport of airborne heavy metals adsorbed to particulate matter (PM). Lower average content of cadmium in soil appeared in Trękusiek and Grom, but the lowest ones were in Szczytno. In most localities, a highly significant negative correlation was verified between cadmium accumulation and distance to the road. The content of this metal in soil in 100 m from the road decreased most considerably (by 78 %) in Grom, versus the road edge. The results suggest that a high content of cadmium in soil is induced by the urban settlements. The lowest average cadmium content in soil was observed in Szczytno, which may be explained by the sampling site lying close to a forest, which acted like a natural barrier to the migration of this metal, especially from other sources than traffic. The permissible cadmium content, 15 mg/kg for land under communication routes and 4 mg/kg of soil for farmland, were not surpassed [32]. Relative to the data from the Monitoring of the Chemical Properties of Arable Lands in Poland [33], the cadmium content along the Olsztyn-Szczytno Road was less than the Polish average soil content of this element (0.56 mg/kg) and typical for Province of Warmia and Mazury (0.13 mg/kg of soil).

Chromium. Analyses of the soil samples collected along State Road 53 showed the highest average soil content of chromium in Szczytno (11.90 mg/kg) and in Klewki (11.58 mg/kg of soil) (Table 1). The lowest average content of this metal appeared in the research sites in Pasym (3.05 mg/kg) and Trękusiek (3.65 mg/kg of soil). In most localities, higher soil concentrations of chromium occurred further away from the road edge (Table 1). The biggest differences were determined in Szczytno, Olsztyn and Klewki. The content of this element at a 100-m distance from the road was higher than near the edge of the road by 77 % in Szczytno, 89 % in Olsztyn and by 219 % in Klewki. The differences were smaller in the other localities, and soil samples from Trękusiek demonstrated reverse dependences. The chromium concentrations were below the acceptable content [32] for soils in the vicinity of roads (500 mg/kg of soil) and in farmland (150 mg/kg of soil). Also, according to Kabata-Pendias and Pendias [34], all the determined content of chromium in soil were within the natural limits of soil chromium content. In most sampling sites along the Olsztyn-Szczytno road the accumulation of chromium in soil was similar to or lower than the average content of this element in Polish soils (10.4 mg/kg) and average content for Province of Warmia and Mazury (14.3 mg/kg of soil) [33].

TABLE 1
Content of lead (Pb), cadmium (Cd) and chromium (Cr) in the surface soil layer near the State Road 53
from Olsztyn to Szczytno (averages \pm standard deviation, in mg kg⁻¹ of soil)

| Towns/villages | Distances from route in m | | | | Average | r |
|---|---|---------------------|---------------------|---------------------|---------|----------|
| | along the roadside | 25 | 50 | 100 | | |
| Lead (Pb) | | | | | | |
| Olsztyn | 50.2 (\pm 2.4) | 46.0 (\pm 2.3) | 39.9 (\pm 1.6) | 39.4 (\pm 1.7) | 43.9 | -0.893** |
| Klewki | 31.4 (\pm 1.5) | 36.7 (\pm 0.6) | 41.3 (\pm 1.4) | 44.1 (\pm 1.6) | 38.4 | 0.949** |
| Trękuszek | 36.1 (\pm 2.0) | 44.9 (\pm 2.3) | 45.6 (\pm 0.4) | 46.3 (\pm 2.2) | 43.2 | 0.758* |
| Pasym | 45.3 (\pm 2.2) | 39.0 (\pm 1.9) | 36.8 (\pm 0.6) | 44.6 (\pm 0.9) | 41.4 | 0.046 |
| Grom | 82.0 (\pm 3.6) | 69.5 (\pm 2.6) | 49.0 (\pm 2.2) | 46.6 (\pm 0.6) | 61.8 | -0.904** |
| Szczytno | 69.6 (\pm 3.2) | 49.0 (\pm 0.8) | 49.8 (\pm 1.0) | 49.4 (\pm 3.2) | 54.4 | -0.674* |
| Average | 52.4 | 47.5 | 43.7 | 45.1 | 47.2 | -0.768* |
| LSD for: | town/village - 9.0**, distance from route - 7.3*, interaction - 18.1* | | | | | |
| Average for Province of Warmia and Mazury | | | | | | 12,3 |
| Cadmium (Cd) | | | | | | |
| Olsztyn | 0.20 (\pm 0.01) | 0.15 (\pm 0.01) | 0.17 (\pm 0.01) | 0.26 (\pm 0.02) | 0.20 | 0.672** |
| Klewki | 0.13 (\pm 0.01) | 0.02 (\pm 0.01) | 0.10 (\pm 0.01) | 0.06 (\pm 0.00) | 0.08 | -0.336 |
| Trękuszek | 0.15 (\pm 0.01) | 0.16 (\pm 0.01) | 0.12 (\pm 0.01) | 0.10 (\pm 0.01) | 0.13 | -0.904** |
| Pasym | 0.05 (\pm 0.00) | 0.05 (\pm 0.00) | 0.06 (\pm 0.00) | 0.06 (\pm 0.00) | 0.06 | 0.845** |
| Grom | 0.27 (\pm 0.02) | 0.12 (\pm 0.01) | 0.09 (\pm 0.01) | 0.06 (\pm 0.00) | 0.14 | -0.847** |
| Szczytno | 0.03 (\pm 0.01) | 0.00 (\pm 0.00) | 0.03 (\pm 0.00) | 0.09 (\pm 0.01) | 0.04 | 0.814** |
| Average | 0.14 | 0.08 | 0.10 | 0.11 | 0.11 | -0.368 |
| LSD for: | town/village - 0.05**, distance from route - 0.04**, interaction - 0.09** | | | | | |
| Average for Province of Warmia and Mazury | | | | | | 0.13 |
| Chromium (Cr) | | | | | | |
| Olsztyn | 7.20 (\pm 0.34) | 5.40 (\pm 0.14) | 10.40 (\pm 0.48) | 13.60 (\pm 0.28) | 9.15 | 0.893** |
| Klewki | 5.80 (\pm 0.23) | 11.80 (\pm 0.42) | 10.20 (\pm 0.14) | 18.50 (\pm 0.41) | 11.58 | 0.933** |
| Trękuszek | 9.40 (\pm 0.54) | 0.50 (\pm 0.02) | 1.50 (\pm 0.04) | 3.20 (\pm 0.11) | 3.65 | -0.452 |
| Pasym | 3.00 (\pm 0.14) | 2.40 (\pm 0.04) | 3.40 (\pm 0.13) | 3.40 (\pm 0.14) | 3.05 | 0.599 |
| Grom | 6.90 (\pm 0.31) | 9.50 (\pm 0.34) | 8.60 (\pm 0.14) | 11.10 (\pm 0.32) | 9.03 | 0.882** |
| Szczytno | 9.60 (\pm 0.14) | 8.00 (\pm 0.23) | 13.00 (\pm 0.28) | 17.00 (\pm 0.26) | 11.90 | 0.915** |
| Average | 6.98 | 6.27 | 7.85 | 11.13 | 8.06 | 0.916** |
| LSD for: | town/village - 2.21**, distance from route - 1.80**, interaction - 4.41** | | | | | |
| Average for Province of Warmia and Mazury | | | | | | 14.3 |

LSD (least squares deviation) significant for: * $p=0.05$, ** $p=0.01$; r - correlation coefficient

Nickel. The highest average content of nickel was detected in soil near the road leading out of Olsztyn (28.2 mg/kg of soil), while the lowest one was determined in Grom (12.8 mg/kg) and Szczytno (13.9 mg/kg of soil) (Table 2). The most unambiguous changes in the content of nickel at an increasing distance from the road were identified in Olsztyn, Klewki and Trękuszek. However, while being positive in Olsztyn and Klewki, these changes had a negative character in Trękuszek. Fluctuations in the content of nickel in soil in the other localities were irregular. Relative to the Regulation of the Minister for the Environment [32], our determinations did not reveal excessive content of nickel versus the concentrations set for communication routes (300 mg/kg of soil) and

agricultural areas (100 mg/kg of soil). The content of nickel in soils along the road connecting Olsztyn and Szczytno was higher than the average for Polish soils and average for Province of Warmia and Mazury, reported in the Monitoring of Chemical Properties of Arable Soils in Poland [33].

Manganese. The content of manganese in soils depended on the locality and distance from State Road 53 (Table 2). The soil samples taken in Pasym contained the highest average content of manganese, i.e. 301.0 mg/kg of soil. Similar content of manganese were determined in the upper layer of soil in Klewki (261.4 mg/kg), Grom (260.9 mg/kg) and Szczytno (258.5 mg/kg of soil). In Grom and Trękuszek, the content of manganese decreased

further away from the road, unlike in Pasy and Olsztyn, where it was lower nearer the road. The lowest average content of manganese (218.7 mg/kg of soil) was determined in Olsztyn. The concentrations of manganese in soils along the Olsztyn-Szczytno Road were lower than the average level for Polish soils (397 mg/kg of soil) and average for Province of Warmia and Mazury (357 mg/kg of soil), reported in the Monitoring of Chemical Properties of Arable Soils in Poland [33].

Zinc. The highest average content of zinc (135 mg/kg of soil), much higher than in the other sites, was observed in Grom (Table 2). Compared to the average zinc content in Polish soils (79.8 mg/kg) and average for Province of Warmia and Mazury (42.7 mg/kg of soil) [33], far more zinc accumulated in soil in Grom. Moderate quantities of zinc were accumulated in soils from Szczytno (76.6 mg/kg) and Olsztyn (70.5 mg/kg), and the lowest ones in Klewki (20.4 mg/kg) and Pasy (26.8 mg/kg). A gradual decrease in content of zinc in the soil at a further distance from the road was determined in Grom, where the highest zinc concentration was observed at the edge of the road (305.8 mg/kg of soil). At a distance of 100 m, the content of zinc decreased in the soil samples from Szczytno, but increased in Olsztyn, Trękuszek and especially in Pasy and Klewki, compared to its content in soil by the road. In Pasy and Klewki, it was 3.2- and 5.6-fold lower, respectively, at 100 m from the road than by the road edge. The use of mineral fertilizers in soil with rich organic matter probably increased the content of zinc in soils with maximum distance (100 m) from road at Olsztyn; Klewki; Trękuszek and Pasy. No cases of zinc concentrations surpassing the acceptable content, set by the Regulation of the Minister for the Environment [32] for soils near communication routes or arable lands, were detected.

Copper. The distance to State Road 53 had stronger impact on the copper content of soil than the locality (Table 3). The highest average content of copper in the topsoil were determined in Olsztyn (36.9 mg/kg) and Grom (40.2 mg/kg of soil). Half these amounts were found in the other localities. The highest copper content nearest to the road was determined in Grom (53.4 mg/kg) and Olsztyn (51.0 mg/kg). As the distance to the road increased, the soil content of copper decreased in Olsztyn, Trękuszek, Grom and Szczytno, with the maximum decline of 39, 39, 46 and 57 %, respectively. In

contrast, positive correlations were demonstrated in Klewki and Pasy. The determined copper concentrations did not exceed the acceptable content of copper set by the Regulation of the Minister for the Environment [32]. The content of copper in soils along the road leading from Olsztyn to Szczytno was higher than the average level in Polish and in Province of Warmia and Mazury soils [33].

Iron. The highest average content of iron in the topsoil along the road from Olsztyn to Szczytno was noted in the research sites in Klewki (11 242 mg/kg) and Pasy (11 219 mg/kg), while the lowest one was in Szczytno (5 464 mg/kg) (Table 3). In Olsztyn, Klewki and Pasy, highly significant positive correlation was observed between the iron accumulation and distance from the road edge. In these three localities, the content of iron was almost twice as high 100 m from the road than at its edge. Unlike these three sites, tendencies in the level of iron in soils in Trękuszek and Szczytno were demonstrably negative. In most of the analyzed samples, the content of iron was lower than the average concentration in Polish (9 600 mg/kg) and Province of Warmia and Mazury (12 510 mg/kg) soils [33].

Cobalt. The content of cobalt in soil was strongly dependent on the locality, but less on the distance from the road edge (Table 3). The highest average cobalt content in the upper soil layer was determined in Szczytno (19.65 mg/kg) and the lowest one - in Olsztyn (2.55 mg/kg). A possible reason could be the high average daily traffic in Szczytno. At a distance of 100 m, the content of cobalt in soil sampled in Szczytno was 18 % lower than at the road edge. In the other localities, except for Klewki, the cobalt content was the lowest at the road edge. Relative to the Regulation of the Minister for the Environment [32], the determined cobalt concentrations did not exceed the acceptable content for land near communication routes (30 mg/kg of soil) and were only slightly above the limit for agricultural land (20 mg/kg of soil) at one site, namely 50 m from the road in Szczytno (21.20 mg/kg). With respect to the average cobalt content in Polish and Province of Warmia and Mazury soils, reported in the Monitoring of Chemical Properties of Arable Soils in Poland [33], the cobalt concentrations in soils along the Olsztyn-Szczytno road were much higher.

TABLE 2
Content of nickel (Ni), manganese (Mn) and zinc (Zn) in the surface soil layer near the State Road 53 from Olsztyn to Szczytno (averages \pm standard deviation, in mg kg⁻¹ of soil)

| Towns/villages | Distances from route in m | | | Average | r | |
|---|--|--------------------|--------------------|--------------------|-------|----------|
| | along the roadside | 25 | 50 | | | 100 |
| Nickel (Ni) | | | | | | |
| Olsztyn | 26.0 (\pm 0.6) | 28.2 (\pm 0.2) | 29.1 (\pm 0.9) | 29.4 (\pm 0.4) | 28.2 | 0.860** |
| Klewki | 16.6 (\pm 0.7) | 16.6 (\pm 0.7) | 17.7 (\pm 0.4) | 22.1 (\pm 0.8) | 18.3 | 0.943** |
| Trękusek | 22.7 (\pm 0.7) | 17.3 (\pm 0.7) | 17.5 (\pm 0.7) | 13.0 (\pm 0.6) | 17.6 | -0.938** |
| Pasym | 14.3 (\pm 0.6) | 26.4 (\pm 0.2) | 38.3 (\pm 0.4) | 10.0 (\pm 0.6) | 22.3 | -0.195 |
| Grom | 10.6 (\pm 0.4) | 11.2 (\pm 0.2) | 9.2 (\pm 0.2) | 20.2 (\pm 0.2) | 12.8 | 0.811** |
| Szczytno | 13.1 (\pm 0.5) | 19.4 (\pm 0.3) | 5.8 (\pm 0.3) | 17.2 (\pm 0.5) | 13.9 | 0.087 |
| Average | 17.2 | 19.9 | 19.6 | 18.7 | 18.8 | 0.302 |
| LSD for: | town/village - 4.9**, distance from route - n.s., interaction - 9.9** | | | | | |
| Average for Province of Warmia and Mazury | | | | | 11.2 | |
| Manganese (Mn) | | | | | | |
| Olsztyn | 167.4 (\pm 5.3) | 209.1 (\pm 6.9) | 242.5 (\pm 5.8) | 255.7 (\pm 7.7) | 218.7 | 0.922** |
| Klewki | 262.1 (\pm 6.4) | 251.5 (\pm 7.9) | 228.0 (\pm 7.5) | 303.8 (\pm 9.8) | 261.4 | 0.574 |
| Trękusek | 272.7 (\pm 8.3) | 215.1 (\pm 6.9) | 236.8 (\pm 7.3) | 218.1 (\pm 7.2) | 235.7 | -0.653 |
| Pasym | 239.3 (\pm 1.0) | 332.5 (\pm 4.4) | 318.8 (\pm 9.7) | 313.4 (\pm 9.2) | 301.0 | 0.543 |
| Grom | 301.6 (\pm 1.3) | 268.5 (\pm 8.9) | 257.9 (\pm 6.0) | 215.5 (\pm 3.3) | 260.9 | -0.988** |
| Szczytno | 234.8 (\pm 2.4) | 382.7 (\pm 9.9) | 274.8 (\pm 5.9) | 141.7 (\pm 5.2) | 258.5 | -0.607 |
| Average | 246.3 | 276.6 | 259.8 | 241.4 | 256.0 | -0.377 |
| LSD for: | town/village - 34.0**, distance from route - 28.6*, interaction - 69.9** | | | | | |
| Average for Province of Warmia and Mazury | | | | | 357.0 | |
| Zinc (Zn) | | | | | | |
| Olsztyn | 56.1 (\pm 1.7) | 51.9 (\pm 1.7) | 73.7 (\pm 0.9) | 100.3 (\pm 3.4) | 70.5 | 0.949** |
| Klewki | 8.0 (\pm 0.3) | 6.9 (\pm 0.2) | 22.1 (\pm 0.7) | 44.6 (\pm 1.3) | 20.4 | 0.964** |
| Trękusek | 29.1 (\pm 0.9) | 44.9 (\pm 1.3) | 37.1 (\pm 1.1) | 61.7 (\pm 1.5) | 43.2 | 0.890** |
| Pasym | 11.3 (\pm 0.3) | 25.2 (\pm 0.6) | 34.4 (\pm 0.6) | 36.2 (\pm 1.1) | 26.8 | 0.880** |
| Grom | 305.8 (\pm 9.3) | 79.9 (\pm 1.6) | 77.7 (\pm 1.6) | 77.5 (\pm 2.3) | 135.2 | -0.689* |
| Szczytno | 84.4 (\pm 1.6) | 104.4 (\pm 2.6) | 79.0 (\pm 3.3) | 38.6 (\pm 1.0) | 76.6 | -0.845** |
| Average | 82.5 | 52.2 | 54.0 | 59.8 | 62.1 | -0.496 |
| LSD for: | town/village - 6.1**, distance from route - 5.0**, interaction - 12.2 | | | | | |
| Average for Province of Warmia and Mazury | | | | | 42.7 | |

LSD (least squares deviation) significant for: * p=0.05, ** p=0.01; r - correlation coefficient

Relations between trace elements in soil.

The calculated correlation coefficients (r) verified the presence of significant dependences between lead and nickel, zinc, copper, iron and cobalt; cadmium and zinc; copper and cobalt; nickel and iron and cobalt; manganese and iron; iron and cobalt (Table 4). All the above relationships were positive, except for the correlations between: lead versus nickel and cadmium; cadmium, nickel and iron versus cobalt.

Figure 2 shows that in the soils along State Road 53, from Olsztyn to Szczytno, the first two principal components represent 56.46 % of the variance of original variables, with 31.21 % of the variance attributed to the first component and 25.25 % to the second one. The vectors illustrating soil concentrations of cadmium, copper, zinc, lead and cobalt nearly touch the edges of the unit circle (a circle with the radius equal 1), meaning that they

are very well represented by the first two principal components which create the system of coordinates. The soil content of nickel and iron were reflected somewhat more weakly. The vectors of lead and zinc were more strongly directed towards the first principal component, which proves that their response to the contamination from motor vehicles was similar and positive. By analogy, weaker correlations were observed for copper and cadmium. Reverse correlations were noted with respect to the soil content of nickel and iron. Vectors reflecting the original variables for lead and zinc, copper and cadmium, as well as iron and nickel in soil are similar to one another, which indicates that the relationships between them were positive. On the other hand, the vectors between lead versus nickel and iron, and especially between cobalt versus cadmium, nickel and iron were considerably distant, which illustrates negative correlations between these elements.

TABLE 3
Content of copper (Cu), iron (Fe) and cobalt (Co) in the surface soil layer near the State Road 53 from Olsztyn to Szczytno (averages \pm standard deviation, in mg kg⁻¹ of soil)

| Town/villages | Distances from route in m | | | | Average | r | |
|---|--|---------------------|---------------------|---------------------|---------|----------|--|
| | along the roadside | 25 | 50 | 100 | | | |
| Copper (Cu) | | | | | | | |
| Olsztyn | 51.0 (\pm 1.5) | 29.3 (\pm 0.7) | 35.8 (\pm 0.6) | 31.3 (\pm 1.2) | 36.9 | -0.633 | |
| Klewki | 22.0 (\pm 0.6) | 23.3 (\pm 0.5) | 22.3 (\pm 0.1) | 27.3 (\pm 0.1) | 23.7 | 0.879** | |
| Trękusek | 27.9 (\pm 0.8) | 19.0 (\pm 0.6) | 16.3 (\pm 0.5) | 16.9 (\pm 0.4) | 20.8 | -0.556 | |
| Pasym | 19.8 (\pm 0.8) | 27.0 (\pm 0.7) | 24.0 (\pm 0.6) | 24.1 (\pm 0.7) | 23.7 | 0.351 | |
| Grom | 53.4 (\pm 0.6) | 41.0 (\pm 0.6) | 37.5 (\pm 1.1) | 28.9 (\pm 1.3) | 40.2 | -0.956** | |
| Szczytno | 28.0 (\pm 0.6) | 25.1 (\pm 0.9) | 14.7 (\pm 0.3) | 12.1 (\pm 0.4) | 20.0 | -0.929** | |
| Average | 33.7 | 27.5 | 25.1 | 23.9 | 27.5 | -0.871** | |
| LSD for: | town/village - 3.8**, distance from route - 3.1**, interaction - 7.6** | | | | | | |
| Average for Province of Warmia and Mazury | | | | | | 8.0 | |
| Iron (Fe) | | | | | | | |
| Olsztyn | 5561 (\pm 122) | 6398 (\pm 198) | 10302 (\pm 309) | 11167 (\pm 328) | 8382 | 0.920** | |
| Klewki | 6823 (\pm 203) | 12578 (\pm 186) | 10077 (\pm 120) | 15490 (\pm 146) | 11242 | 0.848** | |
| Trękusek | 10365 (\pm 99) | 5929 (\pm 65) | 4967 (\pm 129) | 4593 (\pm 131) | 6463 | -0.807** | |
| Pasym | 8408 (\pm 299) | 9823 (\pm 127) | 12421 (\pm 137) | 14225 (\pm 257) | 11219 | 0.977** | |
| Grom | 6655 (\pm 118) | 7468 (\pm 96) | 7860 (\pm 98) | 6385 (\pm 89) | 7092 | -0.260 | |
| Szczytno | 5724 (\pm 97) | 7671 (\pm 124) | 5691 (\pm 142) | 2770 (\pm 42) | 5464 | -0.784* | |
| Average | 7273 | 8311 | 8553 | 9105 | 8310 | 0.932** | |
| LSD for: | town/village - 815**, distance from route - 665**, interaction - 1629** | | | | | | |
| Average for Province of Warmia and Mazury | | | | | | 12510 | |
| Cobalt (Co) | | | | | | | |
| Olsztyn | 1.30 (\pm 0.04) | 3.00 (\pm 0.06) | 3.60 (\pm 0.06) | 2.30 (\pm 0.07) | 2.55 | 0.306 | |
| Klewki | 4.70 (\pm 0.09) | 5.10 (\pm 0.07) | 4.80 (\pm 0.05) | 2.50 (\pm 0.04) | 4.28 | -0.853** | |
| Trękusek | 6.20 (\pm 0.19) | 9.80 (\pm 0.14) | 12.90 (\pm 0.14) | 12.80 (\pm 0.28) | 10.43 | 0.853** | |
| Pasym | 8.20 (\pm 0.18) | 10.70 (\pm 0.13) | 10.00 (\pm 0.18) | 9.70 (\pm 0.20) | 9.65 | 0.361 | |
| Grom | 10.60 (\pm 0.12) | 15.70 (\pm 0.33) | 17.00 (\pm 0.35) | 17.50 (\pm 0.24) | 15.20 | 0.822** | |
| Szczytno | 20.20 (\pm 0.43) | 20.60 (\pm 0.24) | 21.20 (\pm 0.24) | 16.60 (\pm 0.26) | 19.65 | -0.767* | |
| Average | 8.53 | 10.82 | 11.58 | 10.23 | 10.29 | 0.433 | |
| LSD for: | town/village - 1.83**, distance from route - 1.50*, interaction - 3.66** | | | | | | |
| Average for Province of Warmia and Mazury | | | | | | 4.81 | |

LSD (least squares deviation) significant for: * p=0.05, ** p=0.01; r - correlation coefficient

TABLE 4
Correlation coefficients (r) between trace elements in the soils

| | Cd | Cr | Ni | Mn | Zn | Cu | Fe | Co |
|----|-------|--------|----------|--------|---------|---------|----------|----------|
| Pb | 0.186 | 0.067 | -0.462 * | -0.098 | 0.662 * | 0.453 * | -0.328 * | 0.384 * |
| Cd | | -0.098 | 0.159 | -0.256 | 0.445 * | 0.501 * | -0.147 | -0.457 * |
| Cr | | | -0.015 | -0.118 | 0.087 | 0.056 | 0.165 | 0.017 |
| Ni | | | | 0.068 | -0.204 | 0.077 | 0.294 * | -0.454 * |
| Mn | | | | | 0.189 | 0.019 | 0.455 * | 0.181 |
| Zn | | | | | | 0.594 * | -0.200 | 0.214 |
| Cu | | | | | | | 0.081 | -0.229 |
| Fe | | | | | | | | -0.420 * |

significant for p=0,05 *

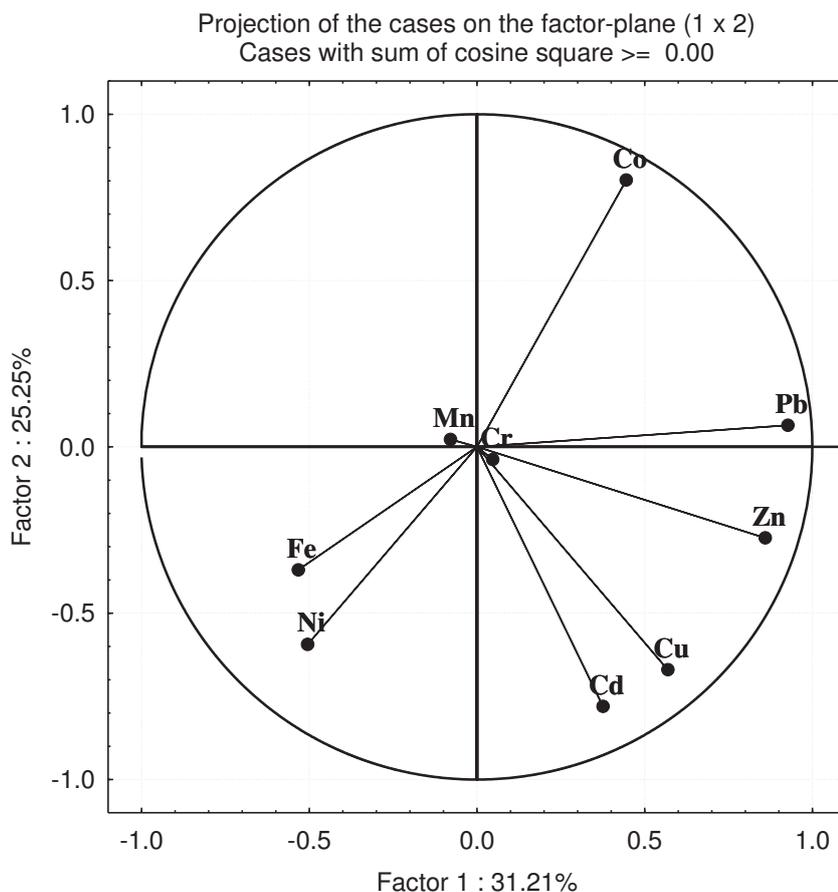


FIGURE 2

Content of trace elements in the topsoil along State Road 53 from Olsztyn to Szczytno illustrated with the PCA method. Key: vectors represent analyzed variable (Pb - lead, Cd - cadmium, Cr - chromium, Ni - nickel, Mn - manganese, Zn - zinc, Cu - copper, Fe - iron, Co - cobalt).

Having determined the percentage of the observed variance with the η^2 coefficient, using the ANOVA method, it was demonstrated that the content of trace elements in soils along State Road 53 between Olsztyn and Szczytno was most strongly dependent on its location in a specific town or village, especially with respect to cobalt and copper, where 89.89 % and 58.81 % of the variance was created by the given variable (Table 5). High

values were also determined for the remaining elements, where from 38.80 % (Ni) to 51.04 % (Cr) of the total variance depended on the location. Manganese was an exception - 20.63 %. In the soil samples taken at an increasing distance from the road, the contribution of this variable was relatively small, ranging from a few to less than fifteen per cent, being the highest for copper (13.20 %) and chromium (14.39 %).

TABLE 5
Per cent contribution of variable factors according to the content of trace elements in soils

| Variables | Trace elements | | | | | | | | |
|--|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Pb | Cd | Cr | Ni | Mn | Zn | Cu | Fe | Co |
| Towns/villages | 40.37 | 49.52 | 51.04 | 38.80 | 20.63 | 44.46 | 58.81 | 48.78 | 89.89 |
| Distances from route | 6.57 | 7.10 | 14.39 | 1.54 | 7.21 | 4.34 | 13.20 | 4.30 | 1.41 |
| Towns/villages · Distances from route | 29.95 | 25.68 | 25.03 | 43.17 | 53.79 | 50.69 | 21.65 | 43.87 | 4.60 |
| Error | 23.10 | 17.69 | 9.54 | 16.49 | 18.37 | 0.52 | 6.34 | 3.05 | 4.10 |

DISCUSSION

The highest concentrations of lead, cadmium, zinc and copper in soils along State Road 53 from Olsztyn to Szczytno appeared in the nearest proximity to the road, but decreased further away from it. Similar results are reported by Kluge and Wessolek [12], Ciec ko et al. [35], Modrzewska and Wyszowski [36].

Most pollutants generated by traffic are accumulated near busy roads, and their highest concentrations are documented at road junctions, where such contributing factors as increased use of brakes and tyres as well as higher emission of exhaust fumes are observed [6-7, 14-16]. Moreover, as reported by Duong and Lee [16], the accumulation of heavy metals in soils is affected by the velocity of vehicles and their number on roads. High values of trace elements in soils are also generated by large urban agglomerations [35, 37]. The accumulation of trace elements is seen to be influenced by average daily traffic and associated emission of exhaust fumes to the environment. Faiz et al. [6] or Duong and Lee [16] noticed that the number of vehicles moving on roads has risen dramatically over the last several years, which is one of the causes of excessive soil pollution.

The presence of humic and fulvic acids in soils, especially in organic substrate, plays an important role in the accumulation of heavy metals and their mobility in soils. This relationship has been confirmed in several studies [38-39], where lead, chromium, zinc, copper and iron were shown to have attached to soil organic matter, thus being less available to plants. The high accumulation of these metals in soils reported herein might have been caused by the close distance to the lakes Linowskie and Machnac, which meant that the soils were more likely to contain more organic matter. Zwonitzer et al. [40] showed the dependence between the presence of zinc in soil and soil contamination by lead and cadmium. The relationship between the occurrence of zinc in soils and their pollution by lead and cadmium has been proven experimentally. In our research, the soil

sampled in Szczytno was poor in organic matter and overgrown by grasses. The soils from all the sampling sites were slightly acid. Knight et al. [41], Wilson and Bell [42] argued that trace elements were more mobile in more acidic soils. The poor binding of metals in soils may be attributed to the above factors because at a low soil reaction metal ions are liberated from the soil sorptive complex, thus making the metals more easily available to plants. The high accumulation of manganese is associated with the presence of iron in soil, the fact supported by Martyn and Niemczuk [43].

The content of iron in soil continued to increase further away from the road, but this finding is not confirmed by Słowik et al. [2], who found that less iron was contained in soil at a larger distance from the road edge. This result may have been caused by the presence of soils richer in organic matter or by the natural content of iron due to the geological structure of the Earth [14]. Regarding nickel and cobalt, their content was observed to rise in soils 25 and 50 m away from the road, but to decrease at 100 m from the road edge, which contradicts studies by Gadziński [44], who suggested that the content of nickel and cobalt in soils should decrease at a growing distance from the edge of a road. Ciec ko et al. [35] attribute this dependence to the airborne road dust, transported by winds over large distances. Chromium acted in a similar manner, with its highest concentration found in soil 100 m away from the road. Apart from the anthropogenic pressure, the soil content of heavy metals is also affected by such as volume, type and duration of atmospheric precipitations, wind strength and spells of dry weather, atmospheric conditions, singled out by Helmreich et al. [15]. All these circumstances have influenced the concentrations of the analyzed metals in soils along State Road 53 from Olsztyn to Szczytno.

CONCLUSIONS

The flow of motor vehicles had a significant effect on the distribution of trace elements in soils

along State Road 53 from Olsztyn to Szczytno, where 4-5 out of 9 examined metals were found in elevated concentrations. The content of lead, zinc, nickel (in some sites), copper and cobalt were higher than their natural content in soils. In Olsztyn, an increase of lead content in soil was detected. These concentrations unquestionably arise from pollution produced by road vehicles, but their accumulation does not threaten the natural environment. The most evident decrease in the content of heavy metals in soil at an increasing distance from the road was identified for copper. The concentration of chromium and iron were the highest in the furthest soils from the road, which may have been caused by the agricultural use of the land or by their higher natural content in the parent rock. The remaining elements appeared on content similar to the natural content. It can be concluded that the rate of average daily traffic is one of the most important factor which increased contamination of heavy metals in soils near the road. The concentrations of trace elements in the analyzed soils were below the allowable limits for lands under transportation routes.

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TiO₂/BiOI COMPOSITE WITH HIGH VISIBLE LIGHT PHOTOCATALYTIC ACTIVITY FOR WATER PURIFICATION

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ABSTRACTS

Novel highly active visible light photocatalysts TiO₂/BiOI composites were prepared by a facile solvothermal approach. The material shows excellent photocatalytic performance towards photodegradation of methyl orange under visible light irradiation. The high photocatalytic activity of TiO₂/BiOI composites under visible light could be ascribed to the opened porous structure, appropriate band-gap, as well as synergistic effect between TiO₂ and BiOI. In addition, TiO₂/BiOI photocatalyst is very promising for water purification as well as other environmental applications.

KEYWORDS:

TiO₂/BiOI composite, Visible light irradiation, Photocatalytic degradation

INTRODUCTION

Since Fujishima and Honda first announced the photo induced evolution of oxygen and hydrogen from a TiO₂ electrode in 1972 [1], semiconductor photocatalysts have attracted great attention and played an important role in splitting of water and the decomposition of harmful pollutants. Among these various photocatalysts, TiO₂ has been widely studied due to its high chemical stability, low cost, and nontoxicity [2]. However, anatase TiO₂ only responds to ultraviolet due to its large bandgap (3.2 eV), which hinders its application in the range of visible light [3]. In order to improve the sunlight utilization efficiency, the development of novel photocatalysts under visible light is necessary [4-7].

Recently, new type of semiconductor materials of bismuth oxyhalides (BiOX, X=Cl, Br, I) have attracted great attention for their high photocatalytic activity under visible light. The layered structures are characterized by [Bi₂O₂] slabs interleaved by double slabs of halogen atoms [7, 8-10].

This layered structure can provide a space large enough to polarize the related atoms and orbitals, and then the induced dipole can separate the electron and hole pairs efficiently, which is beneficial to enhance the photocatalytic activity [8,9]

Previously, many heterostructured semiconductors concerned BiOX, such as NaBiO₃/BiOCl [8], BiOCl/Bi₂O₃ [9], AgI/BiOI [10] and Fe₃O₄/BiOCl [11], have been reported and demonstrated the enhanced photocatalytic activities. Therefore, it is a promising way to improve the photocatalytic activities of BiOX by construction of heterojunction[12-19].

Among the BiOX family, BiOI possesses the smallest band gap ($E_g = 1.73-1.92$ eV), has been widely studied due to their relative high photocatalytic activity. In this work, the TiO₂/BiOI composites were prepared via a hydrothermal method. The structures of samples were well characterized and their photocatalytic performance was evaluated by degradation of methyl orange (MO). Furthermore, a possible photocatalytic mechanism was proposed and the relationship between the activity and the physicochemical properties of the catalysts were also discussed.

MATERIALS AND METHODS

Synthesis of samples. All the reagents were purchased from the Sinopharm Chemical Reagent Co., Ltd. and used without further treatment.

Firstly, 5 mmol KI and Bi(NO₃)₃ · 5H₂O were separately dissolved into 40 mL distilled water under magnetic stirring. And then 0.5 mL acetic acid was into Bi(NO₃)₃ solution to prevent the hydrolyze. The KI solution was slowly added into Bi(NO₃)₃ solution and the stirred for 1 h. After that, 5 mL tetrabutyl titanate was also added into with magnetic stirring. After stirring for 2 h at room temperature, the mixture was transferred into a 100 mL Teflon-lined stainless steel autoclave and maintained at 120 °C for 6 h then was cooled to room temperature naturally. After completion of the

reaction, the precipitates were collected by centrifugation, washed several times with distilled water and ethanol to remove any ionic residue, and finally dried in an oven at 80 °C.

Characterization. The crystal structure of the as-synthesized samples of TiO₂/BiOI was examined by X-ray diffraction (XRD, DX-2600X, Dandong, China) in the 2θ range 10-80° using Cu-Kα X-ray source (λ=0.15418 nm). The scanning electron microscopy (SEM) images were obtained on a SU1510 (Hitachi, Japan) scanning electron microscope. Fourier transform infrared spectroscopy (FT-IR) analysis was carried out on a Nicolet AVATAR 370 spectrophotometer. UV-vis diffuse reflectance spectra (DRS) of the samples were obtained on an UV-vis spectrophotometer (Hitachi U-3310) using BaSO₄ as a reference. Thermogravimetric analysis (TGA) was performed on a STA409 (NETZSCH, Germany) analyzer. Energy dispersive X-ray spectra (EDX) were observed by an EDAX spectrometer (Oxford, UK).

Photocatalytic activity tests. The photocatalytic performance of as-synthesized samples was evaluated by degradation of methyl orange (MO) in a photoreaction apparatus under visible light (λ>420 nm). A 300 W Xe lamp (Instrument Company of Light Source, Beijing) with a 420 nm cutoff filter was used as the light source to provide visible light irradiation. Prior to illumination, the suspension that contained 50 mL MO solution (20 mg/L, neutral condition) and 10

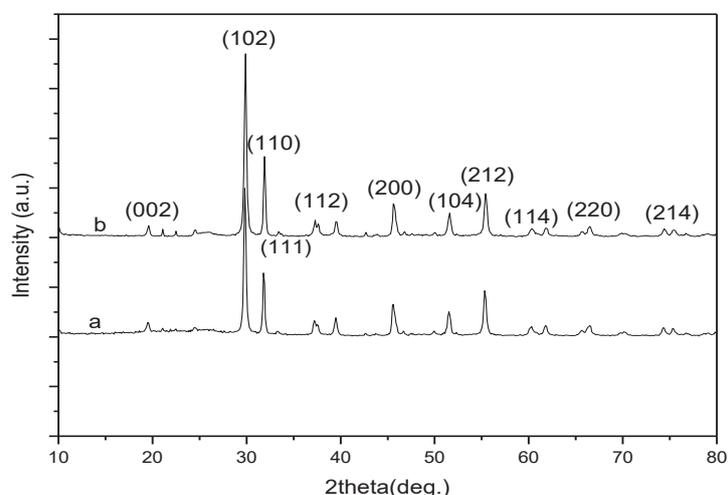
mg catalyst was stirred magnetically for 30 min in the dark to reach adsorption-desorption equilibrium. At irradiation time intervals of 5 min, 2 mL suspension was collected, centrifuged (8000 rpm, 10 min) and filtered through 0.22 μm Millipore filter to remove the photocatalyst particles. Then, the catalyzed phenol solution was analyzed with a UV3310 spectrophotometer (Hitachi, Japan) at the length of 462 nm (the main absorbance peak of MO) with the distilled water as a reference sample.

RESULTS AND DISCUSSIONS

Sample characterization. XRD analysis. Fig. 1 shows the XRD patterns of the as-synthesized samples. Due to the reaction time, the XRD of TiO₂ shows that the structure of TiO₂ is amorphous.

It is observed from Fig. 2b all the peaks of 20°, 29.96°, 31.76°, 37°, 40°, 55°, 67° and 75° coincided with the standard tetragonal phase and those of pure BiOI corresponded to tetragonal phase (JCPDS No. 73-2062). The results agree with reported data. No impurities can be detected indicating the well-crystallized BiOI can be easily obtained under the current synthetic conditions. The added TiO₂ resulted in a gradual increase of diffraction strength for BiOI, which indicates the increase of the composites' crystallinity. TiO₂ formed on the surface of tetragonal BiOI crystalline, indicating that composite well between them.

FIGURE 1
The XRD spectra of as-prepared samples (a: TiO₂, b: BiOI/TiO₂)



The peak shift after addition of different quantities of TiO_2 was also confirmed in the magnifying XRD pattern (Fig. 1), implying a possible incorporation of Ti atoms into the BiOI lattice. The possible reasons are that the size of Ti atom (61 pm) smaller than Bi atom (103 pm).

SEM and TEM. It can be observed from Fig. 2 that the SEM image of TiO_2 (Fig. 2a) is completely different with that of TiO_2/BiOI (Fig. 2b). The TiO_2/BiOI sample appears three-dimensional (3D) nanosheet-constructed microspheres approximately 3 μm in diameter, which agrees with previous report [20]. The sample

displays a 3D hierarchical micro/nano structure, which is composed of numerous nano-flakes. Fig. 2a shows that image of TiO_2 , which contains of irregular particles. However, after the solvothermal reaction, the crystallinity of the samples increased significantly, and pure BiOI phases were obtained. The transmission electron microscopy (TEM) image (Fig. 2c) shows the hollow structure inside TiO_2/BiOI . It can be observed from Fig. 2c that the measured lattice fringe distances are 0.235 nm, corresponding to the (102) lattice planes of TiO_2 . The bright spots in selected area electron diffraction pattern (SAED) of TiO_2/BiOI (Fig. 2d) indicates good crystallinity of TiO_2/BiOI .

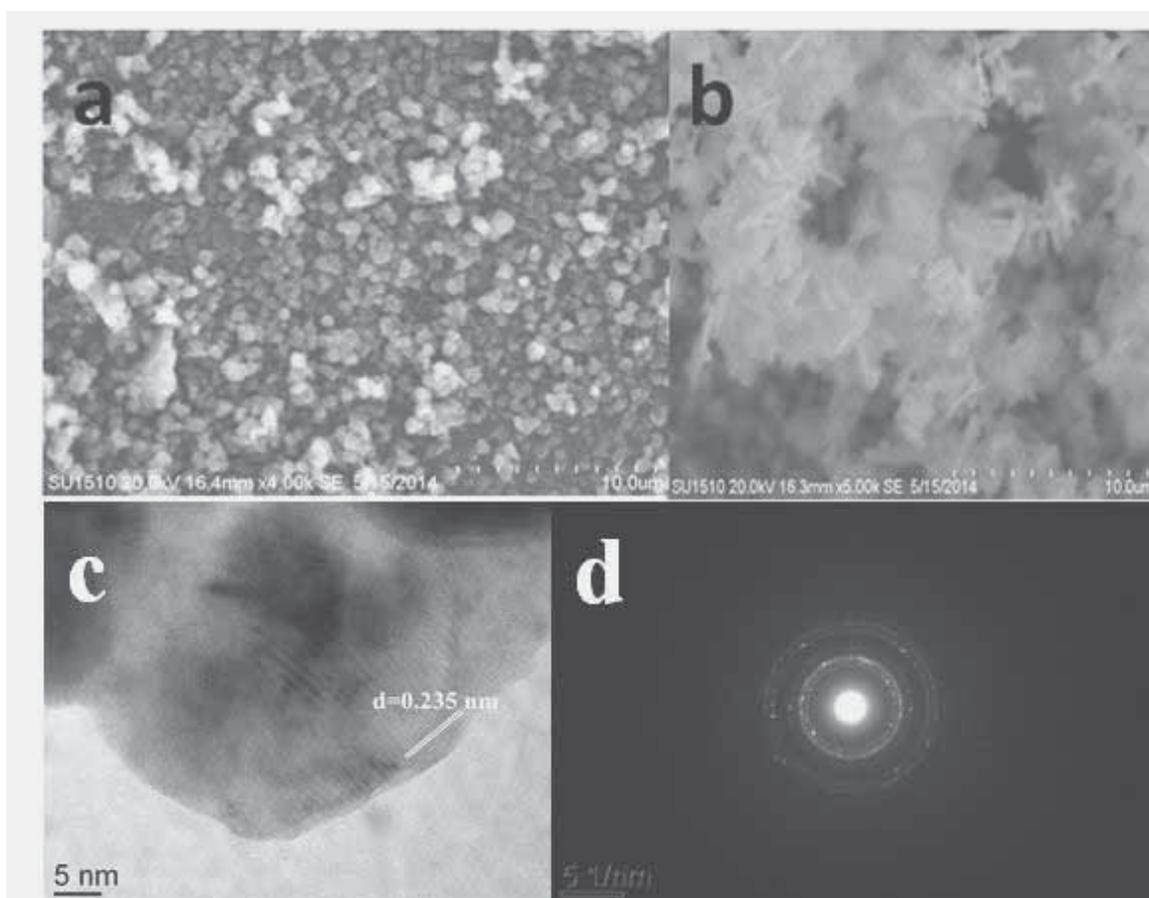


FIGURE 2
The SEM of TiO_2 (a) and TiO_2/BiOI (b), HRTEM (c) and SAED (d) of TiO_2/BiOI .

BET analysis of photocatalysts. The porous structure of TiO_2 and TiO_2/BiOI was further characterized by nitrogen adsorption/desorption isotherm measurements (Fig. 3). It is found that the TiO_2 and TiO_2/BiOI samples a moderate specific surface area. The specific surface areas, pore volumes and mean pore diameters of the samples

are listed in Table 1. TiO_2/BiOI exhibits higher surface area and pore volume, which can facilitate the molecule insertion and rapid diffusion during the photocatalytic reactions.

UV-vis diffuse reflectance spectra analysis. Fig. 4 shows the UV-vis diffuse reflectance spectra

(DRS) of TiO₂ and TiO₂/BiOI composite. TiO₂ exhibits strong absorption in the UV region, absolutely no visible light absorption (430-800 nm). The absorption range of TiO₂/BiOI composite is enlarged to visible light region. Given that TiO₂ and TiO₂/BiOI are indirect gap semiconductors [21-23], the relationship between the absorption edge and photon energy (hv) is given as $\alpha hv = A (hv - E_g)^{n/2}$. In this equation, α , hv, A and E_g are absorption coefficient, Planck absorption energy, proportionality constant and band gap, respectively; n decides the type of the transition in a

semiconductor (n=1, direct absorption; n=4, indirect absorption). For TiO₂/BiOI, the value of n is 4 for the indirect transition [24-27]. Therefore, the band gap energy (E_g value) of the resulting samples can be estimated from a plot of $(\alpha hv)^{1/2}$ versus photon absorption energy (hv). The intercept of the tangent to the X axis would give a good approximation of the band gap energy of the samples. The band gap of TiO₂ and TiO₂/BiOI are evaluated to be 2.67 eV and 1.94 eV, close to the value reported in the literature [27,28].

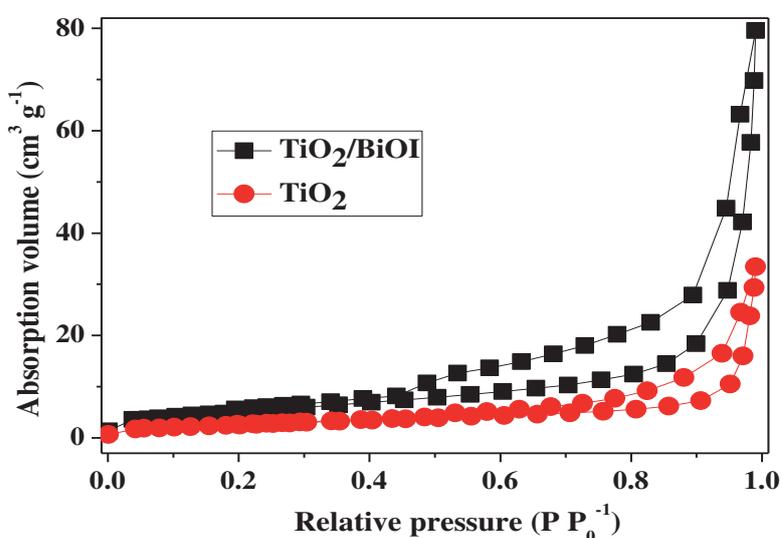


FIGURE 3
N₂ adsorption/desorption isotherms at 77 K for TiO₂ and TiO₂/BiOI samples.

TABLE 2
The absorbed dose rates in air (ADRA) and annual effective dose equivalent (AEDE) in Gerze/Sinop.

| Samples | Specific surface area/m ² g ⁻¹ | Pore volume/cm ³ g ⁻¹ | Mean pore diameter/nm |
|------------------------|--|---|-----------------------|
| TiO ₂ | 31.65 | 1.75 | 32.34 |
| TiO ₂ /BiOI | 10.57 | 0.94 | 21.73 |

Photocatalytic performance. The photodegradation efficiency of MO over TiO₂ and TiO₂/BiOI samples as a function of time under visible light irradiation was investigated (Fig. 4(a) and (b)). Self-photodegradation of MO is almost negligible without catalyst, only 12% MO are decomposed over TiO₂ under visible light irradiation. Fortunately, the hybrid catalyst displays high photocatalytic performance, over 95% of MO was degraded at TiO₂/BiOI under visible light irradiation within 60 min.

Several reasons may account for this enhanced photocatalytic activity. Firstly, as the interphase spacers to TiO₂ nanocrystals, the introduction of 3D amorphous BiOI can provide large surface area and enhance the adsorption of pollutants in the liquid phase. Furthermore, the high surface area of the composites will facilitate efficient transfer of electrons and holes to the surface of the material. Secondly, the opened mesoporous channels will not only make the active surface of the BiOI highly accessible to the MO molecules, but also improve

the mass transfer within the hybrid structure during photocatalytic process. Thirdly, the band-gap of TiO_2/BiOI composites (1.94 eV) made the materials easily be excited by visible light. Finally, the photocatalytic activity of TiO_2/BiOI is higher than that of TiO_2 . This is because of the synergistic effect between TiO_2 and BiOI , which is attributable to the incorporation of Ti atoms into the BiOI

lattice, also result in an effective transfer of the photogenerated carriers during the photocatalytic reaction. Therefore, the large surface area, opened mesoporous structure, appropriate band-gap, as well as synergistic effect between TiO_2 and BiOI result in the high photocatalytic activity of TiO_2/BiOI nanocomposites [4].

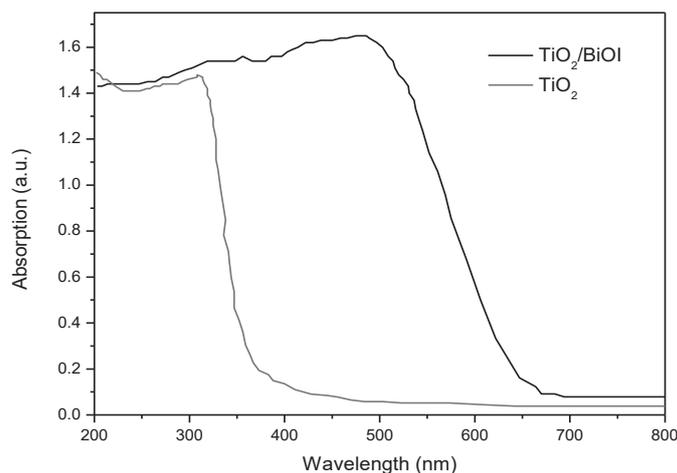


FIGURE 4
The UV-vis diffuse reflectance spectra (DRS) of TiO_2 and TiO_2/BiOI composite.

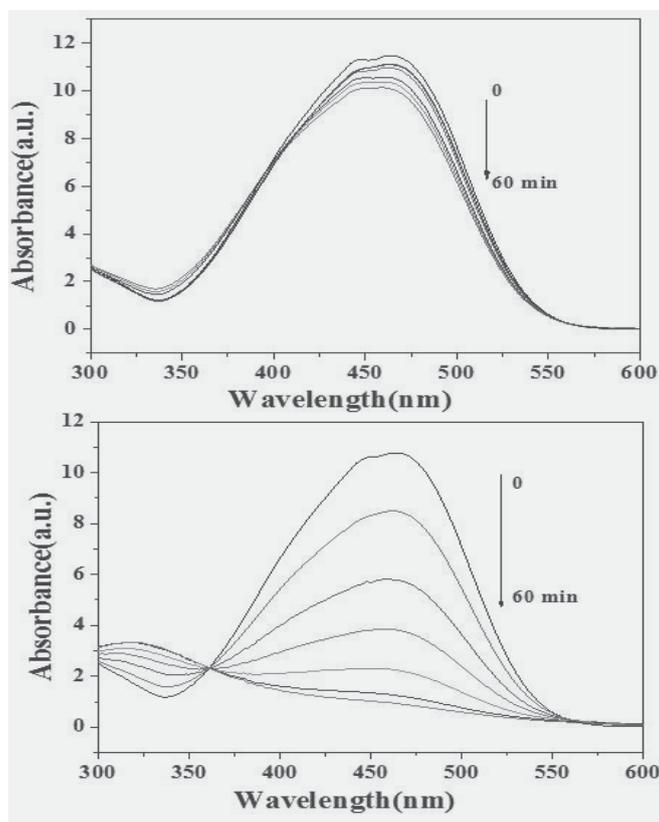
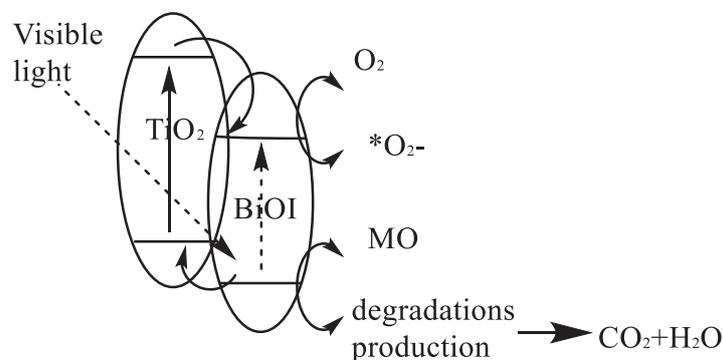


FIGURE 4
The photodegradation efficiency of MO over TiO_2 (a) and TiO_2/BiOI composites (b) samples under visible light irradiation

Photocatalytic mechanism. Generally, the photogenerated hole on the surface of BiOI cannot react with $\text{OH}/\text{H}_2\text{O}$ to form $\cdot\text{OH}$ because the standard redox potential of $\text{Bi}^{\text{III}}/\text{Bi}^{\text{IV}}$ (+1.59 eV at $\text{pH}=0$) is lower than that of $\cdot\text{OH}/\text{OH}^-$ (+1.99 eV) [28]. Besides, if the degradation is carried out by $\cdot\text{OH}$, the effect of dye adsorption is minor. Nonetheless, the adsorption of the dye on the catalyst surface plays a critical role when the photocatalytic degradation is initiated by the direct hole oxidation [29]. And visible light photocatalysis involves dye sensitization and the excited electrons scavenged by O_2 to obtain superoxide radical anions $\cdot\text{O}_2^-$ [30]. Based on the above assumption, a

plausible photocatalytic process was proposed, as illustrated in Scheme 1. Firstly, the dye molecules are easily captured on the surface of the catalyst owing to the hybrid induced high surface area and mesoporous structure. The adsorbed MO can be excited by visible light irradiation and inject electrons into the conduction band of the catalyst to form $\cdot\text{O}_2^-$. Simultaneously, the photo-induced holes are capable of oxidizing MO directly and the photo-generated electrons was also believed to be capable of benefit the oxidation process through reduction of absorbed O_2 into $\cdot\text{O}_2^-$ [30,31]. Thereafter, the $\cdot\text{O}_2^-$ and $h\nu_{\text{B}}+$ can oxidize the MO molecular to CO_2 and H_2O [32,33].



SCHEME 1

Schematic processes of the photodegradation of MO over TiO_2/BiOI composite.

CONCLUSION

In summary, we have developed a facile and efficient method for the preparation of TiO_2/BiOI composite. The as-prepared TiO_2/BiOI composite exhibits more efficient photocatalytic performances for the degradation of MO, compared with the TiO_2 under visible light irradiation. Then high photocatalytic activity of TiO_2/BiOI composite under visible light could be ascribed to opened mesoporous structure, appropriate band-gap, as well as synergistic effect between TiO_2 and BiOI. The results provide a reliable experimental and theoretical model for the development of photocatalyst, and will benefit the practical application in environmental cleaning.

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DETERMINATION OF LEAD REMEDIATION CAPACITY OF LIVING AND DEAD HALOPHILIC BACTERIUM *CHROMOHALOBACTER* SP. BIOMASS

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ABSTRACTS

The objective of the present study was to determine that the remediation capacity of *Chromohalobacter* sp. strains to lead and evaluate the biosorption of the metals in living and dead biomass. In this study sorptions of dead cells were found to be more effective than living cells. The functional groups on *Chromohalobacter* sp. for adsorption and biosorption were characterized by FT-IR. According to FT-IR results, N-H stretching (3400-3200 cm⁻¹), -CH₃ bending or -NO₂ stretching (1450-1375 cm⁻¹ or 1550-1350 cm⁻¹), C-O bending or stretching, vibrations (1200-1050 cm⁻¹) and strong C-Br (750- 500 cm⁻¹) peaks were observed. This is the first study to reveal potential of *Chromohalobacter* sp. for sorption of lead. It was concluded that the biosorption of lead by *Chromohalobacter* sp. can be used as a potential source for the bioremediation. This can be an interesting alternative for bioremediation processes of lead.

KEYWORDS:

Biomass, bioremediation, biosorption, *Chromohalobacter* sp., heavy metal, lead

INTRODUCTION

Heavy metals contamination has been one of the most important environmental problems because of their incremental accumulation in the food chain, toxicity threat to living organisms and continued persistence in the ecosystem [1-2].

Toxic heavy metals are frequently contained in wastewaters produced by many industrial processes, such as those employed in the electroplating, metal finishing, metallurgical,

tannery, chemical manufacturing, mining, and battery manufacturing industries [3-4].

Saline and hyper saline environments are frequently contaminated with heavy metals as a result of industrial and/or agricultural activities [5-7]. Contamination of these habitats constitutes a serious environmental problem mainly due to the high toxicity. Lead (Pb) is one of the most widely used metals in industries and almost in all over the world exposure to Pb continues to be a common problem.

Conventional technologies for the removal of heavy metals, such as ion exchange, chemical precipitation, electrochemical treatment, membrane technologies and adsorption on activated carbon, etc. are often inefficient and very expensive when used for the reduction of heavy metal ions to very low concentrations [8-9]. Alternative methods of heavy metal removal and recovery based on biological materials have received increased attention in recent years because of their potential application in environmental protection [10-11]. The role of microorganisms and plants in biotransformation of heavy metals into nontoxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal-contaminated sites [12-17]. Nevertheless, very few studies on interactions of halophiles with heavy metals have been reported [18-19]. Studies of extreme microorganisms in biotechnology is becoming increasingly important. Especially moderately halophilic bacteria compose a heterogeneous group of microorganisms able to grow in a very wide range of salt concentrations including hypersaline habitats [20].

Members of *Chromohalobacter* are moderately halophilic bacteria, a group of extremophilic prokaryotes that optimally grow in 8-10% NaCl, and may grow in salt concentration up

to 30%. The broader range of temperature and pH observed for growth are 5-45 °C and a pH of 5.0-10.00, respectively. The objective of the present study was to determine the tolerance of moderately halophilic, Gram-negative bacterium *Chromohalobacter* sp. strains to lead, evaluate the biosorption of the metals in living and dead biomass. The functional groups involved in lead biosorption were identified using FT-IR analysis.

MATERIALS AND METHODS

Test microorganism and growth condition. *Chromohalobacter* sp. was a kind gift from Dr. Josefa Anton University of Alicante, Spain. The bacterium was routinely grown on Modified Growth Medium (MGM) with 23% salts.

Optimal growth of *Chromohalobacter* sp. *Chromohalobacter* sp. was a kind gift from Dr. Josefa Anton University of Alicante, Spain. The Optimal temperature and pH of growth of *Chromohalobacter* sp. were determined by culturing bacteria in MGM. Two milliliter aliquots of dense bacterial culture were added to 50 mL fresh MGM and cultured with shaking (150 rpm) at 20 °C, 30 °C, 37 °C, 55 °C and 70 °C. An analogous method was used to determine the pH optimum, employing pH-adjusted MGM and Salt-tolerance assays [21]. Developments of the isolate was observed at different lead concentrations (125, 250, 500, 750, 1000 ppm) and their growth were monitored by measuring OD₆₀₀ on a spectrophotometer. Growth of the isolate was evaluated with the comparisons of control group.

Sorption studies and atomic absorption spectroscopy. Biosorption experiments were conducted by taking different lead concentrations (125, 250, 500). *Chromohalobacter* sp. was grown aerobically with agitation in a growth medium (23% MGM) at 35 °C and set at 200 rpm in a shaking incubator. After reaching the mid-exponential growth phase of cells (OD₆₀₀ nm = 0.9-1.0), the cells were harvested by centrifugation at 6000 rpm for 15 min and washed three times with deionized water. One ml of lead concentration (125, 250, 500, 1000 ppm) were then added and they were incubated 35 °C. Sterile deionized water used as a negative control. Ependorfs were allowed to attain equilibrium on rotary shaker at 200 rpm and samples were collected at regular time intervals. Centrifugation at 6000 rpm was done and the amount of metal ion in the residual solution was analysed by atomic absorption spectroscopy (ICP-OES, VARIAN 720 ES). The supernatant was

discarded and the cells were resuspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface [22].

The biomass was heat killed in a conventional hot air oven at 100 °C for 30 min. This dead biomass was used for the biosorption experiments. Biomass was harvested from the medium by centrifugation at 6000 rpm for 15 min. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface. The sorption of lead was investigated all experiments were performed using standard solutions of this element.

Adsorption isotherm. Adsorption isotherms are plots of dye concentration in the solution against the biosorption capacity (q) at a constant temperature. Langmuir biosorption equilibrium isotherm model was used to evaluate the adsorption of lead.

The general Langmuir equation is commonly presented as:

$$C_e/q_e = 1/Q_b + C_e/Q$$

where q_e is the amount of dye removed (mg/g), C_e the equilibrium concentration (mg/L), Q and Q_b are the Langmuir constants related to adsorption capacity and adsorption energy, respectively [23].

Surface characterization (FTIR spectral analysis). To determine the nature of functional groups on the cell wall for metal sorption, potentiometric titration of an aqueous cellular suspension and IR spectrum analysis of the solid phase biomass were performed. *Chromohalobacter* sp. incubated at 37 °C, 7 days growth on 23% MGM which contains 125, 250, 500 and 1000 ppm lead. To identify functional groups using the IR spectrum procedure, the bacteria were pelleted by centrifugation at 6000 rpm for 15 min and dried overnight at 60 °C in an oven. One mg of finely crushed particles of biomass was encapsulated in 300 mg of KBr. The infrared spectra of the biomass were recorded in KBr disks using an FT-IR spectrophotometer (Perkin Elmer Spektrum BXII). Lead free MGM media was used as a control groups. Infrared spectra of *Chromohalobacter* sp. with or without adsorbed lead ions were obtained.

RESULTS AND DISCUSSIONS

Optimal growth of *Chromohalobacter* sp. According to our study, *Chromohalobacter* sp. grew well at 30 and 37°C and showed minimal

growth at 20°C and didn't growth at 55°C. The optimum growty was determinated at pH 5 and 7. *Chromohalobacter* are one of the broadest salinity ranges found in prokaryotes [24]. Arahali et al. [25] reported that *Chromohalobacter salexigens* is able to grow from 0.15 M to 4.3 M NaCl in complex medium, and from 0.5 M to 3 M NaCl in minimal medium. Salt-tolerance assays showed that the bacterium grew well at salt concentrations up to 30%. It was shown that *Chromohalobacter* sp. was found to be resistant to lead up to 750 ppm using 23% MGM. In this region, all experimand were performant at pH7, and 35°C. Amoozegar et al. [26] reported that ten moderately halophilic bacteria were isolated from saline soils in Iran and susceptibility to copper and cadmium varied among the isolates. Silver had the maximum toxicity, whereas lead and copper showed minimum toxicity. Williams et al. [2] were studied that fifty six halobacterial strains isolated and Halobacterium

saccharovorum, Halobacterium salinarium and Natronobacterium gregoryi showed resistance against zinc, arsenic and cadmium respectively.

Effect of contact times and Pb concentration on sorption. In this study, the cells were deeply colored due to the uptake of the lead (Fig 1). This indicates that the lead removal by *Chromohalobacter* sp. may be largely attributed to sorption. The sorption of heavy metals depends on both sorbate and adsorbent chemical composition and physical characteristics. Zeng [27] reported that hydrophobic ion to diffuse through the polysaccharide chains and adsorb onto the negatively charged outer membrane surface. The hydrophobic ion, with molecular weight 329.4 Da, may then penetrate through the outer membrane either 60 through the porin channels or directly through the phospholipid bilayer.

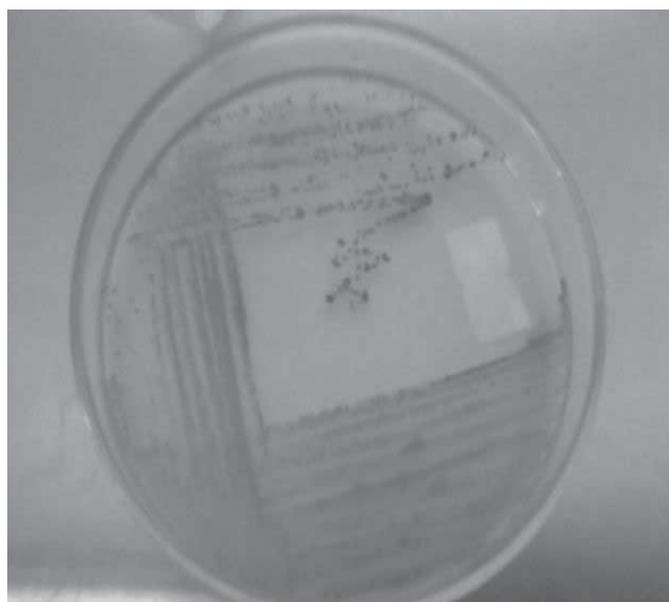


FIGURE 1
Biosorption of *Chromohalobacter* sp.

The biosorption studies were carried out at different concentration of lead and different contact times. In practical operations, living cell as biosorbents does not always qualify for heavy metal removal and recovery from toxic industrial wastewater. The use of dead biomass can avoid the problem of toxicity of heavy metals toward living cells. Another advantage of using dead biomass is

the easy and non-destructive recovery of adsorbed metal ions, which allows regeneration of the biosorbent for reuse [28]. Therefore, both dead and living cells used in the study. Biosorption of *Chromohalobacter* sp. with living and dead cells at 125, 250 and 500 ppm Pb concentration at different contact times are shown in Table 1

TABLE 1
Sorption of *Chromohalobacter* sp. with living and dead cells at 125,250 and 500 ppm Pb concentrations at different contact times.

| Concentration of Pb(ppm) | Ti me (min) | Dead Cell (DC) | | Living Cell (LC) | |
|--------------------------|-------------|----------------------------------|--------------------|----------------------------------|--------------------|
| | | Mean ($\mu\text{g}/\text{gr}$) | Standart deviation | Mean ($\mu\text{g}/\text{gr}$) | Standart deviation |
| 125 | 1 | 21994,66 | 132,34 | 22764,43 | 43,41 |
| | 2 | 19521,12 | 84,48 | 22967,17 | 73,07 |
| | 3 | 21232,69 | 75,27 | 22361,05 | 85,3 |
| | 4 | 24401,34 | 81,71 | 22590,89 | 138,38 |
| | 5 | 23771,53 | 110,48 | 21548,58 | 45,73 |
| | 10 | 27793,74 | 42,74 | 21618,66 | 45,94 |
| | 15 | 28736,70 | 129,11 | 23868,05 | 23,71 |
| | 20 | 23880,09 | 50,07 | 23988,56 | 108,69 |
| | 30 | 22915,34 | 17,29 | 22883,63 | 33,53 |
| | 40 | 25326,13 | 93,94 | 22067,98 | 20,83 |
| | 50 | 33541,76 | 142,29 | 22768,73 | 13,83 |
| | 60 | 27101,51 | 104,68 | 24535,41 | 42,94 |
| 250 | 1 | 52448,50 | 408,01 | 52620,68 | 268,66 |
| | 2 | 47677,71 | 45,18 | 50083,33 | 2,77 |
| | 3 | 48317,50 | 149,33 | 64135,63 | 93,55 |
| | 4 | 39255,42 | 10,66 | 57899,12 | 84,43 |
| | 5 | 50232,79 | 91,65 | 52497,58 | 241,95 |
| | 10 | 42268,09 | 126,75 | 56389,55 | 184,60 |
| | 15 | 48285,22 | 175,80 | 58744,40 | 98,23 |
| | 20 | 41114,98 | 70,77 | 51997,58 | 253,31 |
| | 30 | 43280,52 | 78,70 | 47636,03 | 452,30 |
| | 40 | 47819,30 | 51,49 | 55925,00 | 257,87 |
| | 50 | 45358,13 | 188,48 | 47870,00 | 109,93 |
| | 60 | 48519,66 | 242,91 | 56339,95 | 237,21 |
| 500 | 1 | 75047,26 | 371,07 | 72567,56 | 797,96 |
| | 2 | 75156,88 | 154 | 72236,71 | 350,11 |
| | 3 | 76475,14 | 242,57 | 73667,94 | 189,86 |
| | 4 | 75704,42 | 389,78 | 77380,24 | 202,78 |
| | 5 | 69463,58 | 160,43 | 75747,48 | 377,97 |
| | 10 | 87384,51 | 263,21 | 73060,16 | 306,66 |
| | 15 | 81048,79 | 432,94 | 72556,42 | 377,31 |
| | 20 | 80534,28 | 232,2 | 65280,40 | 247,9 |
| | 30 | 77067,54 | 159,6 | 73728,36 | 155,64 |
| | 40 | 71143,80 | 165,45 | 66760,73 | 85,36 |
| | 50 | 71619,22 | 175,29 | 72428,07 | 144,03 |
| | 60 | 83150,46 | 319,22 | 78391,79 | 387,03 |

In our study, we found that increase or decrease the amount of lead in the cells at regular intervals at all concentrations tested. This may be due to the desorption. The mechanisms of sorption and desorption processes involve complexation, coordination, chelation and, to a lesser extent, ion exchange. There is little information about the desorption process. Tan [30] found that higher concentrations of dissolved lead in the effluent were an indication of lead desorption from sediment into seawater. Desorption of bound metals from the bacterial surface and its reuse (resorption) are important for the practical applicability of the

biomass when water purification is considered. In an optimal situation, the desorbent used should be able to release all bound metal repeatedly without affecting resorption capacity of the biomass.

Adsorption isotherm. For micro surfaces, such as microparticles and emulsion surfaces, the effective surface area can be very large. Hence, N can be comparable to C most of the time and cannot be neglected from the $(C - N)$ term in equation. Therefore, in most cases, the modified Langmuir model should be used to analyze adsorption isotherms obtained for colloidal systems [27]. Kang

et al. [31] investigated that biosorption of the chromium ions Cr (III) and Cr (VI) onto the cell surface of *Pseudomonas aeruginosa*. It was found that the sorption isotherms of *P. aeruginosa* for Cr (III) were described well by Langmuir isotherm models. In this study, Langmuir adsorption isotherm model was used to characterize the interaction of adsorption of lead with the live or

dead *Chromohalobacter* sp. cells (Table 2). The coefficient of determination (R^2) for model was greater than 0.95 and close to 1. This indicates that model adequately describe the experimental data of the biosorption of Pb. Langmuir adsorption isotherm model was suitable for sorption of both living and dead biological cells (Fig. 2)

TABLE 2
The parameters obtained from Langmuir isotherms.

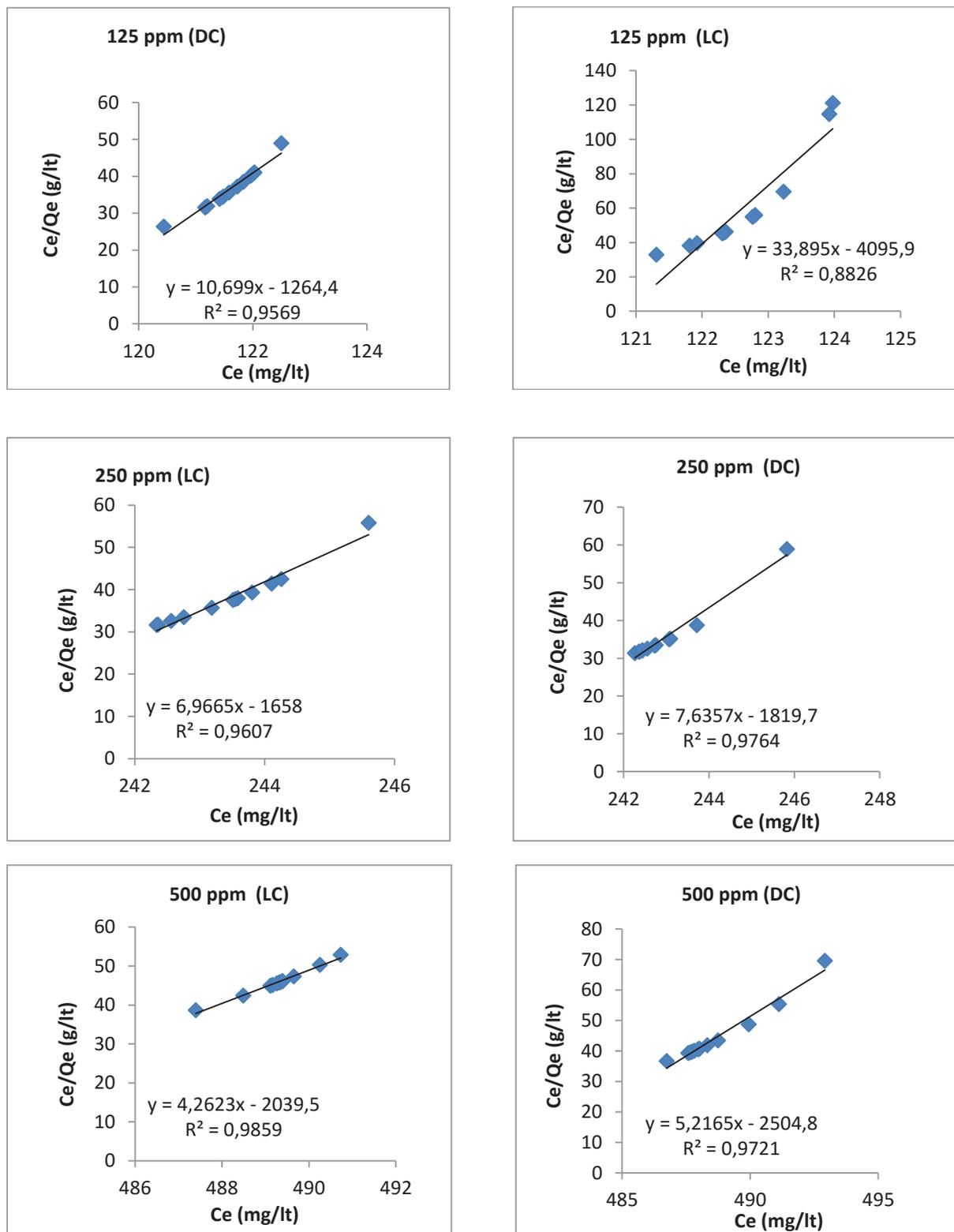
| | Langmuir model | | | | | |
|---------|----------------|----------------|----------------|---------|----------------|----------------|
| | DC | | | LC | | |
| | 1/n | K _f | R ² | 1/n | K _f | R ² |
| 125 ppm | -49,399 | 133,88 | 0,9927 | -45,004 | 122,07 | 0,9963 |
| 250 ppm | -41,986 | 101 | 0,9932 | -40,221 | 96,796 | 0,9897 |
| 500 ppm | -49,399 | 133,88 | 0,9927 | -45,004 | 122,07 | 0,9963 |

FTIR spectral analysis. Biomass of *Chromohalobacter* sp. (control) and biomass treated with lead obtained after sorption were analyzed with FT-IR. Table 3 summarize the functional groups of the bacterium corresponding adsorption frequencies. According to results of control group, N-H stretching (3400-3200 cm⁻¹), -CH₃ bending or -NO₂ stretching (1450-1375 cm⁻¹ or 1550-1350 cm⁻¹), C-O bending or stretching, vibrations (1200-1050 cm⁻¹) and strong C-Br (750-500 cm⁻¹) peaks were observed (Fig.3).

The FT-IR analysis results of MGM media which contained three different lead concentration (125, 250, 500 ppm) N-H stretching (3400-3200 cm⁻¹), -CH₃ bending or -NO₂ stretching (1450-1375 cm⁻¹ or 1550-1350), C-O cm⁻¹ bending or stretching, vibrations (1200-1050 cm⁻¹) and strong C-Br (750-500 cm⁻¹) peaks were observed. Also >C=C<_H (995 ve 910 cm⁻¹) peaks were observed in 125 and 250 ppm lead concentrations. However >C=CH₂ (890 cm⁻¹) peak was evaluated in 250 ppm lead concentration.

Yamaguchi et al. (2012) reported that periplasmic metal binding protein characterized by high histidine content was cloned from moderate halophile, *Chromohalobacter salexigens*. This protein showed typical halophilic characteristics: acidic feature of protein calculated pI is 4.38), abnormally slow mobility, higher stability and melting temperature with increasing salt concentrations and reversible refolding from heat-denaturation at salt concentration up to 1.15 M NaCl.

In this study; according to heavy metals biosorption results in the FT-IR spectrometer, the heavy metal retention of bacteria was alkane CH stretching (2980-2900 cm⁻¹) groups. Lypopolysaccharide can be effective in binding of lead.



LC: Living Cells, DC: Dead Cells

FIGURE 2
 Langmuir model (C_e/q_e versus C_e)

TABLE 3
Comparison of the spectrum.

| $\bar{\nu}$ cm ⁻¹ | Group | Interpretation | C ontrol | 125 ppm Pb | 250 ppm Pb | 500 ppm Pb | |
|---|---|--------------------------------|---|--------------------|--------------------|--------------------|--------------------|
| 3μm region (3600-2500 cm ⁻¹) | O-H,N-H and C-H stretching or vibrations | 3400-3200 | N-H stretching | 3400,50 | 3291.50 | 3290.70 | 3291.78 |
| | | 2980-2900 | C-H stretching alkane | | 2926.95 | 2926.93 | 2926.57 |
| Finger print region (1600-400 cm ⁻¹) | Single bond stretching, bending, vibrations | 1450 and 1375 or 1550 and 1350 | -CH ₃ bending or -NO ₂ stretching | 1405.22 | 1403.73 | 1402.18 | 1402.98 |
| | | 1200-1050 | C-O , C-N, or stretching | 1240.42 1090.43 | 1239.67 1085.90 | 1239.81 1084.88 | 1240.11 1083.50 |
| | | 995 - 910 | >C=C< H ^H | | 933.68 | | 933.85 |
| | | 890 | >C=CH ₂ | | 892.92 | | |
| | | 750-500 | C-Br Strong | 620.61 | 537.35 | 538.32 | 539.80 |

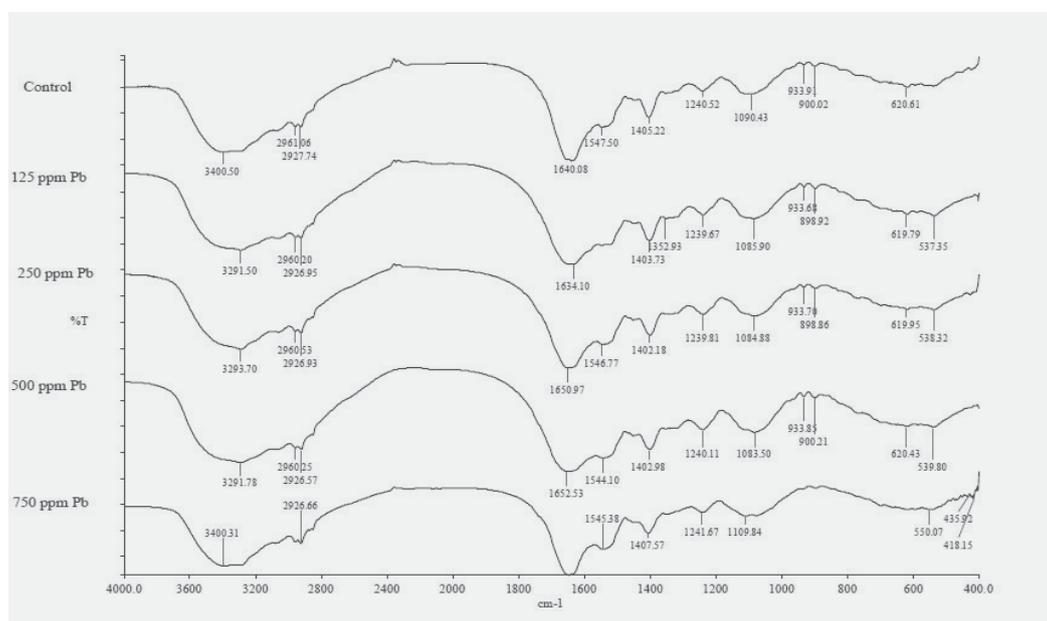


FIGURE 3
FT-IR spectral analysis of *Chromohalobacter* sp. which growth lead free MGM

CONCLUSION

To our knowledge, this is the first report biosorption of the lead by living or dead biomass of moderately halophilic bacterium *Chromohalobacter*

sp. It was concluded from the present study, *Chromohalobacter* sp., which are common in saline biotopes of various ecogeographic zones and show promise for biotechnological application, are especially interesting in this respect. Future studies focus on chemical structure of metal binding

group which is thought to be lipoproteins and increase the metal binding capacity.

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ITALIAN FOUNDRY CONTAMINATION DUE TO CS-137

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ABSTRACT

In late October 2005 at the Beltrame plant in Susa Valley (Italy) a radioactive source was accidentally burned in the blast furnace. The source was not discovered by the detectors at the entrance, evidently because of some shielding effect. This caused the contamination of foundry dust, air intake system, and of the filters. There was no dispersion in the environment and risks to workers. All contaminated dust was collected in big bags and placed within containers waiting to know for their destination. The global activity is now estimated at 4 GBq. Five different contamination scenarios, have been analyzed, i.e., possible ways by which such an amount of radioactive material could have been introduced into the foundry. The considered contamination (4 GBq) is compatible with the total activity of some calibration sources. In both cases, Cs-137 is in the form of a quite small radioactive source capsule, sealed and surrounded by a shielding material assembly, such as Pb. The source capsule would have a quite high radioactive concentration, and then a total mass in the order of grams. It has therefore to be classified – for sure – as a High Radioactivity Material, or, once it is inadvertently thrown away, as High Level Waste (HLW), according to the Italian regulation (III Categoria). The lead assembly shielding explains why the assembly passed through the check of the gamma sensor without creating alarm.

INTRODUCTION

The use of radioactive materials for medical treatment is established and controlled throughout the entire life of the sources. Nevertheless, the Goiânia (Brazil) accident revealed the possibility that unattended sources may become extremely dangerous items. In September 1987, in Goiânia, a teletherapy unit was abandoned after the moving of a private radiotherapy institute and the materials abandoned in the premises were left to the investigation activities of the local population. The capsule containing the caesium chloride salt was stolen and opened with the subsequently release of the radioactive material in the environment. The contamination was both direct and indirect and it was distributed among the population

as the capsule itself was sold as scrap and small particles of the caesium salt were also sold or distributed as they were glowing in a blue light in the dark, thus fascinating the unknowing people.

Four people died for the consequences of the internal and external irradiation, while other 250 were contaminated and treated at local hospitals [1]. Thorough environmental clean up took place in order to remove the potential residual sources of contamination and to bring the area back to the original situation which allowed for normal living conditions.

The remembering of this accident, for a long time indicated as one of the most relevant accidents in the nuclear history, was revamped when in an Italian foundry, in October 2005, an abnormal radioactivity level was measured in the dust and the suspect that an unexpected, and undetected at entrance in the plant, radioactive source was burnt among the other scraps became evident.

BACKGROUND

CAESIUM PROPERTIES AND USE

Caesium is a chemical element in the periodic table that has the symbol Cs and atomic number 55. It is a soft silvery-gold alkali metal and it is liquid at or near room temperature.

Major caesium deposits can be found at Bernic Lake in Manitoba. The deposits in that area are estimated to contain 300,000 metric tons of pollucite at an average of 20% caesium.

Caesium has a great number of isotopes (39 known) and their atomic masses range from 112 to 151. Of these, only one isotope, ^{133}Cs , is naturally stable, while most of the others have half-lives from a few days to fractions of a second.

^{137}Cs is a radioactive isotope which is formed mainly by nuclear fission in power plants and from the detonation of nuclear weapons. Its half-life is 30 years, and it decays by pure beta decay to a metastable state of ^{137}Ba . Barium-137m has a half-life of minutes and is responsible for all the of gamma ray emission. The ground state of barium-137 is stable (Table1).

Ba-137m can be used in food irradiation or in cancer treatment, thanks to the energy of its photon (about 800 keV).

TABLE 1
Data on Caesium-137 (adapted from [1])

| | | |
|-----------|-------|--|
| Emissions | | |
| | Gamma | 0,66 MeV (84%) |
| | Beta | from 0,51 MeV (95%) to 1,17 MeV (5%); mean 0.187 MeV |
| Half-life | | 30 years |

¹³⁷Cs is used for brachytherapy in the form of small spherical sources. Compared to ⁶⁰Co, caesium has the advantage of a lower price, due to its direct derivation from the normal operation of nuclear power plants; besides, ¹³⁷Cs has a longer half-time (30 years) and thus it is not necessary to change the source to maintain a high activity as often as with ⁶⁰Co; however, the energy of its γ photons is lower (0,662 MeV) and its specific activity is 10 times lower: thus, keeping constant the activity, the volume of the source is quite bigger than that of ⁶⁰Co.

¹³⁷Cs can be used in teletherapy machines, it is used in the form of highly soluble caesium chloride salt and it is contained in a sealed radioactive source capsule. This is set in a source wheel, made of lead and stainless steel, to form the rotating shutter mechanism. To produce a radiation beam, the shutter is rotated electrically to align the source capsule with the radiation aperture. As a measure to reduce the risk of uncontrolled contamination, the sealed radiation sources are usually produced in selected laboratories and are identified with a serial number.

CLASSIFICATION OF RADIOACTIVE WASTES

US regulations. In the United States, radioactive waste is classified according to five categories which range from high-level to low-level radioactive wastes. Materials are also classified according to the amount (quantity) of elements heavier than uranium, to their belonging to the uranium milling residues, or to the family of naturally occurring radioactive materials (a class to which also radioactive materials produced in an accelerator are associated).

High-level radioactive waste is spent nuclear fuel or highly radioactive waste produced if spent fuel is reprocessed. Spent nuclear fuel is used fuel from nuclear power plants. If the spent fuel is reprocessed, the materials that are left over after the collection of the reusable material is classified as high-level radioactive waste. The United States is not presently reprocessing spent nuclear fuel.

Uranium mill residues, or tailings, are the materials which remain after uranium has been

extracted from the mined ore: these tailings cannot be classified as low-level radioactive waste.

Certain materials, such as radioactive waste that contains more than specified concentrations of elements heavier than uranium (transuranics), naturally occurring radioactive material or radioactive material produced in an accelerator are not under the jurisdiction of the Federal Nuclear Regulatory Commission (NRC) and cannot be classified as Low-Level Radioactive Wastes.

As a consequence, low-level radioactive wastes include a variety of different materials. Some examples are wastes generated at facilities such as nuclear power plants, hospitals, and research institutions. Many materials and equipments can be associated to the low-level wastes category: these might be radioactive materials used in various processes or contaminated supplies and equipment. Hereafter some examples [2] are provided:

- from nuclear power plants:
 - tools, piping, components, general equipment used e.g. for ordinary maintenance;
 - unrecoverable equipment and spare parts, such as ion exchange resins and filter materials (these are used for processing water);
- from research sites:
 - any research equipment from laboratories which deal with and handle radioactive materials;
 - disposable equipment such as: protective coat, shoe covers, towels and other supplies that might have been used in a place where radioactive material is present;
- from hospitals and medical centres:
 - towels, containers, paper, liquids, etc. and equipment which might have been contaminated during their use for diagnose or treating in hospitals;

- air filters used in areas where airborne radioactive contamination has been tested.

No liquid waste can be received as low-level radioactive waste disposal facility.

Due to the variability of the nature of the low-level radioactive waste, its concentration of radioactive material can belong to a wide range of values. Because of this, low-level waste is divided into four classes with specific regulations for each class.

The four classes of low-level radioactive waste are Class A, Class B, Class C, and Greater Than Class C and are defined in the Code of Federal Regulations, Title 10, Part 61 (10CFR61) [3]. The materials belonging to all classes, except for the fourth, are generally acceptable for near-surface disposal. Section 10CFR61.55 lists the radioactivity concentration limits of specific radioactive materials (Table 2 reports values for the Cs isotope) allowed in each low-level waste class.

TABLE 2
Classification determined by short-lived radionuclides, (excerpt of ^{137}Cs) [curies per cubic meter] [3]

| | Class A | Class B | Class C |
|--------|---------|---------|---------|
| Cs-137 | 1 | 44 | 4600 |

As it can be derived from Table 2, the description of low-level radioactive waste is usually given according its radioactivity i.e. the rate at which radiation is released by the material. Another common way is to provide the volume of the waste itself.

Italian Regulations - Low-Level Waste according to Italian Regulation. In most countries with a nuclear program, the waste management strategies are based on deep geological disposal of High-Level Waste (HLW), while a less sophisticated disposal method, mostly a near-surface type repository, is used for Low-Level or Intermediate-Level Waste (LLW/ILW)[4].

Italian waste management regulations deal with National Laws on radioactive materials [5], and with Technical Guides from the Italian nuclear regulatory committee [6]. Wastes are classified into three

categories (“*I Categoria*” = First category = Very Low-Level Waste, “*II Categoria*” = Second Category = Low-Level Waste, “*III Categoria*” = Third Category = High Level Waste) according to concentration limits for radionuclides.

Second category waste may be defined “Low-Level Waste” since it may be disposed of in surface or near-surface disposal sites, in a similar way to Low-Level Waste eligible for Shallow Land Burial according to the United States Waste Regulation 10CFR61. First category waste may be defined “Very Low-Level Waste” since it decays down to radioactive concentration levels comparable to natural substances in a maximum decay period of some years. Tables 3 and 4 list a series of maximum concentrations for radionuclides either in the case of conditioned wastes or in the case of unconditioned ones.

TABLE 3
Concentration limits for second category radioactive wastes, conditioned (translated from [6])

| Radionuclides | Concentration |
|---|------------------------------------|
| α emitters $t_{1/2} > 5$ years | 370 Bq/g (10 nCi/g) |
| β/γ emitters $t_{1/2} > 100$ years | 370 Bq/g (10 nCi/g) |
| β/γ emitters $t_{1/2} > 100$ years in activated metals | 3,7 K Bq/g (100 nCi/g) |
| β/γ emitters $5 < t_{1/2} \leq 100$ | 37 K Bq/g (1 $\mu\text{Ci/g}$) |
| ^{137}Cs and ^{90}Sr | 3,7 M Bq/g (100 $\mu\text{Ci/g}$) |
| ^{60}Co | 37 M Bq/g (1 mCi/g) |
| ^3H | 1,85 M Bq/g (50 $\mu\text{Ci/g}$) |
| ^{241}Pu | 13 K Bq/g (350 nCi/g) |
| ^{242}Cm | 74 K Bq/g (2 $\mu\text{Ci/g}$) |
| Radionuclides with $t_{1/2} \leq 5$ years | 37 M Bq/g (1 mCi/g) |

TABLE 4
Concentration limits for second category radioactive wastes, unconditioned (translated from [6])

| Radionuclides | Concentration |
|---|-------------------------|
| Radionuclides with $t_{1/2} > 5$ years | 370 Bq/g (10 nCi/g) |
| ^{137}Cs and ^{90}Sr | 740 Bq/g (20 nCi/g) |
| Radionuclides with $t_{1/2} \leq 5$ years | 18,5 K Bq/g (500 nCi/g) |
| ^{60}Co | 18,5 K Bq/g (500 nCi/g) |

CASE HISTORY

In late October 2005, at the Beltrame plant in Susa Valley (Italy) a radioactive source was accidentally burned in the blast furnace. The source was not discovered by the detectors at the entrance, evidently because of some shielding effect. This caused the contamination of foundry dust, air intake system, and of the filters. There was no dispersion in the environment and risks to workers. All contaminated dust was collected in big bags and placed within containers waiting to know for their destination. The global activity is now estimated at 4 GBq. In 2004, there was already a similar incident occurred in Vicenza, caused by an apparatus for gammagraphy: it is likely that the same has happened in this new incident. An investigation was carried out to identify possible originating causes for the contamination.

CONTAMINATION SCENARIOS

Considering the fact that the estimated ^{137}Cs contamination was around 4 GBq, it was possible to consider some (five) different contamination scenarios, i.e., possible ways by which such an amount of radioactive material could have been introduced into the foundry.

A – Lost Radiotherapy Source. Cs-137 is used for teletherapy machines for medical use. In this case, however, the required dose rate intensity (around 5 Gy/h) implies a radioactive source of around 1000 Ci of ^{137}Cs , that is, around 50 TBq. Several orders of magnitude higher than our case. A contamination of around 4 GBq is not compatible with the presence of such a source.

B – Lost Industrial Gammagraphy Source. ^{137}Cs is seldom used for this purpose also, even if its maximum gamma energy (660 keV) is not so high. In this case also, considering the specific gamma ray constant of ^{137}Cs (8.9 mGy/h per GBq at 1 m) it is unlikely that such a source could cause the contamination in exam. 4 GBq would cause a gamma dose rate at 1 m of about 35 mGy/h, insufficient for

any industrial gammagraphy purpose. One could think to a portion of that source, however it would be unlikely that such a source would have been sealed and shielded, in order not to cause alarm when passing through the gamma sensor.

C – Lost gamma source for emoderivatives irradiation. Cs-137 sources are industrially used for irradiation of emoderivatives products for medical use. In this case, the source would have an activity compatible with the considered contamination (some GBq). The source would come inside a sealed shielding assembly, with a source capsule surrounded by a shielding materials such as lead.

D – Lost instrumentation calibration source. ^{137}Cs source are also used as calibration source for several instrumentation devices. Those sources are sealed and included into shielding assemblies too. Once the device is not operative anymore, the source may be thrown away with it and become an inadvertent contaminant inside a metallic waste. The considered contamination (4 GBq) is compatible with the total activity of some calibration sources.

E – Contaminated material – Italian II Categoria. One must consider the possibility of having contaminated waste in which ^{137}Cs is present as a contaminant in a bulky amount of material. However, we know that the material has passed a check through a very sensitive radiation sensor before entering the foundry.

This scenario is unlikely, and to demonstrate it, let us consider the upper limit for second category waste (II Categoria) in the Italian regulation, that is, for ^{137}Cs in unconditioned materials, 740 Bq/g (Table 5). If a contamination level compatible with, however inferior to, this limit (say around 400 Bq/g) is assumed, then we should have had at least 10000 kg (10 Metric tons) of contaminated material, in order to have a total radioactivity of 4 GBq. If the specific radioactivity is 40 Bq/g only, then we would have 100 metric tons of contaminated materials, and so on.

This situation is not possible in practice. The material has passed, without causing alarms, through a sensor (a gamma portal) with a sensibility that

excludes the presence of such a radioactivity concentration in the material.

In order to be diluted in such a way in order to avoid any alarm by the gamma sensor, the quantity would have been of several hundreds of tons of contaminated material: this is not possible. By definition, then, it is not possible to have ^{137}Cs in so low concentrations in order to classify the material in the Italian first category waste (I Categoria).

Further case – Contaminated material – US 10CFR61 Class C Waste. Considering finally the maximum concentration limits for LLW according to the US regulation, we may consider the highest possible concentration limit for ^{137}Cs in a material that can be classified as LLW in the US. According to the previously mentioned 10CFR61 regulation, we may consider the limit for Class C waste: it is - for ^{137}Cs - $5 \cdot 10^4 \text{ Ci/m}^3$ (material still eligible for Shallow Land Burial). This translates into $18.5 \cdot 10^{14} \text{ Bq/m}^3$, that is, $18.5 \cdot 10^8 \text{ Bq/cm}^3$. If we have a material with a density similar to that of steel (7.8 g/cm^3) we then have a maximum radioactivity concentration of around $2.5 \cdot 10^8 \text{ Bq/g}$. If the density is similar to concrete (2 g/cm^3) then we have a maximum radioactivity concentration of around $9 \cdot 10^8 \text{ Bq/g}$.

The amount of material necessary for having 4 GBq is therefore varying from 3 to 16 grams of material. However, if ^{137}Cs is not exactly equal to the upper Class C waste limit, the amount of material becomes higher as the concentration of ^{137}Cs decreases.

This situation may be a likely scenario, and it may be included in cases C and D (see above) as an upper limit.

However, it is quite unlikely that a sealed and concentrated source of ^{137}Cs could be classified as Class C waste in the US and disposed of in Shallow Land Burial (see definitions in the previous paragraph).

It is much more likely that, given its small volume and its nature, the ^{137}Cs source would be classified and disposed of as High Level Waste.

CONCLUSION

In conclusion, we consider as likely scenarios for the contamination source the ones as C and D in our list:

C – Lost gamma source for emoderivatives irradiation

D – Some lost instrumentation calibration sources

In both cases, ^{137}Cs is in the form of a quite small radioactive source capsule, sealed and surrounded by a shielding material assembly, such as Pb.

The source capsule would have a quite high radioactive concentration, and then a total mass in the order of grams. It has therefore to be classified – for sure – as a High Radioactivity Material, or, once it is inadvertently thrown away, as High Level Waste (HLW), according to the Italian regulation (III Categoria).

The lead assembly shielding explains why the assembly passed through the check of the gamma sensor without creating alarm.

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EFFECTS OF RECOVERY OF WETLAND VEGETATION ON ACTIVE NUTRIENTS FRACTIONS IN ALBIC SOILS

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ABSTRACT

Extremely limited studies exist on the restoration of albic soils which are predominantly found in eastern China. These low-yielding soils have been further degraded over the years in the Sanjiang Plain (China) due to intensive agricultural practices. Restoration measures are therefore crucial in ensuring ecological functionality and sustainability. The present study was undertaken to ascertain the effects of restoration practices on active nutrients fractions in the depleted albic soils in the Sanjiang Plain. Four treatments were considered, including only planting *Calamagrostis angustifolias* (xyz), only applying litters (litter), planting *Calamagrostis angustifolias* and applying litters (xyz+litter) and blank (albic soil). The soil nutrient indices that were assessed contained dissolved organic carbon (DOC), total organic carbon (TOC), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). Statistical analysis was performed using SPSS. The results showed that the amount of MBC and MBN increased significantly ($P < 0.001$) in the treatments xyz and xyz+litter relative to the other treatments. Soil microbial biomass of xyz+litter treatment was higher than that of xyz treatment ($P < 0.05$). It was also observed that MBC/MBN in the xyz and xyz+litter treatments was higher than that in the other treatments ($P < 0.05$). Meanwhile, the difference in the MBC/MBN in soil between the xyz and xyz+litter treatments was negligible ($P > 0.5$). Relative to the blank, the DOC and TOC of xyz treatment increased significantly. In the litter treatment, the increase in TOC in the albic soil surface was negligible. Compared with the blank, soil DOC in the litter treatment increased significantly ($P < 0.001$). The DOC/TOC in the litter treatment was highest, followed by xyz, blank and xyz+litter treatments, respectively. It can be deduced that the main source of TOC in 0-20cm of the albic soil was the roots of the *Calamagrostis angustifolias*. The DOC in the soil was mainly as a result of biological and physical activities of litters and microbial residues. The increase in MBC in the xyz+litter treatment indicates that the

microorganisms were in an active condition. Notably, litters and underground biomass enhanced the availability of the microbial energy source in the soil. It is therefore asserted that the combined effects of plant roots and litters on soil microorganisms is significant. This treatment greatly improved the nutrient conditions in the albic soil. The present study therefore concludes that combining wetland plants and litters as a restoration practice in albic soils may be an effective approach in ensuring ecological functionality and sustainability.

KEYWORDS:

active nutrient fractions; albic soils; China; ecological sustainability; restoration measures; Sanjiang Plain

INTRODUCTION

Over the last few decades, land use has changed dramatically across the globe [1]. The overexploitation and intensive use of soil resources as a result of increased anthropogenic activities have resulted in a significant loss in soil organic matter. This in turn has had detrimental effects on soil fertility and water-holding capacity. These pose severe challenges to environmental sustainability. Effective land use management is therefore critical in addressing the issue.

It is important to note that land use management is closely linked with land nutrient cycling. Change in land use management can affect vegetation litters and residual volume [2]. It also influences microbial activities in soil which in turn affects the redistribution of nutrient in the soil system [3]. Management practices which involve less soil disturbance promote the accumulation of organic carbon in soil. In general, it has been observed that agricultural activities can lead to a 16%-77% loss of organic matter content in soil, with an average of about 29% [4]. It must be pointed out that the lighter the soil texture, the higher the loss of organic matter. In addition,

agricultural activities could also decrease nitrogen in soil.

The removal of natural vegetation decreases aboveground biomass, accelerates the mineralization of the organic matter and causes a loss of organic carbon in soil. This subsequently leads to soil degradation [5]. Lal R reported that about 60%-70% of carbon that has been depleted can be recovered by applying appropriate and effective land management practices [6]. Many studies have shown that the restoration of natural vegetation from farmland increases organic matter content in soil. Hughes et al concluded that dissolved organic carbon (DOC) will increase after restoring the land to a forest as a result of the increase in organic A1 compounds and chelating organic acid [7]. Zhou and Jiang conducted a research on red-soil carbon pool and observed that total organic carbon (TOC) and different kinds of active carbons significantly increased after plantation restoration [8]. However, it must be pointed out that it is only limited to the top soil [9-12]. By studying the dynamic variation of carbon in restored farmland soils, Landgraf observed that the content of organic carbon increased gradually in the 0-10cm soil depth while it decreased at soil depth of 10-30cm [13].

It must be noted that the recovery rate is closely linked with the background of organic matter, land use patterns, hydrological conditions of soil and microbial activities in soil [14, 15]. It is important to note that for the same soil type, recovery rate of the main ecological function of the system is highly dependent on the duration of land usage. Knops and Tilman demonstrated that a recovery of 95% of the total organic matter during a restoration process from farmland to wetland would be achieved in a period of about 230 years [16]. However, the active components of soil can largely be recovered within a shorter period. Macedo et al conducted a study on nutrient dynamics in soil after recovery of degraded land using leguminous nitrogen-fixing trees and discovered that the nutrient stocks in the litter of the restored area were similar to those found in native forest after 13 years [17]. The microbial carbon in the 0-20cm soil after 17 years of restoration was 2.95 times larger than that of the soil after only 9 years of restoration [18].

Since the 1990s, reclamation of wetlands in the Sanjiang Plain has been on the increase. In recent times, measures have been implemented that promote reclamation whilst curbing land degradation. This has resulted in a massive change in carbon storage and structure in the ecosystem.

Yuan et al undertook a study on the effects of different land-use patterns on the albic soil in Sanjiang Plain [19]. Zhang conducted a study on the labile fractions and organic carbon change in soil over the restoration period [20]. Yang carried a research on changes of soil organic carbon and nitrogen under different land uses in Sanjiang Plain [21]. Wang carried out a research on organic carbon accumulation in various types of freshwater wetlands in the Sanjiang Plain [22]. However, it must be pointed out that extremely limited studies have been conducted to ascertain the dynamics of carbon and nitrogen in the soil over the restoration period. This study assessed the changes in dissolved organic carbon (DOC), total organic carbon (TOC) as well as the microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in the topsoil during the restoration process.

In the Sanjiang Plain, the major soil types include meadow and albic soils [23]. Albic soils occupy the largest proportion in the cultivated land, which is 33.5%. These soils have poor permeability and are mostly less fertile [24]. The humus layer occupies only 15-20cm depth below which lies the albic layer, whose depth is 20-30cm. The albic layer is largely characterized by low porosity and high impermeability. In the past 50 years, the intensive agricultural activities in the area has depleted the humus layer. It must be noted that the extent of soil degradation is extremely serious. This prompted the reclamation measures. There is therefore a dire need for studies to focus on the changes in the storage and structure of soil conditions to improve soil quality in the Sanjiang Plain.

This study was undertaken to assess the role of *Calamagrostis angustifolias* in the recovery of the albic soil layer. Specifically, the present study ascertained the changes in carbon content of the albic soil layer by planting *Calamagrostis angustifolias*.

MATERIALS AND METHODS

Study Area. The study site was set up at the Sanjiang Mire Wetland Experimental Station, Chinese Academy of Sciences, Sanjiang Plain, China, approximately 47° 35' N, 133° 31' E (Fig. 1). The place has been the largest area of mire wetlands in North China as a result of boreal climatic conditions and low slope grade. The average altitude is between 55.4 and 57.9m, the annual mean temperature is 1.90C and the non-frost period is about 125 days in one year [26-28]. The

study site is in the seasonal frozen zone with annual precipitation between 550 and 600mm, mainly in July and August, accounting for more than 65% of the annual precipitation.

Experimental Design. Albic soil samples at depth of 45–60cm in the experimental station were collected in early May, 2012. The samples were then dried and passed over 0.5cm sieve to remove fine roots and large granular mineral substances. Cylindrical holes of about 20cm in diameter and 50cm in height were made in the experimental

station. PVC tubes of 18cm in diameter and 60cm in height were placed in the holes. The PVC tubes were then filled with albic soil to a depth of 50cm. Four treatments were made. These included only planting *Calamagrostis angustifolias*(xyz), only applying litters (litter), planting *Calamagrostis angustifolias* and applying litters (xyz+litter) and blank (albic soil). Six repetitions for each treatment were made. It must be noted that 25 *Calamagrostis angustifolias* seedlings whose stems/heights were nearly consistent (15cm) were planted in the PVC tubes in mid-May.

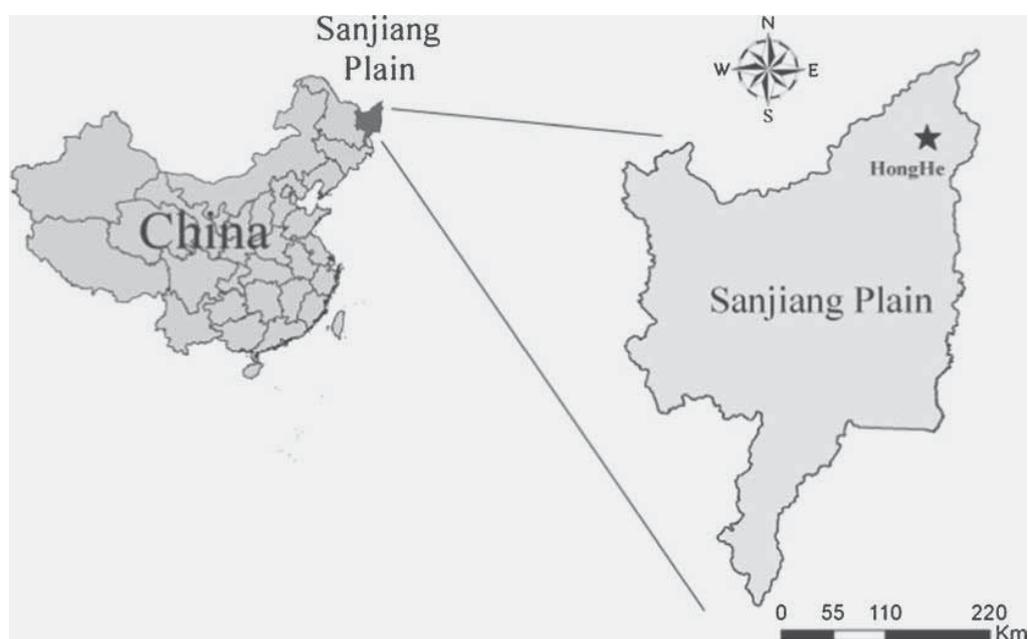


FIGURE 1
Location of Sanjiang Mire Wetland Experimental Station [25]

Sample Treatment. Soil samples were taken and measured in mid-October, 2012. Soil samples were sieved through a 2-mm mesh sieve to remove the litter, dead roots and small animals and preserved in refrigerator at 4°C until analysis for the four soil parameters [29]. The parameters that were measured included TOC, MBC, MBN and DOC.

TOC and DOC Measurements. Soil samples were air dried at room temperature for three days. Samples were grounded and sieved through 0.149 mm sieve. Samples were then tested for TOC using high temperature combustion (TOC-VCPH C analyzer, Shimadzu, Kyoto, Japan).

Moist soil samples (equivalent to 10 g oven dry weight) were weighed into 40-mL polypropylene centrifuge tubes. The samples were

extracted using 30 mL of distilled water for 30 min on an end-over-end shaker at 30 rpm and centrifuged for 20 min at 8,000 rpm. The entire supernatant was filtered through a 0.45- μ m filter into separate vials for C analysis [30]. The extracts were analyzed for C using high temperature combustion (TOC-VCPH C analyzer, Shimadzu, Kyoto, Japan).

MBC and MBN Measurements. The soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using a chloroform fumigation-extraction method. Fumigated and non-fumigated soils were extracted with 0.5 mol/L K₂SO₄ for 30 min (soil/extractant ratio=1:5), and the extracts were analyzed for C using a TOC analyzer (TOC-VCPH, Shimadzu). The total dissolved N concentrations were

determined using a continuous-flow analyzer (Skalar, Breda, Netherlands). The C/N obtained from the fumigated samples minus that from the non-fumigated samples was assumed to represent

the microbial-C/N flush and converted to microbial biomass C/N by using the following relationships: microbial biomass C=microbial-C flush/0.38, and microbial biomass N=microbial-N flush/0.45 [31].

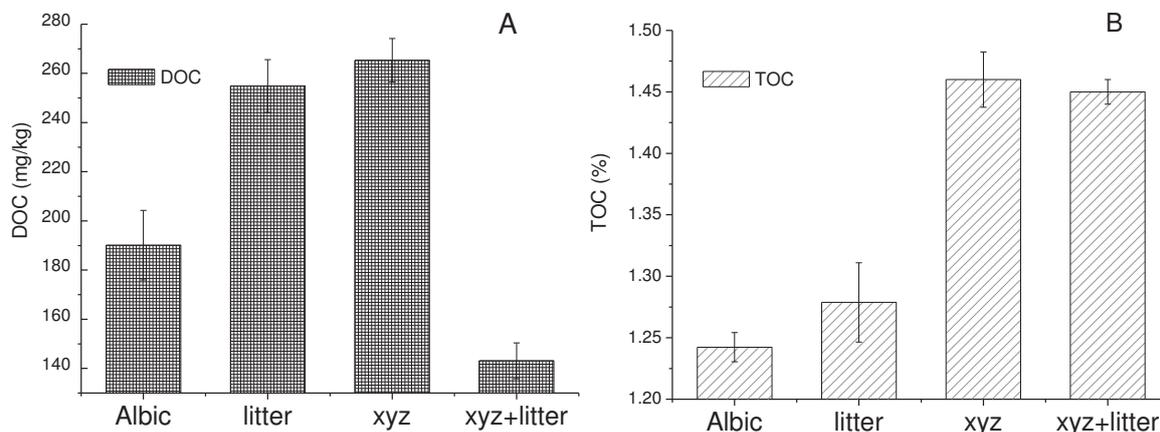


FIGURE 2
Changes of TOC and DOC in the respective treatments

Statistical Analysis. Statistical analysis was done using the SPSS software package for Windows (SPSS 17). One-way ANOVA was used to test for significance. For all analyses where $P < 0.05$, the factors tested and the relationships were considered to be statistically significant.

RESULTS

With respect to DOC and TOC in the soil, the present study revealed that they are highly influenced by the rooting of *Calamagrostis angustifolias* (Fig. 2). Relative to the blank, soil DOC and TOC of xyz treatment increased significantly in one growing season. Comparing xyz and xyz+litter treatments, there was no significant difference ($P > 0.5$) in terms of change in TOC. In the case of the litter treatment, the increase in TOC in the albic soil surface was negligible. It was also observed that the soil DOC in the xyz+litter treatment was significantly lower than that in the xyz treatment ($P < 0.001$). Compared with the blank, soil DOC in the litter treatment increased significantly ($P < 0.001$).

The results obtained in the present study showed that the difference in the DOC/TOC among the respective treatments was significant (Fig. 3).

The DOC/TOC in the litter treatment was the highest, followed by the xyz treatment. The DOC/TOC in these treatments were observed to be significantly higher than that in the other two treatments ($P < 0.05$). The DOC/TOC in the xyz+litter treatment was the lowest.

It was observed in the present study that the amount of MBC and MBN increased significantly ($P < 0.001$) in the xyz and xyz+litter treatments relative to the other treatments (Fig.4). Soil microbial biomass of xyz+litter treatment was also observed to be higher than that of xyz treatment ($P < 0.05$).

The results showed that the roots of plants and litters have a combined effect on the increase of soil microbial biomass. However, in the litter treatment, the increase in MBC and MBN in soil was not statistically significant ($P > 0.5$).

The present study showed that the MBC/MBN changed greatly after planting *Calamagrostis angustifolias* in albic soil (Fig.5). In the xyz and xyz+litter treatments, MBC/MBN in soil was observed to be higher than that in the two other treatments ($P < 0.05$). Meanwhile, the difference in MBC/MBN in soil between the xyz and xyz+litter treatments was negligible ($P > 0.5$).

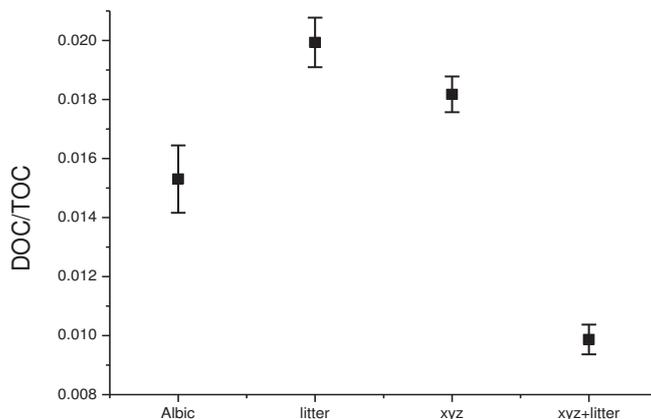


FIGURE 3
DOC/TOC of the respective treatments

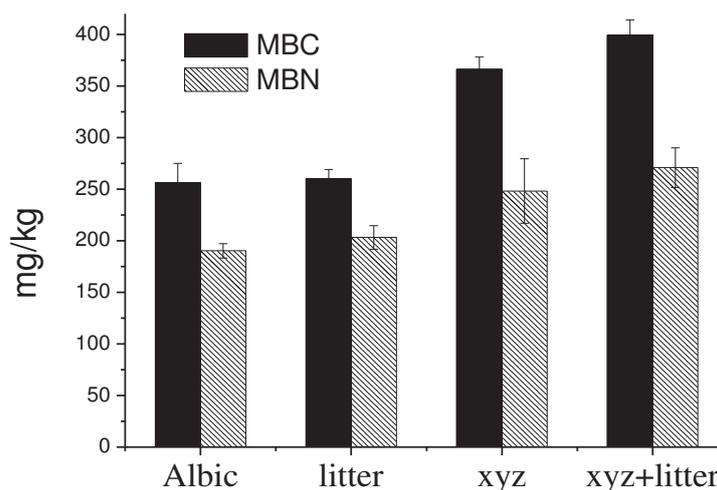


FIGURE 4
Changes of Microbial Biomass in the respective treatments

DISCUSSION

The increase in TOC in the albic soil in the xyz treatment was obviously from the roots of the *Calamagrostis angustifolias* since there was no aboveground plant biomass. Comparing xyz treatment with xyz+litter treatment, there was no

significant difference in the TOC. This means that litters on the surface contributed little to the increase of TOC in albic soil during the process of organic carbon accumulation. It can be deduced that the roots of *Calamagrostis angustifolias* served as the main source of TOC in the 0-20cm of the albic soil.

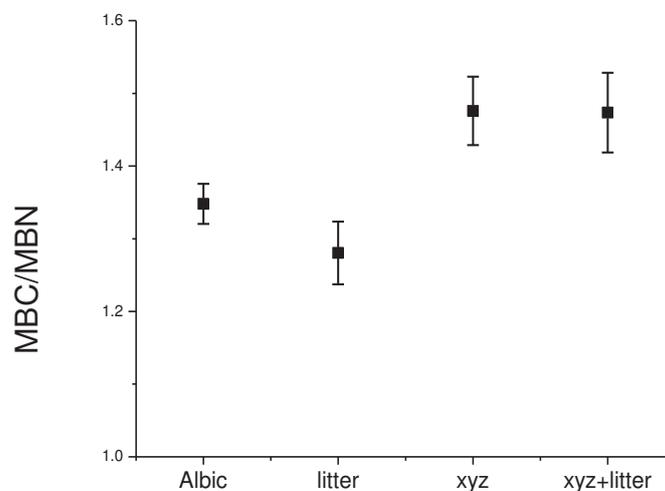


FIGURE 5
MBC/MBN in the respective treatments

The DOC in soil was mainly from the biological decomposition of litters, physical fragmentation as well as microbial residues. Moreover, root debris was another major source of DOC in soil. It must be noted that the DOC in soil increased significantly as a result of the presence of underground biomass after planting *Calamagrostis angustifolias* in the albic soil. Litters on the soil surface produced significant dissolved substances due to microbial degradation. This led to significant increase in DOC in the albic soil surface after the application of litters. However, DOC in soil decreased considerably due to the combined effect of litters on the surface and plant roots. This may be closely related to microbial biomass. In the xyz+litter treatment, MBC of albic soil increased significantly. This indicates that the microorganisms were in an active condition. The prevalence of soil microorganisms resulted in the significant consumption of DOC which in turn led to the reduction of the DOC measured in the soil.

Per the characteristics of albic soils which include low nutrient content and poor permeability, microorganisms in this soil type typically have limited nutrient. As such, the soil microbial biomass was found to be very low. However, it must be noted that the root debris of *Calamagrostis angustifolias* changed the condition of nutrient in the albic soil after one growing season. Microorganisms in the soil gained more energy which resulted in the increase of microbial biomass in soil. It is important to point out that the litters and underground biomass of *Calamagrostis*

angustifolias enhanced the availability of the microbial energy source in the soil. This might have accounted for the reason why the microbial biomass in the xyz+litter treatment was significantly greater than that in the xyz treatment. It was also found that the microbial biomass increased negligibly in the litter treatment.

It is also important to note that due to the impermeable nature of albic soils, the nutrients in decomposed litters are only used by the microorganisms present within the soil layer where these litters are found. Hence, the microorganisms beyond this layer do not get access to these nutrients. As a result, there was negligible increase in the microbial biomass in the soil. On the other hand, the combined effects of plant roots and litters on soil microorganisms was significant. It must be noted that this treatment caused significant increase in the soil microorganisms. This may be due to the fact that the drainage condition of the albic soil greatly improved. As a result, the leaching of the decomposed litter into lower layers was possible. Furthermore, improvement in the soil condition provided suitable environment for the microorganisms to flourish and increase in population. Hence, it was noted that the interaction between wetland plants and litters effectively increased the microbial biomass in soil and greatly enhanced the microbial environment in soil. It must be pointed out that this process ultimately lead to increase in carbon and nitrogen in albic soils.

CONCLUSION

The present study revealed that the restoration of albic soils is greatly influenced by the kind of management practice that is adopted. It must be pointed out that *Calamagrostis angustifolias* was observed to significantly change the active nutrient indices such as microbial biomass and DOC in the albic soil. Furthermore, it was revealed in the present study that *Calamagrostis angustifolias* greatly enhances active carbon and nitrogen components in albic soils. The present study showed that the use of litters alone as a restoration practice has little effect on all the soil nutrient indices. Contrarily, it was shown in this study that combining wetland plants and litters could effectively improve active carbon and nitrogen components in albic soils. This points out that combining wetland plants and litters as a restoration practice in albic soils is the most effective approach. The study provides vital information for recovery of active nutrient indices in albic soils during wetland restoration processes. The study is therefore of extreme importance for the effective management of albic soils.

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LAGRANGIAN MODELING OF PARTICLE RESIDENCE TIME IN THE OUJIANG RIVER ESTUARY, CHINA

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ABSTRACT

Residence time (RT) is widely used as an indicator for estimating the health of a water body. However, it is difficult to obtain meaningful RT predictions from high-resolution hydrodynamic models without the additional parameter inputs needed to integrate the model results. In this study, changes in the RT resulting from large-scale coastal projects in the Oujiang River Estuary (ORE) were determined by coupling a high-resolution hydrodynamic model, the Environmental Fluid Dynamics Code, with a Lagrangian transport model. The ORE was subdivided into 7 boxes filled with Lagrangian particles. The Lagrangian transport model was also used to assess the water renewal process and determine the effects of freshwater flows and geomorphologic indices on RT. Freshwater inflow was the main factor that influenced RT. The upstream section of the study area was mainly influenced by runoff, and the downstream section of the study area was mainly influenced by tidal forces. The water exchange characteristics of the midstream section were influenced by both runoff and tidal current, with complex energy and repeated water body exchange. Particles in the water body were moved repeatedly in this section, with relatively slow exchange velocities. The construction of several large projects in the ORE influenced the RT within 3 days; however, this influence was far weaker than the influences of seasonal changes. The method proposed in this paper can be used to accurately simulate the RT changes caused by coastal projects in the ORE.

KEYWORDS:

Lagrangian transport; residence time; estuary; freshwater inflows; geomorphologic factors

INTRODUCTION

Due to rapid economic development and the promotion of industrialization and urbanization, an increasing number of coastal projects are being established in estuaries. These projects change the hydrodynamic features of the estuaries, such as the

flow rate, speed, mixing ratio of freshwater and seawater, flushing rate, and sediment accumulation type. Consequently, the material cycle in the estuary is disturbed, which can change the concentrations of contaminants and their distribution to the open sea [1, 2].

The residence time (RT) of water refers to the average time that a water parcel spends in a water body, such as an estuary, lagoon, or harbor. In general, estuaries with a short RT have higher water quality because discharged substances spend less time in the system and contaminants are less likely to remain there. Thus, RT is an important index for estimating the health of a water body subjected to human-induced stress [3]. However, the concept of characterizing the transport processes of water bodies over a specific time scale has not been defined or named [4]. Different terms can be found in the literature that refer to different types of time scales (residence time, age, flushing time, turnover time, and transient time). Bolin and Rhode [5] highlighted the need to overcome confusion regarding the use of different terms to indicate timescales and reviewed the concepts of “age”, “transit time”, and “turnover time” to introduce more rigorous definitions. Zimmerman [6] introduced the concept of RT as the average time that a water parcel spends in an estuarine system and applied the concept to various estuarine systems in the Netherlands. Based on the definition of RT proposed by Zimmerman [6], Takeoka established a link between RT and other time scales and developed the concept of RT [7]. Through analysis, he noted that the average transient time equals the average turnover time and the average RT is equal to the average age; in addition, he concluded that the average RT is more suitable for describing the time scale of the exchange characteristics of a water body (or material) [7]. Liu et al. analyzed time scales and arrived at a conclusion that was consistent with the analysis of Takeoka [8]. These authors noted that the average RT defined by Takeoka can be described by different types of water exchange. Therefore, we selected RT to use as the time scale in this study.

Nevertheless, substantial disagreement exists regarding the optimal methods for calculating RT,

and excellent discussions on this topic have been published in recent years [9-11]. In the past, RT has been calculated using empirical methods based on various criteria, such as the movement of individual particles [12], the salt budget [13], freshwater fraction models [14], and the use of salinity as a tracer [15]. The empirical formula used for parameter selection is arbitrary, and the results vary substantially depending on the formula used [16]. Freshwater fraction models have been criticized for omitting the effects of flushing by seawater [17]. However, methods based on high-resolution hydrodynamic models using salinity transport require additional analysis to remove the effects of tide-induced oscillations. These methods also assume that all freshwater originates in the river and that the salinity distribution is time-dependent; thus, the validity of this assumption and the uncertainty in the salinity distribution may affect the calculation results [18].

Another approach is to determine RT by using numerical models to derive Lagrangian circulation and examine the motion of water parcels. This approach makes it easier to label a water mass and relate its position at any instant of time with its release points because the particles can carry information regarding the origin of the water mass. Thus, accurate, detailed spatial and temporal information regarding particle trajectories and dispersion histories can be obtained. Several researchers have used Lagrangian implementations of the age concept [19-21], but the memory characteristics of Lagrangian tracers have rarely been explored. One shortcoming of the Lagrangian approach is that the renewal time scales are difficult to define in an unequivocal way. For instance, the RT of a region is generally calculated as the time it takes for all of the water in that region (corresponding to all the tracers released at instant zero) to be replaced by new water. This criterion may lead to long RTs. Regarding practical applications, RT is considered complete when a specific portion of the original water mass, which is referred to as residual water, remains in the region. Subjectively, residual water is described as a specific portion of the original water mass.

The objective of this research was to develop an improved method for calculating RT by using a Lagrangian transport model combined with the

Environmental Fluid Dynamics Code (EFDC) hydrodynamic model [22]. The integrated renewal time scale and integrated water fraction were needed to understand the history of renewal. This method was used to determine the influences of several large coastal construction projects on the Oujiang River Estuary (ORE), China. Next, the determined influences were used to create a dependency matrix to understand the fates of water masses inside the estuary under the assumption that the Lagrangian tracers explicitly convey information regarding their origins. Thus, RT and water history were considered together and the influences of seasonal freshwater inflow and geomorphological modifications on RT were determined.

The Oujiang River is located in the southern part of Zhejiang Province, China, and is the second largest river in Zhejiang, with a length of 388 km and a drainage area of 18100 km². The ORE is a typical continental and marine facies estuary, with a tidal reach of 78 km [23]. Lingkun Island divides the ORE into north and south regions. The discharge from the upper stream of the Oujiang River varies seasonally, and the discharge during the flood season (April to September) accounts for approximately 70% of the annual discharge. According to a statistical analysis of the data collected over many years, the maximum, average, and minimum discharges from the upper streams of the ORE are 22800 m³/s, 470 m³/s, and 10.6 m³/s, respectively, and the total discharge from the ORE to the sea is approximately 16.95 billion m³ per year. The ORE is a typical macro-tidal estuary, with an average tidal range greater than 4 m and a maximum tidal range of 7 m [24, 25]. The area outside of the ORE is complex, with many islands, dense beaches and interlaced swales.

In recent years, the local government has built many large offshore projects to meet the demands of economic development. To accelerate the sedimentation of sand at Wenzhou Shoal, the Lin-Kun Submerged Dike project (with a dike length of 2.5 km) was implemented at the southern mouth of the Oujiang River in 2001 [26]. The Ling-Ni Embankment was completed in 2003, was first used in April 2006, is 14.5 km long, and connects Lingkun and Niyu Islands. The first reclamation project of Wenzhou Shoal was completed in 2010, which increased the land area by 21 km² [27]. The locations of these three projects are shown in Fig. 1.

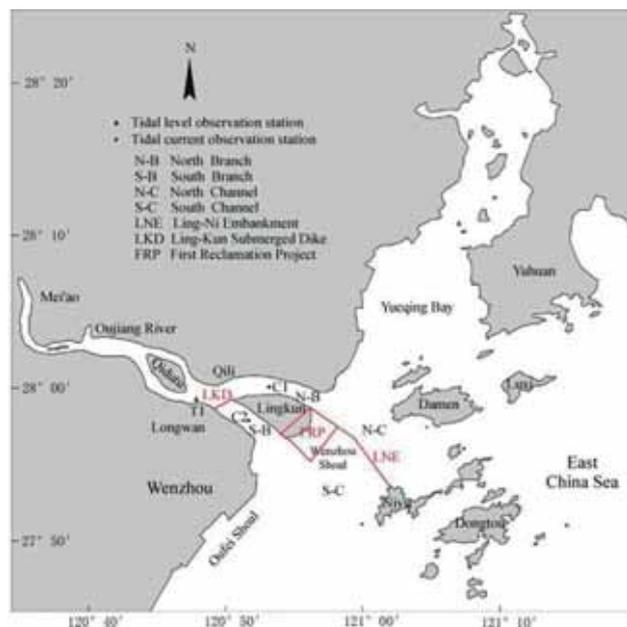


FIGURE 1
Layout of the observation stations in the ORE.

Few studies of the RT in the ORE have been conducted, and the exchange capacity of the ORE water body remains unclear. In recent years, the relief characteristics of the ORE have substantially changed due to the implementation of large coastal projects. RT is closely associated with upstream water conditions, relief characteristics and hydrodynamics. Thus, it is important to study the seasonality and differences in the RT in the ORE before and after projects were implemented.

MATERIALS AND METHODS

FIELD DATA

Field observations of tides and currents are important because they provide the data needed to drive, correct and verify numerical models in coastal hydrodynamics studies. Tide and current measurements (Fig. 1) were obtained from the Shanghai East Sea Marine Engineering Survey and Design Institute and the State Oceanic Administration of China. A hydrology test was conducted from June to July 2005 that involved synchronous observations of two vertical flows during neap, moderate and spring tides. The spring tide occurred from 09:00 on June 23 to 12:00 on June 24, the moderate tide occurred from 11:00 on June 26 to 15:00 on June 27, and the neap tide occurred from 15:00 on June 30 to 19:00 on July 1. All tides lasted 27 hours, with two high tides and two low tides. Hourly tide

measurements were conducted at the tide gauge stations from June 20, 2005, to July 19, 2005. These data were used to correct and verify the numerical models.

According to previously published research data, the discharge from the upper stream of the Oujiang River exhibits significant seasonality [18]. Therefore, the following two typical scenarios were considered in this paper: summer, when the discharge from the upper stream is large, and winter, when the discharge from the upper stream is lower. According to the measured hydrological data, the average seasonal discharges of the upper stream of the Oujiang River are 1109 m³/s in summer and 124 m³/s in winter.

MODELING SYSTEM

Hydrodynamic model. The EFDC water modeling system has been widely adopted in research, including studies of the tidal James River [28], the York Estuary [29] and the Cape Fear Estuary [30]. The Hydrodynamic Module forms the core of the EFDC water modeling system and can be used for 3-D and 2-D applications. Little difference is observed in the water in the vertical direction because the ORE is shallow. Because the Lagrangian model is based on the average vertical velocity results, we used the 2-D hydrodynamic model in this study. First, the water level and flow were simulated using the EFDC model. Next, the simulated water level and flow were used as velocity field results for the Lagrangian model. In the EFDC model, the

horizontal momentum diffusion coefficient is calculated using the Smagorinsky formula [31], in which the horizontal mixing constant is set to 0.1. The horizontal viscosity coefficient of mass transport for the eddy is the same as the momentum diffusion coefficient.

Improved Lagrangian method. The Lagrangian particle model was used to obtain water particle tracking results, which reflected the unevenness within the marine area and described the designation and particle source. The Euler flow at each time step was calculated using the EFDC model, and the Lagrangian movement of the water particles was calculated based on the Euler flow.

The study region was cut into several boxes full of Lagrangian particles of different colors based on the biological and physical features of the region. The total volume of the particles was equal to the total volume of water in the estuary. These boxes were used to deliver the Lagrangian tracers and supervise the paths of the particles passing through them. Each tracer was identified to obtain the location where the particle was released. Using the Lagrangian approach, the moisture content ($f_{i,j}$) of box (i) at time (t) originating from box (j) is estimated using Eq. (1) as follows:

$$f_{i,j}(t) = \frac{V_{i,j}(t)}{V_{i,i}(0)} \quad (1)$$

where $V_{i,j}(t)$ is the amount of the tracer delivered from box (j) to box (i) at time (t) and $V_{i,i}(0)$ is the water volume of box (i) at $t=0$.

The average RT for a given box can be determined when $i=j$. Specifically, the water in all of the boxes has been renewed when $V_{i,i}(t)$ reaches zero and, consequently, the average RT can be obtained. In some regions, the residual fraction of particles generally remained inside the box for a long time. Consequently, $V_{i,i}(t)$ approached zero very slowly, and the strict definition of the RT likely resulted in excessively high values. One fast and efficient way to solve this problem is to consider the water within a box as completely renewed when a given residual fraction of the original particles remains. However, some subjectivity is inherent when choosing the value of this residual fraction. In this study, an alternative approach based on the method presented

by Tartinville et al. was adopted [32]. By applying this method, the results were adjusted using Eq. (2), which is assumed to obey the exponential law. The RT was obtained from Eq. (2) without any need for subjective parameters.

$$m(t) = m(0) \exp(-t / \tau) \quad (2)$$

The RT was calculated by adjusting Eq. (2) to the model result and then defining the result as the RT. The spatial resolution of the analysis increased when the residence-time parameter was applied in different boxes instead of across the entire estuary.

Integrated water fraction. Generally, the water renewal history is important for understanding the reciprocal effects of nearby water masses in an estuary. In this research, the water renewal history was estimated using the integrated water fraction [33], (F), which is defined in Eq. (1), and was normalized by time as follows:

$$F_{i,j}(T) = \frac{1}{T} \int_0^T \frac{V_{i,j}(t)}{V_{i,j}(0)} dt \quad (3)$$

where $F_{i,j}$ represents the influence of box j on box i during time interval (T). When $i=j$, this index is associated with the RT. At the beginning of the simulation, the value of F is 0, and the initial water contributes less to the total water discharge in the box. The F value gradually approaches 0 as the water inside the box is renewed. When $F=0$, the time corresponds to the RT. The renewal history of box i is calculated using the value of $F_{i,j}$. In this study, the integrated water fractions (F) were used to build a matrix of dependencies between the boxes.

Hardware equipment. In the study, all of the numerical simulations were completed using a computer with an Inter(R) Core(TM) i7-2600 CPU processor with a frequency of 3.40 GHz and 8.00 GB of memory. Serial calculations were performed using Lagrangian particle simulations.

MODEL SETUP

The ORE is limited by a complicated shoreline with many islands and different types of terrain. Thus, a curvilinear-orthogonal grid containing 17286 cells ranging from 10 m to 1000 m in the horizontal direction was used to reflect this complicated geometry (Fig. 2).

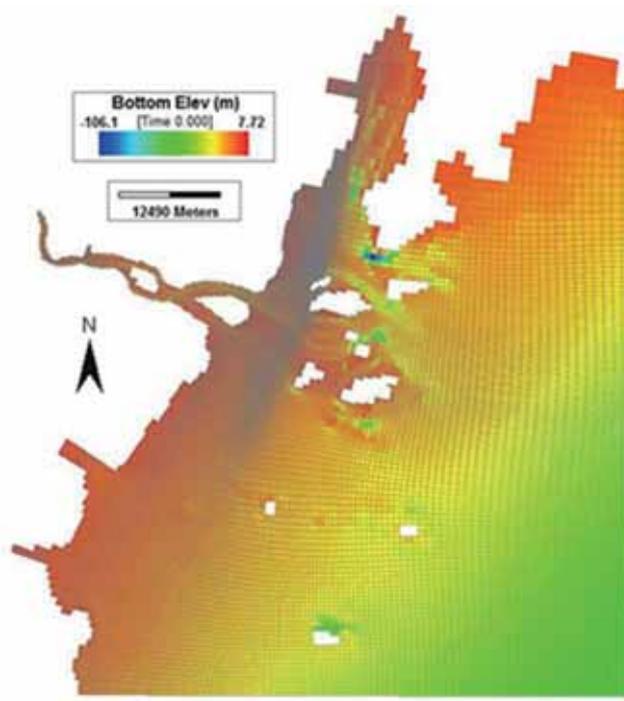


FIGURE 2
Depth of the domain and grid.

This model covered the entire ORE and the coastal area. The borders at high sea were broad enough to cover the important outlets of the Oujiang River and its plumes. The east border was defined as longitude $121^{\circ}23'N$, and the west border was defined as $120^{\circ}28'N$. The model extended to nearly -30 m isobaths. Ample grid resolution was used to fully systematize the important channels and islands. Moreover, the model contains many details and can support specific modeling of certain regions of interest. The shallowness of the ORE was justified, and the average depth of the estuary was approximately 5 m. The elevation of the water surface at the open border was forced by the following 8 tidal constituents: Q1, O1, P1, K1, N2, M2, S2 and K2. Most of the tidal energy in the South China Sea results from these constituents.

The daily river discharge was defined as the inflowing fringe conditions in the upstream portions of the Qujiang River, which was consistent with the discharge observed in practical operations. To account for the influences of freshwater inflows and seasonality on the RT, scenarios were established for winter and summer conditions. To test the influences of geomorphologic changes on the RT, the history of the tracers' fraction in the estuary was estimated by considering the following two geomorphologic configurations: (1) before the Ling-Ni Embankment, Ling-Kun Submerged Dike and First Reclamation

projects (Fig. 1) and (2) after these three projects.

The velocity field of the hydrodynamic model and the stochastic component of speed influenced the tracer movement. The tracers that reached the offshore open border vanished from the domain. The free slip boundary condition was applied at the land boundary to account for the different times at which the beaches were exposed.

Tracer boxes were filled with Lagrangian particles so that the total volume associated with the particles matched the total volume of the estuary. In all simulations, the particles were initially distributed uniformly using 30-m spacing in the north-south and east-west directions. Statistically, the domain contains a total of 92,358 particles. Bilgili et al. [34] used 50-m spacing in the north-south and east-west directions and found their method was suitable for simulating the Lagrangian trends. Thus, using 30-m spacing meets the accuracy requirements.

MODEL CALIBRATION AND VERIFICATION

To ensure the accuracy of the numerical modeling results, the model was verified by the observed data collected from 2005/6/20 to 2005/7/19. Manning's roughness coefficient was applied to correct the hydrodynamic model by trial and error based on the observed data. Large roughness coefficients were used for the upper part of the

estuary because coarse sediments were found in the upper area, and small roughness coefficients were used for the downstream portion of the estuary because fine sediments were found in the downstream area. The roughness coefficients ranged

from 0.012 to 0.04 from downstream to upstream in the estuary.

The observed and modeled water level changes are compared in Fig. 3.

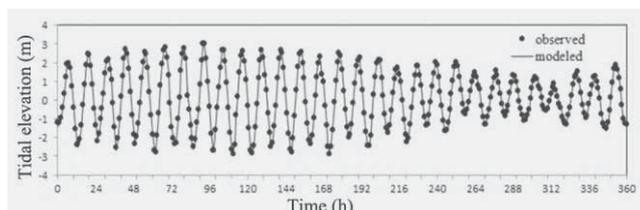


FIGURE 3
Verification of the tidal level.

The model results agreed with the observations, with an average root mean square error (RMSE) of 0.12 m. Most of the discrepancies occurred during the

small flood–ebb tides.

Figure 4 compares the observed and modeled flow velocities.

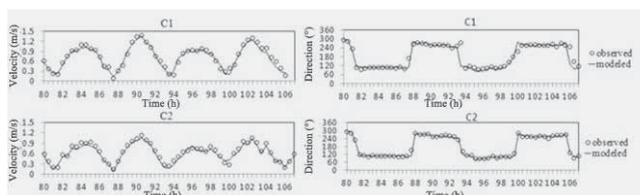


FIGURE 4
Verification of the flow velocity and direction (C1 and C2 current stations).

The modeled flow speed and direction agreed well with the measured results. The RMSEs for the modeled and observation speeds were 0.11 m/s (at C1) and 0.17 m/s (at C2), respectively.

RESULTS

RT. To understand the water body exchange capacities in different areas of the ORE, the research area was divided into 7 boxes. Each box was filled with Lagrangian particles of a different color (Fig. 5). Figure 6 shows the average distribution of the

Lagrangian particles after 15 days. Most of the particles in the Oujiang River moved out of the mouth of the Oujiang River. This finding indicated that the freshwater inflow from the upstream Oujiang River had a strong influence on the movement of Lagrangian particles, especially when runoff was high during the summer. When the particles arrived in the estuary, they were influenced by runoff and the tidal current and had complex dynamics with significant reciprocating water flow movement. The particles were repeatedly moved in this location, with a subsequent decrease in movement speed.

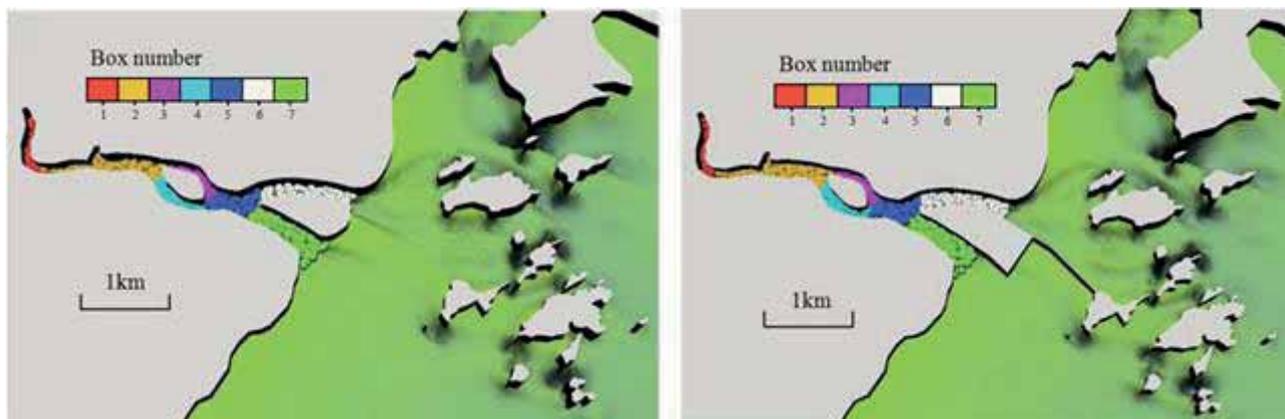


FIGURE 5
Estuary filled with Lagrangian tracers at the beginning of the simulation.

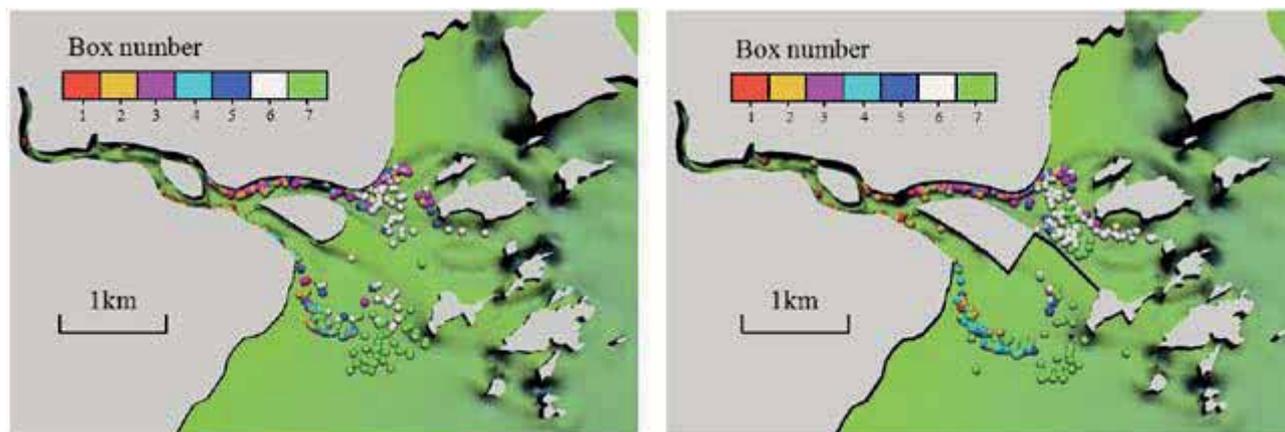
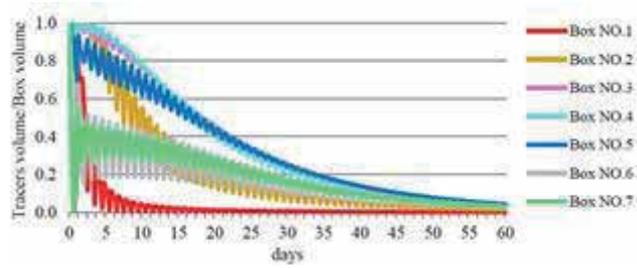


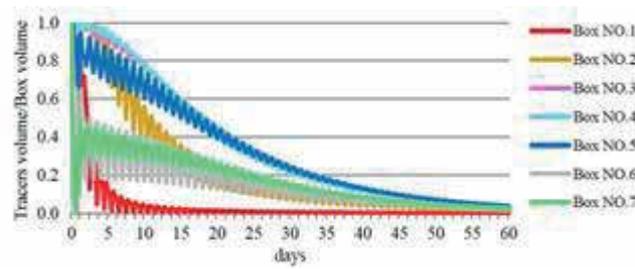
FIGURE 6
Seasonal variations of the Lagrangian tracers before (A) and after (B) the projects (after 15 days).

Figure 7 shows that the number of residual Lagrangian particles decreased over time in each box under different situations. Tables 1 and 2 provide specific proportions of the residual Lagrangian particles in each box with respect to time in these various situations. For all boxes, it was assumed that the proportion of residual Lagrangian particles was 1 under the initial condition. The numerical results showed that the RT was less than 15 days in the summer and less than 60 days in the winter. This difference indicates that the water body exchange capacity was stronger in the summer than in the winter. The RT was proportional to the discharge runoff from the upper stream of the Oujiang River. The larger the discharge of water from the upper

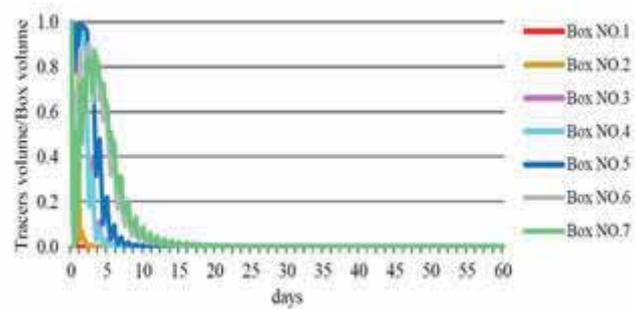
stream, the shorter the RT, and vice versa. Within either season, the northernmost box, Box 1, was mainly influenced by the discharge runoff of the upper stream. The water body essentially participated in one-way exchange, and larger exchange rates corresponded with shorter RTs. Boxes 6 and 7 were located near the mouth of the river and were mainly influenced by the tidal waves of the open sea with a broad water surface. The RT decreased when the water body exchange rate increased. The middle boxes 2, 3, 4 and 5 were obviously influenced by discharge runoff from the upper stream and the tidal waves of the open sea, with the two lengthwise currents increasing the RT in these areas.



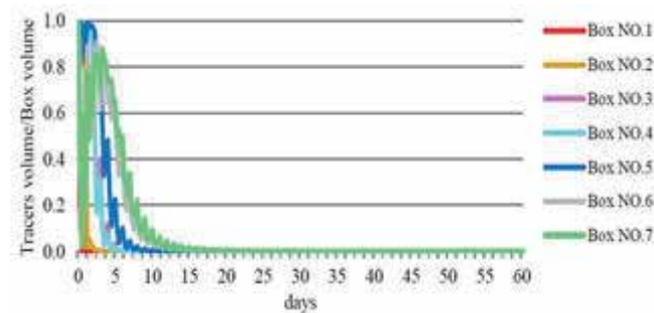
A



B



C



D

FIGURE 7

Temporal evolution of the ratio between the tracers' volume and the estuary volume. A: winter, before the projects. B: winter, after the projects. C: summer, before the projects. D: summer, after the projects.

TABLE 1
Temporal evolution of the ratio between the volume of tracers and the estuary before and after the projects in the winter scenario.

| (a) Winter, before the projects | | | | | | | (b) Winter, after the projects | | | | | | |
|---------------------------------|-------|-------|-------|-------|-------|-------|--------------------------------|-------|-------|-------|-------|-------|-------|
| T (days) | 10 | 20 | 30 | 40 | 50 | 60 | T (days) | 10 | 20 | 30 | 40 | 50 | 60 |
| Box 1 | 0.039 | 0.013 | 0.006 | 0.003 | 0.002 | 0.001 | Box 1 | 0.036 | 0.012 | 0.006 | 0.003 | 0.002 | 0.001 |
| Box 2 | 0.553 | 0.234 | 0.115 | 0.060 | 0.032 | 0.018 | Box 2 | 0.559 | 0.243 | 0.121 | 0.063 | 0.033 | 0.018 |
| Box 3 | 0.763 | 0.459 | 0.255 | 0.140 | 0.077 | 0.043 | Box 3 | 0.767 | 0.454 | 0.248 | 0.134 | 0.072 | 0.039 |
| Box 4 | 0.780 | 0.452 | 0.248 | 0.135 | 0.074 | 0.041 | Box 4 | 0.783 | 0.450 | 0.243 | 0.130 | 0.070 | 0.038 |
| Box 5 | 0.618 | 0.411 | 0.240 | 0.135 | 0.075 | 0.042 | Box 5 | 0.601 | 0.391 | 0.222 | 0.122 | 0.066 | 0.036 |
| Box 6 | 0.183 | 0.144 | 0.094 | 0.057 | 0.034 | 0.020 | Box 6 | 0.170 | 0.126 | 0.078 | 0.045 | 0.025 | 0.014 |
| Box 7 | 0.281 | 0.218 | 0.139 | 0.083 | 0.048 | 0.028 | Box 7 | 0.277 | 0.205 | 0.126 | 0.072 | 0.040 | 0.022 |

TABLE 2
Temporal evolution of the ratio between the volume of tracers and the estuary before and after the projects in the summer scenario.

| (c) Summer, before the projects | | | | | | | (d) Summer, after the projects | | | | | | |
|---------------------------------|-------|-------|-------|-------|-------|-------|--------------------------------|-------|-------|-------|-------|-------|-------|
| T (days) | 1 | 3 | 5 | 10 | 20 | 30 | T (days) | 1 | 3 | 5 | 10 | 20 | 30 |
| Box 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | Box 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Box 2 | 0.873 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | Box 2 | 0.877 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 |
| Box 3 | 0.994 | 0.401 | 0.033 | 0.000 | 0.000 | 0.000 | Box 3 | 0.995 | 0.386 | 0.031 | 0.000 | 0.000 | 0.000 |
| Box 4 | 0.993 | 0.296 | 0.018 | 0.000 | 0.000 | 0.000 | Box 4 | 0.997 | 0.298 | 0.019 | 0.000 | 0.000 | 0.000 |
| Box 5 | 0.871 | 0.837 | 0.227 | 0.006 | 0.000 | 0.000 | Box 5 | 0.850 | 0.833 | 0.220 | 0.005 | 0.000 | 0.000 |
| Box 6 | 0.187 | 0.617 | 0.550 | 0.091 | 0.008 | 0.002 | Box 6 | 0.251 | 0.638 | 0.549 | 0.088 | 0.007 | 0.002 |
| Box 7 | 0.190 | 0.689 | 0.655 | 0.094 | 0.006 | 0.002 | Box 7 | 0.167 | 0.683 | 0.632 | 0.085 | 0.007 | 0.002 |

After the Ling-Ni Embankment, Ling-Kun Submerged Dike and First Reclamation Project were implemented in the ORE, the RT was relatively similar to the RT before the projects were implemented. Thus, the influences of the construction projects on the RT were small. During the winter and summer, the RT values for boxes 1 and 2 remained basically unchanged after the projects were implemented. During the winter, the RT values of boxes 4 and 5 were 1-3 days longer after the projects were implemented, and the values for boxes 6 and 7 were 1-2 days shorter after the projects were implemented. In the summer, the RTs of boxes 3 and 4 remained relatively unchanged after implementation of the projects, and the RTs of boxes 5, 6 and 7 were shortened by 1-2 days. Implementation of the projects had a small influence on the RT in the ORE for the following reasons. First, the splitting ratio of the discharge from the north and south branches was 7:3 before the project. The Ling-Kun Submerged Dike was located in the South Branch, which is not the main water channel of the ORE. The flow pattern in the ORE involves

reciprocating movement vertical to the bank. Lingkun Island and Niyu Island were areas of weak current before the construction projects, and the characteristic properties of the reciprocating flow movement were not altered by the implementation of the Ling-Ni Embankment and First Reclamation Project. In addition, the characteristic properties of the water flow changed very little after the three projects were implemented. Therefore, no significant change in the RT occurred following implementation of the projects. These findings indicate that the potentially adverse effects of coastal projects can be effectively mitigated according to natural laws by carefully choosing the project locations.

Integrated water fraction. A dependency matrix for the variations of water among the boxes was established for the integrated water fraction. The dependency matrix of the ORE before and after the projects is shown in Table 3, where T equals 15 days. The values in the first row of the matrix (*i*) represent the overall contributions of the water masses from all boxes *j* over box *i*. The difference between 1 and the

sum of the first column reflects the water fraction renewed in box j during the time interval (T). The table for the summer scenario is not included because the water in the boxes was fully renewed within 15

days before and after the projects. Moreover, all of the values in the matrix were near zero. These values show that river discharge was the main factor controlling water renewal for these boxes.

TABLE 3
The values in row i of the matrix provide the integrated contributions of the water masses from all boxes j over box i in a dependence matrix before and after the projects for the winter scenario.

| Before the projects | | | | | | | | | |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-----------------------|-------------------------------|
| T = 15 days | Box 1 | Box 2 | Box 3 | Box 4 | Box 5 | Box 6 | Box 7 | % of water from boxes | % of water from other sources |
| Box 1 | 0.076 | 0.034 | 0.005 | 0.004 | 0.000 | 0.000 | 0.000 | 11.9% | 88.1% |
| Box 2 | 0.082 | 0.115 | 0.043 | 0.040 | 0.011 | 0.004 | 0.003 | 29.8% | 70.2% |
| Box 3 | 0.086 | 0.097 | 0.042 | 0.013 | 0.004 | 0.001 | 0.001 | 24.4% | 75.6% |
| Box 4 | 0.077 | 0.089 | 0.017 | 0.041 | 0.003 | 0.001 | 0.001 | 22.9% | 77.1% |
| Box 5 | 0.062 | 0.073 | 0.029 | 0.032 | 0.056 | 0.018 | 0.002 | 27.2% | 72.8% |
| Box 6 | 0.062 | 0.075 | 0.012 | 0.013 | 0.004 | 0.005 | 0.021 | 19.2% | 80.8% |
| Box 7 | 0.061 | 0.073 | 0.014 | 0.011 | 0.005 | 0.023 | 0.004 | 19.1% | 80.9% |
| After the projects | | | | | | | | | |
| T = 15 days | Box 1 | Box 2 | Box 3 | Box 4 | Box 5 | Box 6 | Box 7 | % of water from boxes | % of water from other sources |
| Box 1 | 0.076 | 0.034 | 0.005 | 0.004 | 0.000 | 0.000 | 0.000 | 11.9% | 88.1% |
| Box 2 | 0.095 | 0.159 | 0.054 | 0.048 | 0.016 | 0.007 | 0.001 | 38.0% | 62.0% |
| Box 3 | 0.095 | 0.107 | 0.046 | 0.014 | 0.004 | 0.001 | 0.001 | 26.8% | 73.2% |
| Box 4 | 0.096 | 0.112 | 0.013 | 0.067 | 0.006 | 0.002 | 0.001 | 29.7% | 70.3% |
| Box 5 | 0.057 | 0.061 | 0.025 | 0.027 | 0.032 | 0.013 | 0.011 | 22.6% | 77.4% |
| Box 6 | 0.043 | 0.057 | 0.007 | 0.008 | 0.003 | 0.001 | 0.015 | 13.4% | 86.6% |
| Box 7 | 0.067 | 0.082 | 0.019 | 0.014 | 0.008 | 0.028 | 0.001 | 21.9% | 78.1% |

According to Table 3, a small part of the water in each box after 15 days was originally from other boxes, and a large part of the water was originally from other sources, including freshwater inflows from the Qujiang River and tidal water from open seas.

After project implementation, the proportion of water from other boxes and water from other sources in box 1 remained unchanged. The proportion of water from other sources in boxes 2, 3 and 4 decreased by 8.2%, 2.4% and 6.8%, respectively. Meanwhile, the proportion of water from other sources in boxes 5 and 6 increased by 4.6% and 5.8%, respectively, and the water from other sources in box 7 decreased by 2.8%. A reduction in the proportion of water from other sources indicates that the exchange capacity of the water body decreased. In contrast, an increase would indicate that the water exchange capacity increased. Based on the statistical results, the implementation of the projects did not affect the water body exchange capacity in box 1. The water body exchange capacity was slightly reduced in boxes 2, 3, 4 and 7, and the water body exchange capacity in boxes 5 and 6 was slightly

strengthened, with the proportion of water from other sources remaining less than 10%.

DISCUSSION

The Eulerian method considers the effects of convection diffusion and is more extensive than other methods. To describe particle movement and know where particles came from and move to, Lagrange's point of view should be applied because it simulates the heterogeneity of water exchange in different regions of the estuary and describes the pathways of the particle; however, Lagrange's point of view cannot account for water diffusion [10]. Thus, in this paper, one method for calculating RT is based on using renewal time scales computed with a high-resolution hydrodynamic EFDC model that solves depth-averaged advection and diffusion equations and is coupled with a Lagrangian transport model. This method was used to study the changes caused by implementing several large coastal projects (Ling-Ni Embankment, Ling-Kun Submerged Dike

and the First Reclamation Project) in the ORE. The influences of the seasonal freshwater inflow and geomorphological modifications on RT were surveyed. The calculated results reasonably and accurately reflected the water exchange rules in the ORE [35]. Next, the results were used to objectively assess the effects of implementing large-scale ocean engineering on the RT, which ultimately confirmed that the seasonality of the upstream water discharge had an important influence on the RT [36].

Lagrangian transport models can easily label water masses and relate their positions at any instant of time with their release points. Thus, these models can provide accurate, detailed spatial and temporal information on particle trajectories and dispersion history. One shortcoming of the Lagrangian approach is that the renewal time scales are difficult to define in an unequivocal way. For example, the RT of a region is generally calculated as the time it takes for all of the water in that region (corresponding to all the tracers released at a time of zero) to be replaced by new water. This criterion can result in a very long RT. In practical applications, the RT can be considered as the time at which only a residual amount of water remains in the region. The residual water is generally described in a subjective way as a specific portion of the original water mass [16]. Tartinville et al. [32] has shown that this problem can be overcome by assuming that the evolution of the water mass in the region follows the following exponential law: $m(t) = m(0) \exp(-t / \tau)$. Using this equation, the RT can be determined by adjusting this equation, which is assumed to obey exponential law, where τ is defined as the RT. This solution was used in this study.

It is important to study the effects of varying river discharges and the instant of tracer release in the future. Other relevant relationships, such as light intensity, average depth and temperature, should be considered to obtain a more precise relationship between physical and biological parameters. Due to the use of serial processors in Lagrangian particle simulations, 19 hours are required to complete the calculations when the model is used to simulate 60 days. If a 3D model is used in the future and more influence factors are considered, parallel processors should be used to improve the computational efficiency.

CONCLUSIONS

The water RT in the ORE was calculated by combining a Lagrangian transport model with a high-resolution hydrodynamic model (EFDC). The mathematical model was verified using on-site measurement data, such as tidal level, flow speed and

direction. To understand the water exchange capacity in different areas of the ORE, the research area was divided into 7 boxes, and each box was filled with differently colored Lagrangian particles. The change in the water exchange capacity and the difference in the RT before and after implementing the Ling-Kun Submerged Dike, Ling-Ni Embankment and First Reclamation Project were investigated.

The results showed that the RT in the research area was less than 15 days in the summer and less than 60 days in the winter. The water exchange capacity in the summer was significantly stronger than that in the winter because the discharge runoff from the upper stream in the summer was much larger than that in the winter. The RT of the research area was proportional to the discharge runoff from the upper stream of the Oujiang River. After implementing several projects in the ORE, the RT of some of the areas increased, and the RT in other areas decreased, with a maximum RT change of 3 days. Project construction had less influence on the RT than the seasonal river discharge changes.

The upper stream section of the research area was mainly influenced by upstream discharge runoff, and the water body was basically involved in one-way exchange. Although the water body exchange characteristics in the midstream section were influenced by both runoff and tidal current, the water body exchange was not. The water body particles were moved repeatedly with a relatively slow exchange. The water body in the downstream area of the estuary with a broad water surface was mainly influenced by the tidal dynamics, and the water body exchange was accelerated.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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USE OF DATA OF THE GEOSTATISTICS PORTAL IN SUSTAINABLE RURAL LAND DEVELOPMENT: TWO CASE STUDIES IN POLAND

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ABSTRACTS

Statistical data constitute one of the elements providing the basis for the establishment of indicators and factors facilitating the assessment and monitoring of spatial development. The primary objective of the study was to analyse statistical data collected in the Geostatistics Portal (GP) from the perspective of integrated land management, and particularly crop production. GP satisfactorily fulfilled its role with respect to data obtained from the 2010 General Agricultural Census. GP is an extremely useful tool in strategic decision making process at almost every level of management, also in terms of sustainable land management.

KEYWORDS:

statistical data, land management, agricultural production, geographic information system

INTRODUCTION

The analysis of the position of new EU member states in the global economy is important for their development. Increasing economic differentiation has been observed in the developing world already since the 1970's. It is also reflected in the results of the regional analysis of economic development over the last 25 years [1].

Due to the increasing deficit of soil resources, certain changes have become necessary concerning land management and the related policies and key decision-making processes [2]. A new vision of sustainable development of land management is required [3]. Crop production is the fundamental function of soils in rural areas. Sustainable economic and social development, including suitable soil exploitation, constitutes one of the greatest challenges for the modern world. Statistical data constitute one of the elements providing the basis for the establishment of indicators and factors facilitating the assessment and monitoring of spatial

development [4] [5]. The integration of various statistical data, including, without limitation, demographic, economic, agricultural, and environmental data [6], as well as presentation of such data on maps, facilitate optimal land management [7].

A key element in objective decision-making regarding land-related issues is access to relevant data. These include statistics at policy-relevant scales, on natural resources endowment in a given country or region, as well as on how these resources are used within a given socio-economic setting i.e. the land use [8] [9]. Here, land use refers not only to the socio-economic purpose of activities undertaken in order to obtain desired benefits from the land (e.g. crop or livestock production), but also to information on the land management (e.g. rain fed vs. irrigated, use of fertilizers, etc.) applied in order to obtain such benefits [10]. Various geographic information systems processing statistical data exist around the world. Agro-MAPS is an interactive web-based information system on land use containing statistics on primary food crops, aggregated by sub-national administrative districts, on crop production, area harvested, and crop yields [10].

The Australian Collaborative Land Use and Management Program (ACLUMP) promotes the development of nationally consistent land use and land management practices information for Australia. ACLUMP including the Multi-Criteria Analysis Shell for Spatial Decision Support [11]: (i) insightful desktop combination and study of different types of mapped information, (ii) understanding of the relationships between the decision-making process and the available spatial data, (iii) interactive 'live-update' and mapping of alternative project scenarios, (iv) GIS programming is not required, removing the usual technical obstacles to non-GIS users.

The primary objective of the study was to analyse statistical data collected in the Geostatistics Portal from the perspective of integrated land

management, and particularly crop production. The analysis provides the basis for drawing conclusions regarding the current status, as well as forecasting changes potentially resulting from the contemplated strategies, for the purpose of integrating land management. The research also focused on the assessment of quality, detail, and usefulness of statistical data for the purposes of spatial development in rural areas.

MATERIALS AND METHODS

The Geostatistics Portal (GP) was launched in Poland in 2013. It is a tool for interactive cartographic presentation and dissemination of data obtained from general censuses and periodical thematic reports. GP is designed to collect, present, and disseminate statistical data and geospatial data. The data are made available to a broad audience – i.e. public administration, research institutions, as well as individuals and entrepreneurs.

GP facilitates monitoring of certain areas, analysing changes, as well as forecasting and simulating various risks. The portal is a useful source of information about the current socio-economic situation. It applies GIS technology. The data are presented in a depersonalised form. Statistical confidentiality is respected. Choropleth maps and diagram maps in GP are particularly drafted with the application of ArcGIS software. The tasks in GIS environment can be completed by means of these applications combined, from simple ones to the most advanced, including, without limitation, cartographic studies, data management, geographic analysis, data editing, and geoprocessing [12]. An important argument in favour of the establishment of this portal are the international obligations resulting from the INSPIRE Directive. It also constitutes a part of the project of development of a complete and coherent system of e-Administration [13].

GP includes in particular the following data: (i) 2010 General Agricultural Census up-to-date information about Polish agriculture [14], (ii) 2011 General Population and Housing Census up-to-date information about living and housing conditions of the population [15], (iii) Local Data Bank (BDL) up-to-date information about the social, economic, and demographic situation, as well as about the condition of the natural environment in voivodships, poviats and communes, constituting state social and administrative organisational units

[16]. The results of the censuses are presented in the Analytical Microdata Base (ABM), consisting of the following subsystems: (i) information analysis, (ii) data processing, (iii) data dissemination, (iv) quality monitoring, (v) information security, (vi) management and administration of ABM metadata, (vii) needs and products management.

The following parameters can be set individually by the users for choropleth maps: (i) aggregation level, (ii) standardising attribute, (iii) number of intervals, (iv) classification method, (v) colour scale, (vi) transparency, and (vii) visibility of layers (labels and borders). GP also offers additional tools and functions, such as: (i) locality search engine, (ii) address search engine (a tool available for internal users only), (iii) attribute-spatial search engine, (iv) selection of objects, (v) identification of objects, (vi) presentation of statistics and charts.

Data from the 2011 General Population and Housing Census facilitates the development of approximately 2000 choropleth maps and diagram maps regarding the following subjects: education, migration, housing, or economic activity.

The two analysed case studies include: voivodships Podkarpackie – with capital city Rzeszów, and Zachodniopomorskie – with capital city Szczecin, representing different levels of agricultural development and different farm structures, and located in different regions of Poland (Fig. 1). An analysis of five factors was performed with the application of GP, namely income structure, equipment use, crop production – cereals, forestation level, and fertilization.

The Podkarpackie voivodship (PV) is located in South-East Poland, and comprises montane and sub-montane regions highly prone to natural threats, and particularly erosion. The Zachodniopomorskie voivodship (ZV) is located in North-West Poland in the littoral zone of the Baltic Sea. These two investigated areas are characterised by different conditions of plant production resulting from their geographical location, climate, and farm structure, also resulting from historical conditions. The spatial analysis of statistical data for such different areas will facilitate rational exploitation of soil resources for the purposes of food production considering the requirements of multifunctional development.

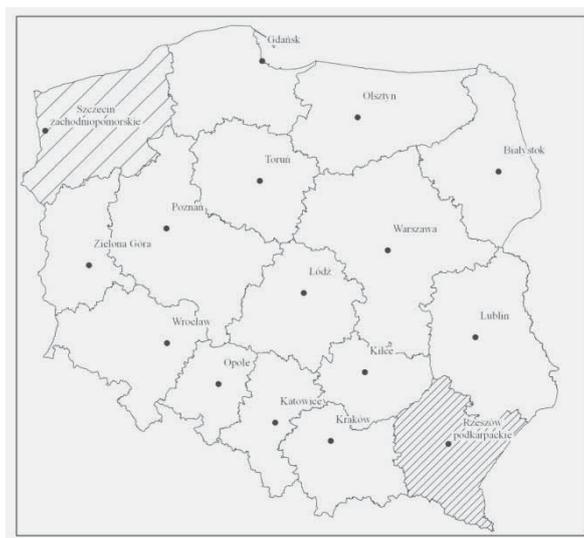


FIGURE 1
Study areas – Zachodniopomorskie voivodship (ZV) and Podkarpackie voivodship (PV)

RESULTS AND DISCUSSIONS

GP is a tool used for interactive cartographic presentation and publication of statistical and geospatial data. For research purposes, data on all of the analysed factors were classified according to the following parameters: farm location, aggregation level – poviats, percentage of farms characterised by a given factor. For direct comparison of data obtained from GP, identical intervals were applied.

Changes in land ownership have shown a tendency to concentrate Polish agriculture in terms of the size structure of farms. Larger farms are located in areas where the average size tended to be greater from the outset. Areas experiencing over-fragmentation of farms for a long time have undergone no more recent major agrarian reforms regarding farm size [17].

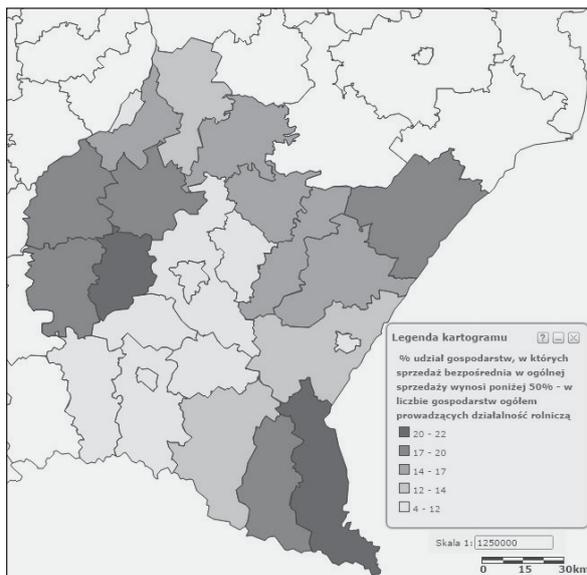
The analysis of this structure was performed based on the following data: types of farms involved in direct consumer sales - farms where proceeds from direct sales to consumers constitute more than 50% of the total turnover. As a result of the geostatistical analysis, choropleth maps were developed depicting the income structure of the study areas (Fig. 2).

ZV includes considerably more farms than PV with direct sales to consumers representing more than 50% of the total turnover. Such income structure results from a larger contribution of large-scale farms (average farm area exceeding 30 ha) in

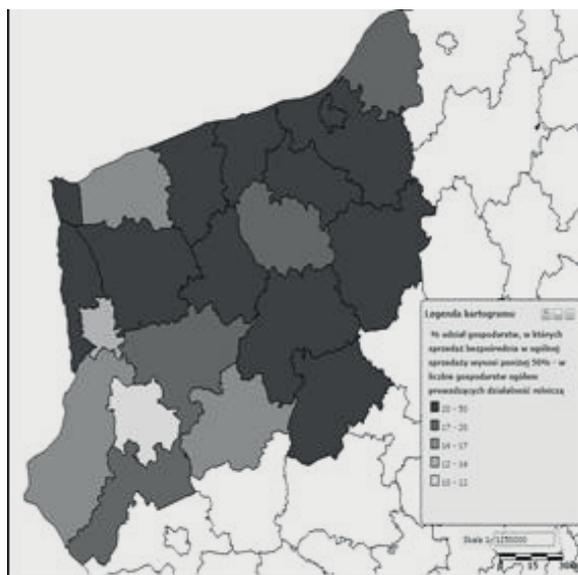
ZV than in PV (where the average farm area amounts to approximately 4.5 ha). The results correspond with the study by Goraj (2010) according to which approximately 20% of Polish farms produce agricultural products solely for their own needs and use, while only 43% of farms focus on direct sales. The spatial differentiation of farm area results from the mutual interaction of contemporary and former social and economic processes.

The evidently different structure of production factors also results in differences in land and labour productivity [17] [19]. Relative savings of fixed costs increase with better technical equipment of labour [20]. Therefore, the next factor reflecting the level of development of rural areas is the use of agricultural equipment by farmers. A choropleth map was developed (Fig. 3) presenting the use of the following machines: farmers' own tractors up to 14.99 kW (i.e. 20.4 hp).

PV includes more farms whose owners use tractors up to 14.99 kW (i.e. 20.4 hp) than ZV. This results from the excessive fragmentation of farms in PV, where smaller farms do not require powerful tractors. The opposite situation is observed in ZV. Equipment of large-scale farms with more powerful tractors than smaller farms (tractors up to 20.4 hp) improves work efficiency in larger farms, and reduces the number of people employed in agricultural production.

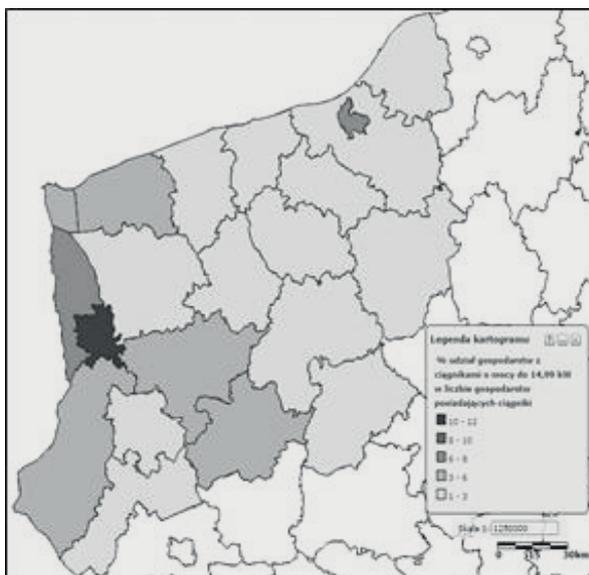


Zachodniopomorskie voivodship (ZV)

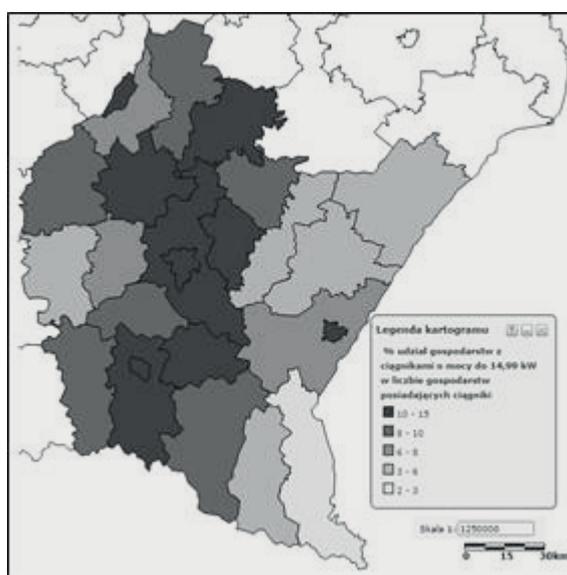


Podkarpackie voivodship (PV)

FIGURE 2
Choropleth maps: income structure [18]



Zachodniopomorskie voivodship (ZV)



Podkarpackie voivodship (PV)

FIGURE 3
Choropleth maps: use of equipment – farmers’ own tractors up to 14.99 kW (i.e. 20.4 hp) [18]

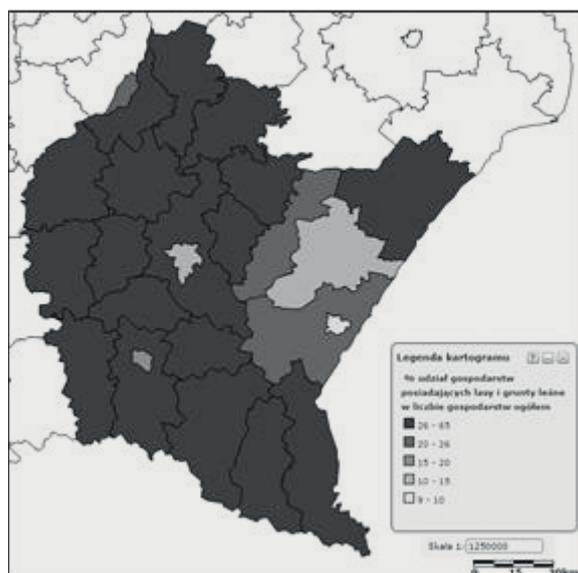
The contribution of forests and forestry lands in the land use structure is also important for integrated and sustainable land management. Such land constitutes an instrument for counteracting erosion, and is extremely important in rational

management of land of poor agricultural suitability. Tools for space protection should limit the risk of economic expansion, as well as strengthen the original functions and diversity of the natural environment [21]. A choropleth map was developed

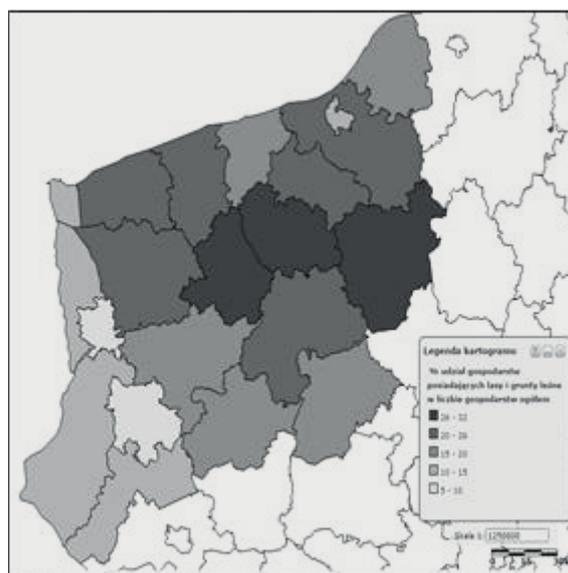
(Fig. 4) presenting land use – forests and forestry lands.

PV includes more farms comprising forests and forestry land than ZV. In certain poviats such farms constitute 65% of the total number of farms. In the case of ZV, the percentage of farms including forests and forestry land does not exceed 32%. Such structure reflects the fact that land of poor

agricultural suitability is more abundant in PV, and that plots are smaller in that voivodship and are therefore more prone to natural forest succession if not cultivated. Such tendencies are in line with commonly applied solutions and the assumption that agriculture and silviculture belong to the most important sectors responsible for protection and shaping of the natural environment [20].



Zachodniopomorskie voivodship (ZV)



Podkarpackie voivodship (PV)

FIGURE 4
Choropleth maps: land use – forests and forestry land [18].

In PV (Fig. 5), increased sowing of cereals is observed in the northern and north-eastern part of the voivodship compared to its central and southern part. This is probably caused by the presence of uplands and mountains in the southern part of the area. In such areas, the scope of cereal sowing is limited due to difficulties faced by agricultural activity, caused by the relief and considerable elevation differences. Any efforts to cultivate these areas would trigger huge expenses that could render such project unprofitable.

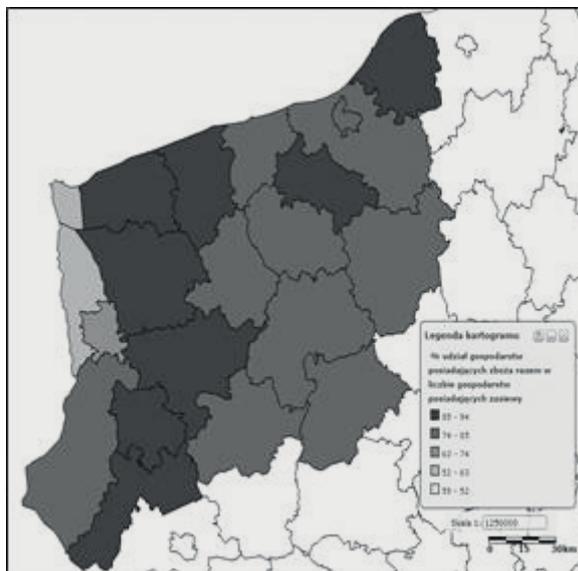
In the case of ZV (Fig. 5), the most cultivated area is its western part (stretching meridionally) except for the Odra River valley. Due to the inconsiderable degree of the observed differences, it is difficult to establish the factors causing them.

The use of mineral fertilizers per 1 ha of arable land was considerably higher in ZV (close to national average) compared to PV, where it was the lowest in the country (Fig. 6). The intensity of plant production has a huge impact on the level of use of mineral fertilizers. Intensive agriculture results in

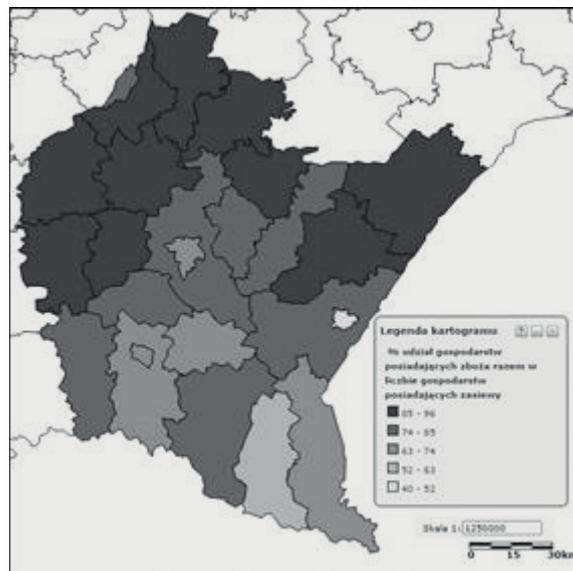
increased use of fertilizers, necessary to obtain high crop yield [23]. It is also important that ecological plant production is popular in the sub-Carpathian region, and in the case of such production fertilizers cannot be used [24]. Both of the voivodships show an evident tendency for larger farms to use more mineral fertilizers per 1 ha of arable land. Therefore, the use of fertilizers is less intensive in PV because of the smaller size of the farms. Notice also that given such differentiation in the use of mineral fertilizers, the cereal sowing structure (fig. 5) and crop yield dt/ha are comparable in both voivodships. In ZV less fertile soils occur, and a higher contribution of large farms is involved in intensive agricultural production focused on profits and economic results. This leads to increased use of fertilizers. The results of the analysis are in line with studies by other authors [25], classifying voivodships in terms of their agrarian structure similarity, and concluding that the individual approach to each region provides the basis for

forecasting and programming of sustainable

development of rural areas in Poland.



Zachodniopomorskie voivodship (ZV)



Podkarpackie voivodship (PV)

FIGURE 5
Choropleth maps: sowing area – cereals [18].

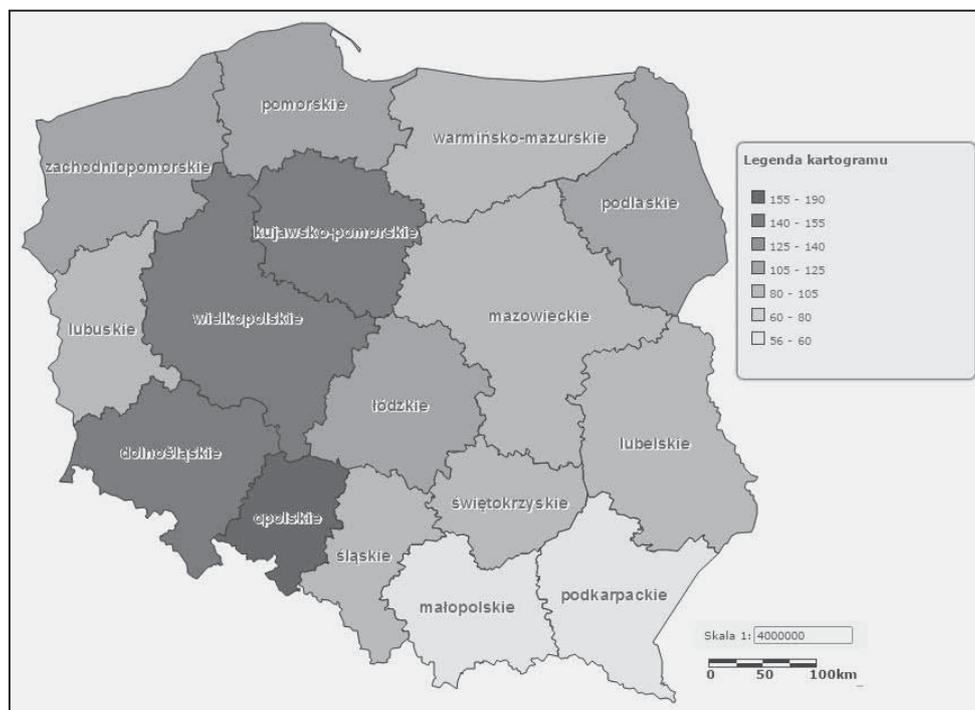


FIGURE 6
Choropleth map: use of fertilizers per 1 ha of arable land [22]

GP satisfactorily fulfilled its role with respect to data obtained from 2010 General Agricultural Census. It permitted the development of choropleth maps regarding all territorial administration units and with respect to each phenomenon. BDL does not produce statistics at the poviát and commune level with respect to all of the phenomena, but only some of them. Therefore, it was not possible to develop a choropleth map presenting the use of mineral fertilizers per 1 ha of arable land at the poviát level.

Nevertheless, GP constitutes an extremely useful tool in strategic decision making process at almost every level of management, also with respect to sustainable land management. It facilitates the analysis of the existing situation, and assists in devising development scenarios for a given region. The obtained data constitute useful information for self-government decision making bodies at the voivodship and/or poviát level, supporting actions undertaken in the scope of rational land management in rural areas.

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MAGNOLIA GRANDIFLORA FOR MONITORING TRICHLOROETHYLENE AND TETRACHLOROETHYLENE CONTAMINATION OF GROUNDWATER

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ABSTRACT

The potential use of *Magnolia grandiflora* for phytomonitoring trichloroethylene (TCE) and tetrachloroethylene (PCE) contamination in groundwater was studied. Four replicate tree samples with one control (without TCE and PCE treatment) were planted in growth chambers from April to June, 2013. Tree cores were sampled and extracted employing Headspace-solid phase microextraction at 25°C for 10mins. Both qualitative and quantitative test of the target compounds was done using Gas chromatograph-Mass Spectrometer. Statistical analysis was performed using SPSS (Statistical significance was accepted at $\alpha=0.05$). The results showed that the experimental period (April to June) favored the trees' ability to take up large volumes of the target compounds because of its enhanced transpiration rate during the period. The concentration of TCE at the initial stages of the experiment were significantly higher than PCE but this changed with time ($P<0.5$). Meanwhile, there was no consistency in the measured concentrations of both compounds at the various heights along the tree (10, 30, 50cm) as was expected. Notably, there was no indication of VOC toxicity to the plants. It is therefore concluded that *Magnolia grandiflora* can be employed in phytomonitoring TCE and PCE in groundwater. The results from the study provides essential and relevant information for policy makers and other environmentalists. This is a new finding and as far as the authors are aware, this is the first manuscript that has understudied this and hence, this study is of great benefit for the efficient management of groundwater resources.

KEYWORDS:

Contamination, GCMS, phytomonitoring, SPME, tree cores

INTRODUCTION

Phytoremediation, has over the years been employed for the cleaning up of both organic and inorganic contaminants from air[1], soil[1] and groundwater[2][3] among other environmental media. The choice of plant often depend on its physical, chemical and morphological properties without looking down on their aesthetic and commercial functions as well. Specific plant characteristics such as size, biomass, growth form, aerial surface, rooting type and depth, duration (annual, biennial or perennial) and dormancy as well as resistance to pest and diseases enable researchers to make informed decisions on the choice of plant to be used. Notable research works in this sector have often shown positive outcomes with regards to significant reduction in concentrations and degradation of contaminants into less toxic forms[5]. On a daily scale, environmentalists desire to identify many plant species that can ensure a successful phytoremediation and thus increase the applicability of the technology in different areas[6][7].

Magnolia grandiflora is a common tree species used in urban landscape designs in many areas because of its evergreen leaves and fragrance. It belongs to the family MAGNOLIACEAE. The species is native to the south-eastern and northern parts of the United States of America but grows well in many other areas. *Magnolia* can reach a height of about 27.5m. Other characteristics can be seen in Duncan and Duncan [8] and Gardiner [9]. *M grandiflora* is tolerant to ozone, highly resistant to pests and diseases due to its antimicrobial toxins [9].

Trichloroethylene (TCE) and tetrachloroethylene (PCE) are volatile organic compounds of extreme importance because of their presence in snow, food products, lubricants, groundwater and rainwater [10] [11] [12] and subsequent adverse impacts on the environment. It must be noted that the two contaminants have been identified to be acutely toxic and is classified by the

International Agency for Research on Cancer as a group 2A carcinogen [13]. Since the 1900's, their presence have been reported in various groundwater related researches; thus proving vivid increase in the risk of exposure and hence the need for continuous, easy and sustainable way of monitoring them.

The objective of this study was therefore to determine at a laboratory scale, the potential of using *Magnolia grandiflora* to qualitatively monitor TCE and PCE in contaminated groundwater.

MATERIALS AND METHODS

Experimental site. The experimental site was located beside the Water Research Greenhouse on the Jiangning campus of Hohai University, Jiangning Economic Development zone of the Jiangning District, Nanjing. It is located at latitude

31°95'N and longitude 118°83'E Nanjing, China. The area is located in the North sub-tropical monsoon climate zone. The weather is hot and humid in summer whereas winter often sees snowfall while rain is likely to happen at any time of the year, not just in the rainy season. The annual mean temperature is 16°C whereas the absolute maximum and minimum temperature of the area is 43°C and -16.9°C respectively and annual precipitation is around 1062mm. Weather data for the experimental period are shown in TABLE 1

Weather data during the experimental period. The experiment took place from November 2012 to June 2013, spanning three of the major seasons of the area. Sandy-loam soil and gravels were purchased. The physical and chemical properties of the soil was tested and shown in TABLE 2

Tested soil properties

TABLE 1
Weather data during the experimental period

| Parameter | Nov | Dec | Jan | Feb | Mar | April | May | Jun |
|-----------------------|-----|-----|-----|-----|-----|-------|-----|-----|
| Relative Humidity (%) | 76 | 74 | 76 | 74 | 74 | 73 | 74 | 78 |
| Rainfall (mm) | 51 | 24 | 37 | 47 | 82 | 73 | 102 | 193 |
| High temperature (°C) | 16 | 10 | 7 | 8 | 13 | 20 | 26 | 29 |
| Low temperatures (°C) | 6 | 0 | -1 | 0 | 5 | 11 | 16 | 21 |

TABLE 2
Tested soil properties

| Property | Results |
|-----------------------------------|---------|
| Organic matter (%) | 8.06 |
| pH | 7.68 |
| CEC (meq) | 13.2 |
| Total P (mgkg ⁻¹) | 330.9 |
| Available P (mgkg ⁻¹) | 10.13 |
| Total N(%) | 0.1 |
| Available N (mgkg ⁻¹) | 65 |
| Total K(mgkg ⁻¹) | 208 |
| Available K (mgkg ⁻¹) | 21.32 |

Plant material and growth conditions. Healthy four year old *Magnolia grandiflora* trees of heights between 3-4m were purchased from GreenTrees Company in Pukou, Nanjing, PRC. A total of five (5) trees with average circumference of 20.3cm were used. Five test beds of 2m depth by 1m width and 2m length were constructed. They

were then filled with gravels to 0.7m length and then sandy-loam soil to 1.7m (

FIGURE 1). This was to imitate the underground environmental conditions. The test bed had influent and effluent wells consisting of T-pipes, allowing the controlled application of TCE

and PCE. Bed slope was 1:10 to facilitate flow. The design was according to James et al[4]. There were

four replicate chambers (C1 to C4) with one control (Cc). Shallow contamination was assumed.

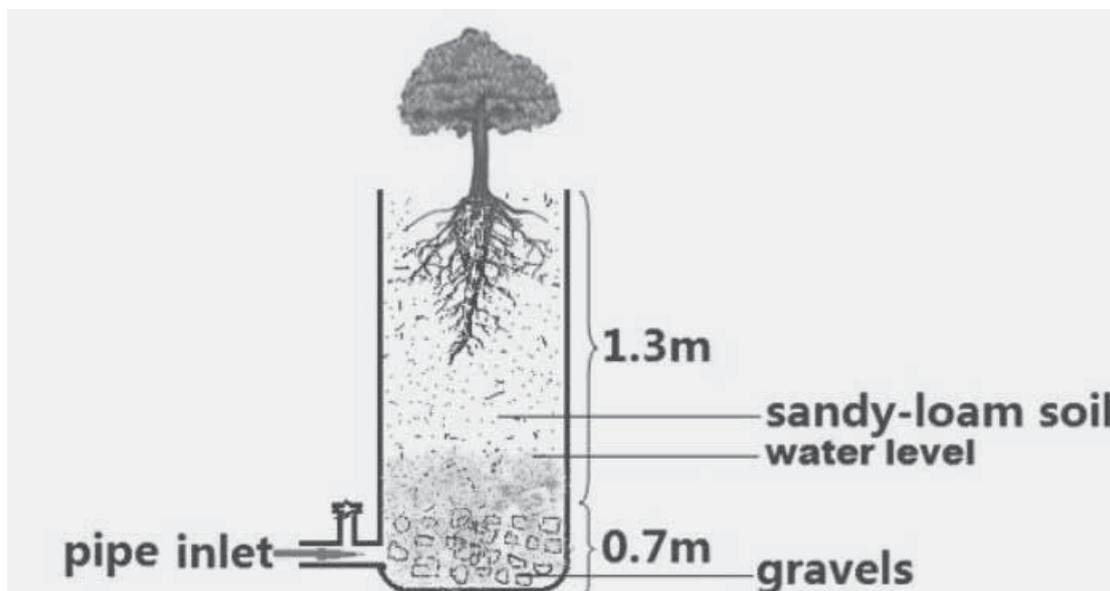


FIGURE 1
Experimental setup

Potassium containing fertilizer was applied at the initial stages to help trees to photosynthesize, enable the regulation of water within the plants, strengthen the plants to overcome drought stress and the cold winter conditions as well as to aid in the trees' ability to resist diseases that might have been in the planting area.

Chemicals and reagents. Pure trichloroethylene and tetrachloroethylene sealed in glass ampules were purchased from AccuStandard International, USA and kept at a temperature of -25°C until use. Methanol 99% grade was purchased

from Ur Chemical Co. Ltd, Shanghai, PRC. Potassium containing fertilizers were from Weamax, China.

Chemical dosing. Chemical dosing was only started after trees were observed to adapt to the new environment and new leaves begun to grow. Water mixed with various concentrations of PCE and TCE were introduced into the chambers through the influent by using an electric pump. Chemical dosing was from the 23rd of April to June, 2013 at a two (2) week interval. The concentrations for the various treatment days were as shown in TABLE 3.

TABLE 3
Treatment doses of the VOCs for the experimental period

| Dosing dates | Concentration of TCE and PCE in water (mg/L, H ₂ O) |
|--------------|--|
| 23rd April | 50 |
| 7th May | 50 |
| 21st May | 100 |
| 4th June | 100 |

Sample collection. Tree cores were obtained and tested for PCE and TCE on fortnights. Tree cores of length 2cm were obtained at 10cm, 30cm and 50cm height from the base of the tree. Haglöf

increment borer, which is of 0.169" core diameter by 8" length was used for sample collection. Composite samples were obtained by taking two cores approximately 0.5cm away from each other.

Replicate samples were acquired from the trees of the same species. Core samples were placed in a standard 10mL vials fitted with a Teflon-lined septum cap and tightly screwed. Sampling and storage were done as quickly as possible in order to reduce volatilization of the VOCs. The samples were kept at a temperature of 10°C in chest coolers prior to analysis. Data collection was done between 6th May and June 17th 2013. On days that it rained, data collection was postponed to the next day.

Sample analysis. Headspace-Solid Phase Microextraction (H-SPME) was performed on the tree cores using 75µm carboxen/polydimethylsiloxane (CAR/PDMS) fused silica fiber purchased from Supelco analytical, USA. The samples were allowed to equilibrate for 10mins after which headspace extraction at 25°C for 10mins was carried out. Afterwards, the fiber was immediately withdrawn and transferred into the chromatograph injection port for thermal desorption at 250°C for 4mins.

Standard preparation and creation of calibration curve. To prepare the stock solution, TCE and PCE standards were dissolved in methanol and five levels of standard solutions prepared. These standards were injected into previously tested tree cores and the SPME used in extracting the analytes of interest. This was then

tested in the GCMS for creation of the calibration curve.

Instrumentation. Gas Chromatography-Mass Spectrometer (GC-MS) QP2010 Plus from Shimadzu Corporation (Tokyo, Japan) was used for chromatographic analysis. Splitless injection was employed. A fused-silica capillary column Cd-5ms 60mx0.25mm. 0.25µm was used. Initial column oven temperature was 60°C for 1min and ramped to 140°C at 10°C/min, then to 250°C at 25°C/min and held for 3min. The MS was operated in the electron ionization (EI) mode. Helium (99.999%) was used as a carrier gas at a flow rate of 1.99mL/min.

Recovery. Recovery tests were performed to test the efficiency of extraction. Untreated tree cores were spiked with 10µg/ml TCE and PCE standard solutions and tested as described above. The recoveries of TCE and PCE were 74% and 80% respectively.

Data analysis. Qualitative and quantitative analysis of target compounds were achieved by comparing the spectral responses and retention times recorded by the MS to the National Institute of Standards and Technology (NIST 08) mass spectral library database. The concentration of the VOCs in the tree core are expressed as in Equation 1.

EQUATION 1

$$\text{Concentration of VOCs (per core)} = \frac{\text{Detected concentration of VOC } (\mu\text{g})}{\text{Weight of tree core (kg)}}$$

Statistical analysis. The resulting data was analyzed using descriptive statistics and One-way ANOVA in Statistical Package for Social Scientists (SPSS). The data are presented as mean±standard error, R² and P from the replicated treatments. Statistical significance was accepted at α=0.05.

RESULTS

On the first day that measurements were made (6th May) (

FIGURE 2), uptake of TCE was higher than PCE. TCE readings were between 1.152 to 27.293µg/kg and PCE 0.171 to 0.966µg/kg. F-value

obtained was 31.79 (Fcr=4.196; P= 4.87E-06; n=15) (TABLE 4) indicating a significant difference between the concentration of TCE and PCE. In C1, the concentration of the VOCs at the different heights (10, 30 and 50cm), did not differ so much from each other. However, in C2 and C4, highest concentration of both TCE and PCE were recorded at the 30cm height, whereas C3's was at the 50cm height.

Alternatively, for the subsequent measurement days, PCE levels were higher than that of the TCE in all the replicates. On 20th May, TCE and PCE levels ranged from 0.402 to 15.040µg/kg and 1.263 to 38.238µg/kg for TCE and PCE respectively

(FIGURE). F-value obtained was 10.956 (Fcr=4.195; P= 0.003; n=15) (With respect to the target compounds; TCE and PCE, qualitative and quantitative analysis were done at peak profile retention times of 5.42 and 7.16mins respectively. ANOVA results are as shown in TABLE 4.

This was statistically significant. Highest concentrations in C1 and C3 were at 50cm whereas C4 was at the 10cm height. That of June 3rd was similar (Figure 4). On June 17th the TCE and PCE concentration ranges were 0.543 to 2.047µg/kg and 0.8580 to 24.142µg/kg respectively (

FIGURE). The mean±S.E were 7.885± 1.833ppm and 1.579± 0.127ppm for PCE and TCE respectively (TABLE 4). F-value was 11.776 (Fcr=4.195; P= 0.002; n=15). It was thus statistically significant.

The highest concentration of both TCE and PCE in C1, C2 and C4 were recorded at a height of 30cm whereas that of C3 was at a height of 10cm. It must be well noted that some levels of the analytes of interest, were observed even in the controls.

TABLE 4
Results from the statistical analysis

| Sampling date | Mean±S.E (µg/kg) | P-value | R ² | F | Fcr |
|---------------|---------------------------------------|----------|----------------|----------|-------|
| 6th May | TCE=15.944±0.0337 PCE= 4.301±1.132 | 0.003 | 0.81 | 10.956 | |
| 20th May | TCE=0.308±0.0567 PCE=10.725±1.8467 | 4.87E-06 | 0.70 | 31.78969 | 4.195 |
| 3rd June | TCE= 6.530±0.771 PCE= 38.137±0.6 | 5.4E-05 | 0.627 | 22.623 | |
| 17th June | TCE= 1.579±0.127 PCE= 7.885±1.833 | 0.002 | 0.627 | 11.776 | |

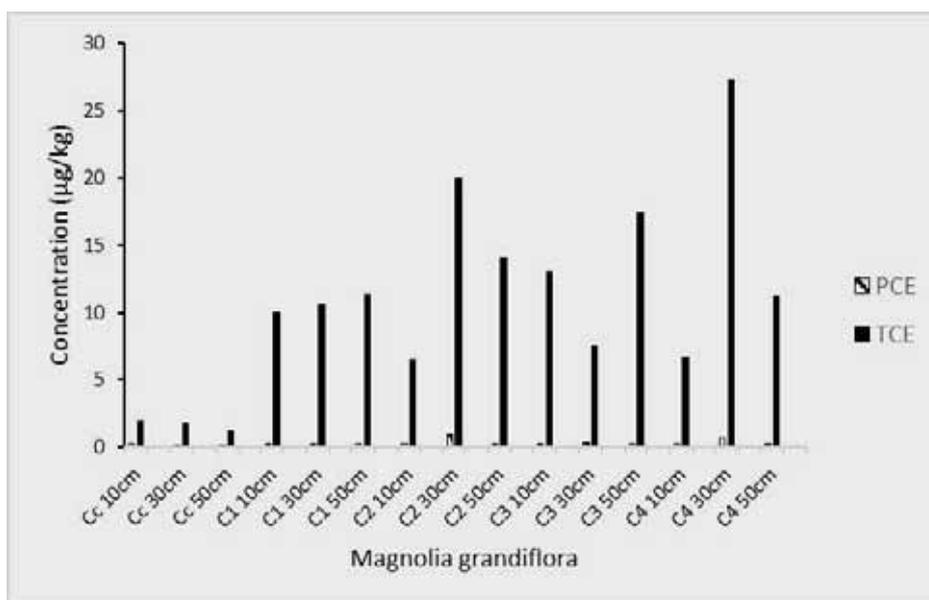


FIGURE 2
Concentration of TCE and PCE in *Magnolia grandiflora* cores on 6th May (Statistical analysis: n=15; confidence level=95%, Descriptive statistics; α=0.05

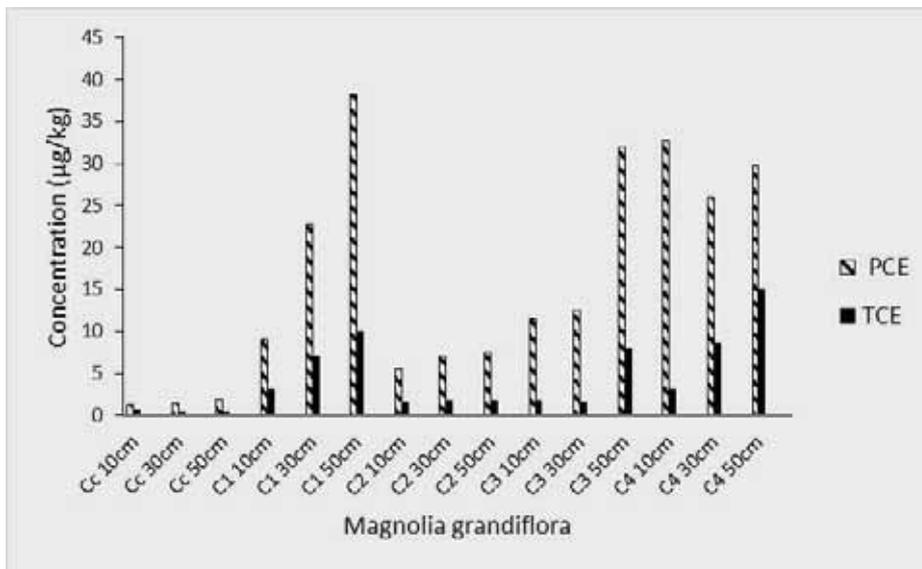


FIGURE 3
 Concentration of TCE and PCE in *Magnolia grandiflora* cores on 20th May (upper left) (Statistical analysis: n=15; confidence level=95%, Descriptive statistics; $\alpha=0.05$)

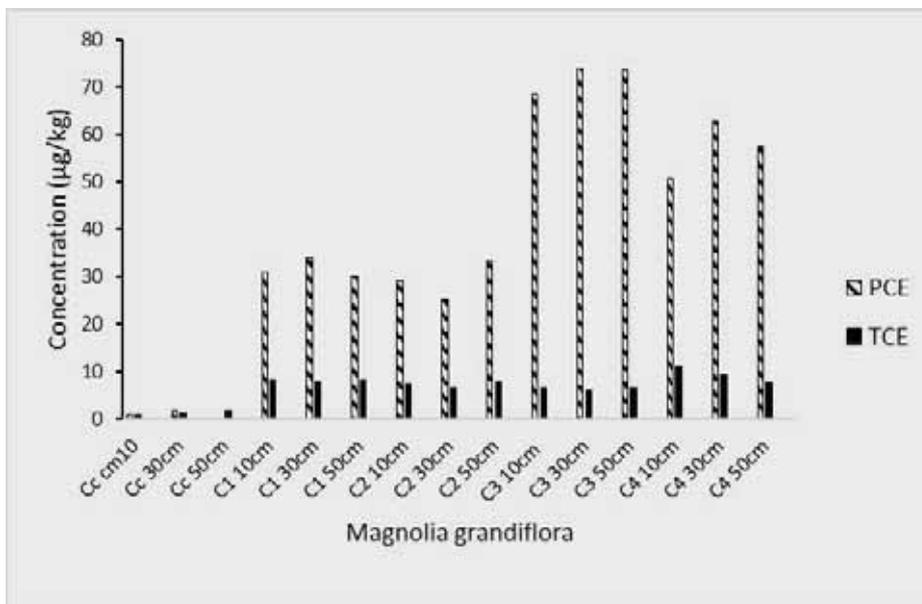


FIGURE 4
 Concentration of TCE and PCE in *Magnolia grandiflora* cores on 3rd June (Statistical analysis: n=15; confidence level=95%, Descriptive statistics; $\alpha=0.05$)

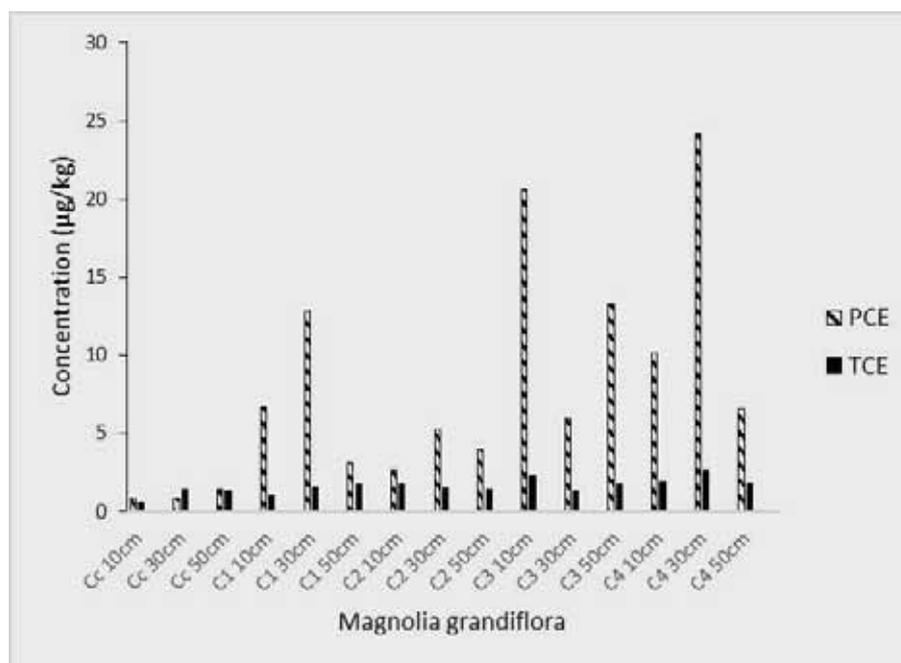


FIGURE 5

Concentration of TCE and PCE in *Magnolia grandiflora* cores on 17th June (Statistical analysis: n=15; confidence level=95%, Descriptive statistics; $\alpha=0.05$)

DISCUSSION

From the results, some amount of the target compounds; trichloroethylene and tetrachloroethylene were observed in the controls. It is worth noting that although the concentrations measured were low in comparison to the treated chambers, they cannot be ignored. TCE and PCE in the controls, might have been from the surrounding environment through atmospheric deposition, volatilization from the nearby treated groundwater or soils or pore to pore transfer between the trees, via the stomata or stem. Radial distribution in stem tissues have been reported as some of the potential sources of VOCs in untreated or uncontaminated areas in similar studies[15][16]. This therefore might have caused the transfer of some amount of the VOCs from the treated chambers into the controls. The control chamber was located at approximately 0.5m from the treated ones and this might have increased their susceptibility to compound transfer from the neighbouring treated chambers. Also worthy of noting is the fact that VOC levels in the controls did not increase nor decrease significantly throughout the experimental period.

Trees have been identified to have high concentration of lignin that enhance the absorption of organic contaminants into their tissues[16]. The lignin composition as well as high transpiration rate

of *Magnolia grandiflora* enhanced its absorption of the VOCs from the soil into the plants to be processed. The results from this study, showing the higher uptake of the VOCs with the increase in treatment concentrations confirms the observations previously reported. Therefore, the identification of the target compounds in the sampled tree cores, is no surprise.

The initial concentration of trichloroethylene in the *M. grandiflora* trees was higher than tetrachloroethylene. However, as the number of days increased, the concentration of PCE, increased while that of TCE decreased. Some properties of TCE such as low solubility in water and weaker adsorption in soil might have enhanced its absorption and made it readily available to be taken up from the groundwater media into the tree root media for uptake into the trees. The chemical characteristics of TCE such as its lipophilic nature and weaker adsorption in soils tend to influence its absorption into other media[17]. Initial smaller concentration of PCE may be related to its slow sorption into the roots and lower stem as found in other studies[18]. On the other hand, the high volatility of TCE probably also increased its level of escape from the trees and hence, the reduced levels with time. Additionally, within the tree system, if diffusion was the main pathway through which the TCE was taken up into the stem, then the subsequent smaller concentrations measured are not

unlikely. Studies by Ma and Burken[14], showed similar findings; especially at the early stages of treatment.

Conversely, the period of the research might have caused a reduction in the concentration of TCE in the tree cores. Concentration of TCE has been identified by Koppmann et al[19] to be higher in winter and spring. The results also confirms the studies by Yokouchi et al[20] and Dimmer et al[21][21] where it was revealed that the period between May and September are where the concentrations of TCE are low.

The uptake of trichloroethylene by trees are often inhibited by the presence of other chlorinated ethenes[22] and therefore shows that the lower concentrations of TCE measured in the study was perhaps as a result of the presence of its primary co-contaminant, PCE. Nonetheless, a study by Wang et al[23], revealed that there is no substantial competitive effect of co-contaminant PCE on the uptake and transport of TCE by trees.

From this study, it can be said that although both TCE and PCE can be measured in tree cores of *Magnolia grandiflora*, the uptake and storage of the latter in the tree trunks, are higher than that of the former. The findings contrast results from another study[18] where it was identified that the concentration of TCE in woody plants are often higher than PCE.

Additionally, it was observed that there was no consistency in the uptake of the VOCs per the sampling at different heights. Whereas highest concentrations of both TCE and PCE were often measured at the 30cm height, in most cases, the concentrations were roughly the same at all the heights. This observation is in contrast with findings by Wang et al[23] where they found out that the concentration of VOCs decreased in stems along the height of trees.

Lastly, the use of the headspace-solid phase microextraction (H-SPME) technique for sample extraction, ensured maximum concentration of the analytes. The studies therefore further proved the reliability of this extraction technique for monitoring VOCs in tree cores.

CONCLUSION

The use of *Magnolia grandiflora* for monitoring TCE and PCE contamination in groundwater was studied. From the results, the experimental period (April to June) favoured the trees' ability to take up large volumes of the target compounds because of its enhanced transpiration rate during the period. The concentration of TCE at

the initial stages of the experiment were higher than PCE but this changed with time.

It must be pointed out that there was no consistency in the measured concentrations of both compounds at the various heights as was expected. The present study also indicated that the VOCs used were not toxic to the plants. Therefore, the results from the experimental study demonstrated that *Magnolia grandiflora* which is an evergreen species of both economic and ecological importance, can be used as an alternative to other woody plants; where available, for the phytomonitoring of TCE and PCE contamination of groundwater.

The results from the study provides essential information and an additional tree species to select from, for policy makers and other scientists who are looking at phytomonitoring as an alternative to other conventional methods of monitoring VOCs in groundwater. As far as the authors are aware, this is the first manuscript that has understudied the potential use of *Magnolia grandiflora* for phytomonitoring, hence, this study is of great benefit for the efficient management of groundwater resources.

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CONCENTRATION OF HEAVY METALS AND INVESTIGATION OF BACTERIAL TOXIC METAL RESISTANCE IN COASTAL CITY SEDIMENTS (EASTERN AEGEAN SEA)

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ABSTRACT

Izmir is located on the Eastern Coast of the Aegean Sea in Turkey. In present study, it was aimed to determine the toxic metal resistance of bacterial isolates from coastal city sediments. Twenty-six isolates (*Bacillus*, *Marinibacillus*, *Halobacillus*) were identified and these strains were investigated in respect of the minimum inhibitory concentrations of metals, susceptibility of some antibiotics (Ampicillin, Tetracycline, Chloramphenicol, Tobramycin). The MICs of metals were different for each strain but the general order of resistance to the metals was found to be as Ni>Pb>Cu>Cr>Cd>Hg and the toxic effects of these metals increased with increasing concentration. Bacterial isolates were highly resistant to Ni (13 mM), Pb (6 mM). Additionally, it was found that the strains were resistant to Ampicillin. The highest concentrations of metals were found in the sediments of the inner part of the Bay, which is intensely industrialized compared to the middle and outer parts of the Bay. This study and several other studies suggest that sediment bacteria in an area polluted with heavy metal can be biological indicators.

KEYWORDS:

Heavy metals, toxicity, bioindicators, metal resistance, *Bacillus*, Aegean Sea

INTRODUCTION

Various human activities such as industrial and domestic wastes and agricultural inputs cause contaminants enter aquatic environments. These contaminants disrupting the natural balance are

industrial wastes, petrol derivatives, pesticides, artificial agricultural chemicals, detergents, radioactive substances, inorganic salts, organic substances etc. Heavy metals in industrial wastes and some pesticides may become a threat to the ecological balance. Entrance of metals to aquatic environments may occur by natural or human resources. These inputs result from atmospheric transport, mining applications, usage of fossil fuel, veterinary drugs, discharge of urban and industrial wastes, and vessel applications. Heavy metals in marine environments may be found in dissolved (free ions, compound ions etc.) or solid phase (colloid form, adsorbed on particle surfaces, in mineral matrix), inorganic or organic forms [1, 2, 3, 4]. Metals might be accumulated by aquatic organisms and be highly toxic even at low levels. Metal pollution has toxic effects on organisms at biochemical, cellular, population and community levels. Sediment is a significant accumulation area for heavy metals and therefore, it is used to determine the level of metal pollution in aquatic environments [5]. However, sediment is also a significant living and feeding ground for various organisms. Heavy metals lead to problems by 56 accumulating in sediments instead of water column. Thus, bacteria in sediment adapt to the environment in terms of survival and transfer characteristics and gain resistance. In other words, they are able to tolerate high concentration of heavy metals. If there are heavy metals in the sediment, microorganisms affect the catabolism of organic chemicals and ecology of the environment. As a result, microorganisms develop various mechanisms in order to tolerate the metals. These mechanisms include converting into volatile form, extracellular precipitation, adsorbing cell surface, and intercellular accumulation. Also, bacteria perform chemical transformations of these metals. Heavy

metal resistant microorganisms may be used in genetic transfer studies of the heavy metal resistance mechanism [6]. They may also be used as indicators of potential toxicity for other viable forms [7]. Heavy metals have a significant role as selective agents in the evolution of microbial communities [8]. Briefly, microorganisms have a significant role in biogeochemical cycles of metals and they can be used for bio-regeneration in polluted environments [9, 10]. However, correlation is frequently observed in antibiotic resistance besides metal resistance because resistance genes in chromosome or plasmid are close to each other. Increases in antibiotic resistance lead to problems in the treatment of infectious diseases worldwide. Therefore, it is needed to be careful when pesticides, antimicrobials containing metals and antibiotics entering the environment are used [11, 12]. Thus, biological accumulation and bioavailability potentials of heavy metals in aquatic environments are becoming a matter of concern globally. Screening of heavy metal resistance patterns of bacteria and heavy metal contamination in areas with industrial input such as bay are important for the identification of metal effects on the location of flora and fauna in that area and their survival [8]. In present study, heavy metal resistant bacteria species in sediment samples collected from the harbor and other areas of Izmir Bay which were intensely polluted were investigated and heavy metals in the sediment were measured.

MATERIALS AND METHODS

Study Area. Izmir Bay is one of the biggest bays in the Mediterranean Sea with totally 200 km² areas and 11.5 billion m³ water capacity profoundly inserted to Anatolia Coasts of Aegean Sea. Izmir, a significant city of commerce, industry and harbor, is the biggest residential area with approximately 88000 ha areas surrounding the bay. Izmir Bay has been polluted since 1960 due to the disembovement of unrefined domestic and industrial waste waters directly to the bay as a result of increases in population and the number of industrial foundations. This pollution became worse in early 2000s. Especially intensive harbor activities inside the bay and pollution due to domestic and industrial wastes became rampant and ecological balance was disrupted difficult to fix. Pollution was taken under control by Cigli Waste Water Treatment Plant becoming a part of activity in 2000 and recovering in the bay has been observed until now. Izmir Bay is divided into 3 main groups as inner, middle and outer bay due to its hydrological and ecological features. The outer bay is the area between Karaburun peninsula and Gediz delta which extends in the northwest-southeast direction and has the larger and deeper part is. The length of the external gulf is 45 km while its width is about 24 km. The depth of the external bay ranges between 45m and 70m. The middle bay starts from the southeast part of the external bay, extends in the west-east direction to the inner bay. The total length of this area is 38 km while its width is 20 km in west and becomes narrower to east [13, 14].

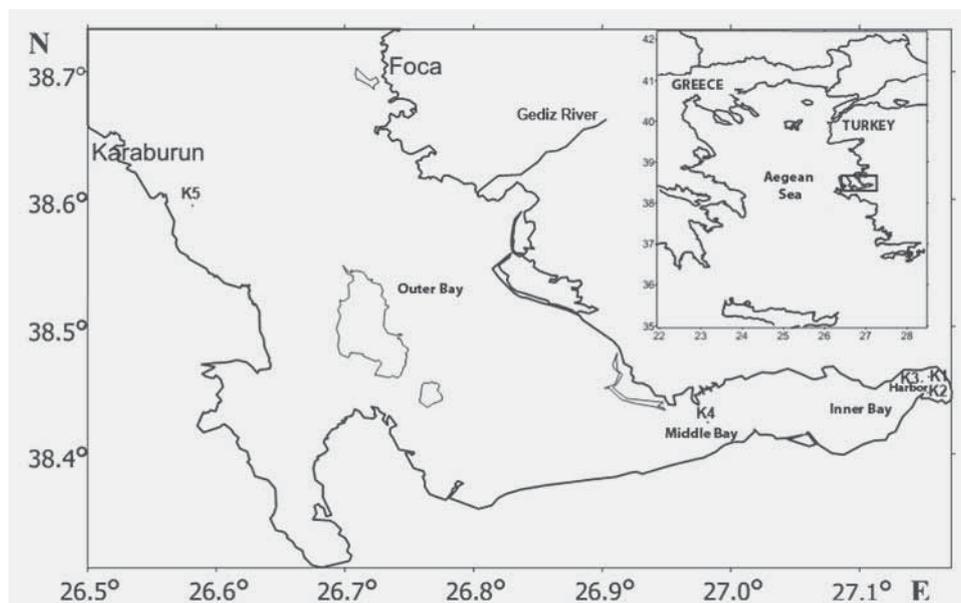


FIGURE 1
Sampling station of Izmir Bay.

Analysis of Heavy Metals in Surface Sediments. The surface sediment samples were collected using by Van-Veen grab for heavy metals from 5 sampling stations as part of a cruise of the R/V K. Piri Reis in 2011 (Figure 1). Immediately after their collection, the samples were placed in acid cleaned polyethylene bottles and were kept frozen at -20°C until analysis. Samples of the surface sediment were freeze-dried then sieved to pass a $63\ \mu\text{m}$ sieve and homogenized. Later, triplicate samples were digested in a microwave digestion system (Milestone 1200) with acid mixture solutions [15, 16, 17, 18]. A Varian Spectra Model AA280FS and AA280 Zeeman atomic absorption spectrophotometer (AAS) were used for the analysis of heavy metals. The Hg measurements were performed by using cold vapour atomic absorption technique. The concentrations of Pb, Cr, Cu, Mn, Ni, Zn and Fe in the sediment were measured by flame atomization (air-acetylene flame). Also, Cd levels were detected by graphite furnace with AAS. The detection limits for heavy metals are Hg: $0.05\ \mu\text{gkg}^{-1}$, Cd: $0.10\ \mu\text{gkg}^{-1}$, Pb: $0.10\ \text{mgkg}^{-1}$, Cr: $0.06\ \text{mgkg}^{-1}$, Cu: $0.03\ \text{mgkg}^{-1}$, Zn: $0.01\ \text{mgkg}^{-1}$, Mn: $0.02\ \text{mgkg}^{-1}$, Ni: $0.10\ \text{mgkg}^{-1}$, Fe: $0.06\ \text{mgkg}^{-1}$. Intercalibration sediment (IAEA-433) samples (from the International Laboratory of Marine Radioactivity, IAEA) were used as a control for the analytical methods. The

values obtained (in mgkg^{-1} dry wt) for the analysis of six replicates of this sample (certified: observed values in mgkg^{-1} dry weight \pm standard deviation) were as follows: Hg, 0.168 ± 0.017 : 0.168 ± 0.00 ; Cd, 0.153 ± 0.033 : 0.140 ± 0.025 ; Pb, 26.0 ± 2.7 : 27.0 ± 3.1 ; Cr, 136 ± 10.0 : 136.7 ± 2.0 ; Cu, 30.8 ± 2.6 : 30.8 ± 2.3 ; Zn, 101 ± 8.0 : 101.1 ± 1.9 ; Ni: 39.4 ± 3.1 ; 39.4 ± 0.27 .

Bacteriological Analysis of Surface Sediments. Surface sediment samples obtained from five various points by Van-Veen grab (Figure 1) were transferred to the laboratory at $+4^{\circ}\text{C}$ in sterile falcon tubes, and isolation and identification of the microorganisms were performed for microbiological analyses. Sediment samples were diluted to 10^{-7} and inoculated onto the plates containing Zobell Marine Agar by the spread plate method and incubated at 26°C for 3-4 days. Approximately 50 bacterial colonies were selected but 26 of these colonies were stored by inoculating pure colonies for further identification. 16S rDNA based molecular analyses were performed to identify bacteria species. For this purpose, genomic DNAs of bacteria were obtained by Genomic DNA Isolation Kit (Zymo Research, USA) and their 16S rRNAs were amplified by PCR using conventional bacterial primers (27F and 1522R). For this test, the Fast Start Taq DNA Polymerase dNTPack Kit

(Roche, Germany) was 58 used. Primers were 0.2M and template DNA was 10 ng, and the PCR protocol was performed 30 cycles (first cycle at 95°C for 5 min, denaturation at 95°C for 55 s, annealing at 52°C for 40 s, elongation at 72°C for 1.5 min, and final elongation step at 72°C for 7 min) [6, 17, 18]. The DNA sequence analyses of purified PCR products were performed using automatic sequencer ABI Prism 3100 from REFGEN Biotechnology (Turkey). After the sequence results were checked by Chimera program, accession numbers to gene bank numbers were taken by comparing data base in which genes are found (<http://www.ncbi.nlm.nih.gov>) by Blast program. Phylogenetic tree of the species were generated by using the MEGA 6 program [21, 22]. The Minimum Inhibition Concentration (MIC) analysis was performed in order to determine the resistance that the bacterial isolates whose species were identified showed to heavy metals. Thus, heavy metals such as Hg, Cr, Cu, Ni, Pb, Cd were tested. Stock solution of the metals were sterilized by 0.2 µm membrane filtering and then added into

Mueller Hinton Agar (Difco, USA) medium at various concentrations ranging between 0.01 and 15.0 (mM). After the bacteria were inoculated into the mediums containing heavy metals, the plates were incubated at 26°C for 24-48 hours and minimum concentration in which no growth was observed was determined [19, 23]. Besides heavy metal resistance analyses, antibiotic resistance of the bacteria was also determined by the disc diffusion method using Mueller Hinton Agar medium. In this analysis, 4 different antibiotics were used: Ampicillin (AM, 10 µg), Chloramphenicol (C, 30 µg), Tetracycline (TE, 30 µg) and Tobramycin (TM, 10 µg) (Bioanalyse, Turkey). After the bacteria were cultured for 24h, 0.1 ml was inoculated into Mueller Hinton Agar. After 1 h incubation at 26°C, the antibiotic discs were placed on the agar and the plates were incubated again at 26°C for 24-48 hours. The results were evaluated as resistant or sensitive according to inhibition zone diameter measurements [12, 19, 23].

TABLE 1
The mean ± SD values of metals in sediments from Izmir Bay (mg kg⁻¹ dry wt.) and background values.

| Station | Hg | Cd | Pb | Cr | Cu | Zn | Mn | Ni | Fe |
|--|----------------|-----------------|---------------|--------------|---------------|---------------|--------------|---------------|----------------|
| K1 | 0.59± 0.06 | 0.17± 0.01 | 47.9± 0.43 | 116± 1.66 | 46.8± 0.71 | 159± 3.78 | 456± 0.89 | 76.4± 1.08 | 16190± 839 |
| K2 | 7.04± 0.15 | 0.21± 0.01 | 52.3± 1.70 | 168± 1.52 | 72.3± 0.60 | 278± 1.71 | 442± 0.84 | 65.8± 3.49 | 31922± 1015 |
| K3 | 0.40± 0.01 | 0.18± 0.002 | 50.4± 1.40 | 114± 3.13 | 54.4± 0.39 | 193± 1.91 | 481± 4.33 | 125± 0.55 | 40133± 14.1 |
| K4 | 0.19± 0.014 | 0.07± 0.01 | 20.3± 1.29 | 140± 6.00 | 23.4± 0.56 | 101± 1.53 | 314± 5.04 | 74.2± 1.08 | 29035± 609 |
| K5 | 0.28± 0.01 | 0.13± 0.01 | 13.3± 0.88 | 122± 5.16 | 22.6± 0.29 | 77.4± 2.15 | 649± 11.5 | 98.2± 0.13 | 35698± 419 |
| Backgr. values(a) | 0.40 | 0.30 | 20 | 90 | 45 | 95 | 850 | 68 | 47000 |
| Mediterranean Backgrd^(b) | 0.34 | 0.10-2.3 | 25 | 15 | 15 | 50 | | | |

(a) Turekian and Wedepohl[36] b UNEP [37], Whitehead et al.[38] and MAP [39]

TABLE 2
The enrichment factors of heavy metals in sediments from Izmir Bay

| Station | Hg | Cd | Pb | Cr | Cu | Zn | Mn | Ni |
|------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| K 1 | 4.29± 0.39 | 1.60± 0.14 | 6.97± 0.45 | 3.74± 0.13 | 3.04± 0.21 | 4.89± 0.41 | 1.56± 0.09 | 3.29± 0.18 |
| K 2 | 26.0± 0.39 | 0.98± 0.04 | 3.91± 0.02 | 2.73± 0.07 | 2.38± 0.09 | 4.31± 0.17 | 0.77± 0.02 | 1.42± 0.06 |
| K 3 | 1.16± 0.04 | 0.72± 0.01 | 2.99± 0.07 | 1.47± 0.05 | 1.42± 0.01 | 2.37± 0.02 | 0.66± 0.01 | 2.16± 0.04 |
| K 4 | 0.75± 0.07 | 0.32± 0.07 | 1.59± 0.12 | 2.48± 0.17 | 0.85± 0.01 | 1.74± 0.03 | 0.60± 0.00 | 1.78± 0.04 |
| K 5 | 0.91± 0.01 | 0.41± 0.00 | 1.16± 0.07 | 1.67± 0.05 | 0.28± 0.01 | 0.64± 0.03 | 0.52± 0.01 | 1.45± 0.03 |

RESULTS AND DISCUSSION

Concentrations of Heavy Metals in Surface Sediments. The means of heavy metals concentrations in sediment from Izmir Bay and background levels were presented in Table 1. The comparison of the metal concentrations with background values revealed that the stations K1, K2 and K3 (Harbor) in the Inner Bay were polluted with Hg, Pb and Cu. Additionally, Cu levels at K4 and K5 were higher than these background levels. The values of Cr and Ni were generally higher than those for background levels in all sampling stations (except K2 for Ni). Although the concentrations of Zn in sampling stations (except the station K5) were detected to be higher than those at background levels, this metal level detected at K5 was higher than those in the Mediterranean. On the contrary, Cd, Mn and Fe concentrations were lower than these values (Table 1). The metal enrichment factor (EF) is utilized as an index to evaluate anthropogenic influences of metals in sediments. The EF is generally defined that ratio of the observed metal to conservative elements (Fe or Al). Al and Fe are the main components of the elements in sediments, and they are not affected by contaminants. The mean heavy metal enrichment factors of the sediments in Izmir Bay were given in Table 2. While EF values between 0.5 and 1.5 indicate that the heavy metals originate from the earth's crust, EF values > 1.5 indicate that the heavy metals have anthropogenic pollution origin [24]. According to the enrichment index, enrichment values of Cd and Mn were less

than 1.5 in all the stations except for the station K1. Pb, Cr and Ni enrichment values were greater than 1.5 in all the stations. Conversely, the maximum enrichment of Hg, Pb, Cu and Zn existed at stations K1, K2 and K3 in the inner bay. Besides, Hg, Pb, Cr, Zn and Ni were generally enriched. It means that a significant portion of Hg, Cr, Zn, Ni and Pb were provided by anthropogenic sources. The comparison of bibliographical data on metal concentrations of sediments from various regions of the Mediterranean and Aegean Sea was given in Table 3. In this study, the concentrations of Cd were found relatively higher than those found in other studies except the conducted on Venice [25], Izmit [26] and Izmir Bay [27, 28], while Hg values were higher than those determined in all the other areas. Generally, the Pb, Cr and Mn contents of the sediments were lower compared to those obtained in other areas (Table 3). The Pb, Cr, and Zn pollution at all the sampling areas and Hg, Pb, Cr, Cu, Ni and Zn pollution at K1, K2 and K3 are anthropogenic sources. In the past various studies were conducted measure the heavy metals pollution in the sediments of Izmir Bay (Table 3). The highest Cd, Pb, Cr and Cu values were determined as 0.82, 119, 316 and 109 mgkg⁻¹, respectively [27]. In another study by Bergin et al. [29] in Izmir Bay, the highest Ni concentration was found as 157 mgkg⁻¹. The Gediz River usually transports high concentrations of metals due to the inputs of textile, pigment, metal plating, beverage and paper factories and domestic wastes to the bay. The inner bay generally was polluted by beer industries, antifouling paints, iron, paper, chlorine-alkali plants,

cement factories, and intense harbor activities. In addition, the streams and hundreds of small domestic discharge outlets flow into the inner bay. Until the municipal treatment system was introduced in 2000, domestic and industrial

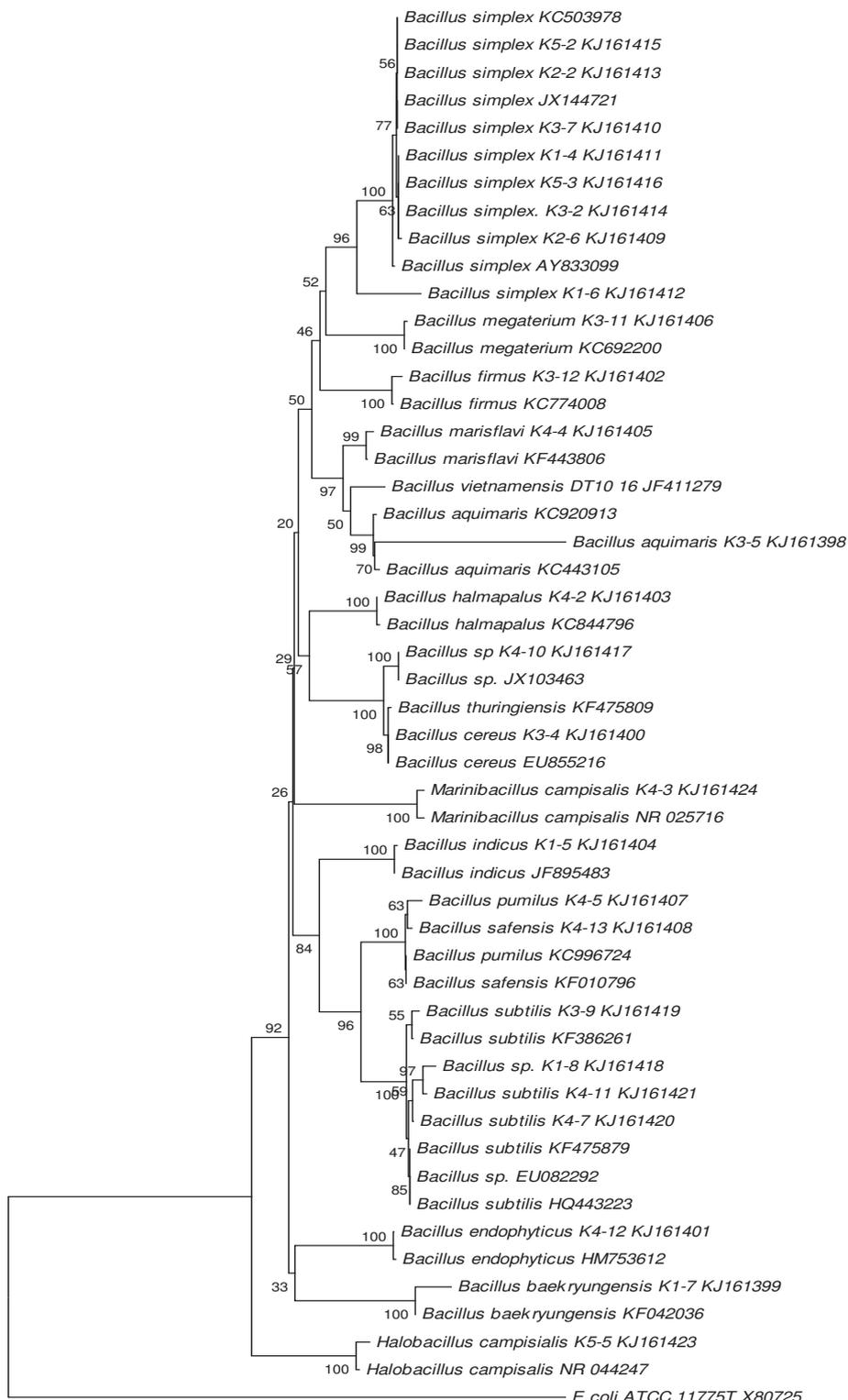
wastes were discharged into the Izmir Bay without treatment. Consequently, in the Izmir Bay, the sediment which was continuously in contact with water was intensely polluted.

TABLE 3
Metal concentrations (mg kg⁻¹, dry weight) in surface sediments in various coastal ecosystems.

| | Hg | Cd | Pb | Cr | Cu | Mn | Ni | Zn | Reference |
|---------------------|------------------|------------------|------------------|----------------|------------------|----------------|---------------|---------------|-----------|
| This study | 0.17-7.21 | 0.05-0.22 | 17.0-54.0 | 110-169 | 8.26-72.8 | 309-485 | 62-126 | 45-280 | |
| Izmir Bay | 0.05-1.3 | 0.01-0.82 | 3.10-119 | 19-316 | 2.2-109 | 14-412 | - | - | [14] |
| Izmir Bay | - | 0.13-0.42 | 5.0-62.0 | 64-300 | 12-70 | 74-586 | 29-148 | 23-260 | [28] |
| Izmir Bay | 0.05-0.39 | 0.01-0.14 | 14.0-69.0 | 40-165 | 2.6-50 | 227-777 | 25-157 | 20-249 | [29] |
| Saros Gulf | 0.07-0.19 | 0.01-0.04 | 3.9-48.2 | 18.8-216 | 12-42 | 351-4718 | 47-152 | 49-117 | [40] |
| Gökova Gulf | 0.07-0.17 | 0.01-0.05 | 10-21.8 | 63-332 | 10-41.1 | 399-963 | 283-426 | 48-91 | [40] |
| Marmara Sea | 0.04-3.0 | - | 10-85 | 11-654 | 3.0-107 | 100-2610 | 8.0-1731 | 33-410 | [41] |
| Izmit Bay | - | 3.3-8.9 | 24-178 | 58-116 | 61-139 | - | 38-71 | 510-1190 | [26] |
| Aegean Sea | 0.01-0.08 | 0.003-0.05 | 1.75-51 | 22-163 | 3.2-32 | - | - | 8.9-84 | [42] |
| Taranto Bay | 0.09-0.41 | - | 44.74.8 | 77.1-102.8 | 42.4-52.3 | 552-2826 | 47.9-61 | 87-129 | [43] |
| Northern Aegean Sea | - | - | 10-218 | 7-172 | 19-165 | - | - | 74-358 | [44] |
| Northern Aegean Sea | - | - | 131-2233 | 213-364 | 51-206 | - | - | 159-927 | [45] |
| Venice | 0.7-1.6 | 0.59-3.1 | 19-56 | 28-107 | 12-51 | 222-379 | 11-50 | 71-433 | [25] |

Heavy Metal Resistant-Bacteria. Of the isolates obtained from surface sediments of five sampling stations (K1, K2, K3, K4 and K5) in Izmir Bay, 26 were identified. Phylogenetic analysis using 16S rDNA indicated that the 26 sediment strains belong to genus *Bacillus*, *Halobacillus* and *Marinibacillus* (*Bacillus aquimaris* K3-5, *B. baekryungensis* K1-7, *B. cereus* K3-4, *Bacillus endophyticus* K4-12, *B. firmus* K3-12, *B. halmपालus* K4-2, *B. indicus* K1-5, *B. marisflavi* K4-4, *B. megaterium* K3-11, *B. pumilus* K4-5, *B.*

safensis K4-13, *B. simplex* strain K2-6, *B. simplex* K3-7, *B. simplex* K1-4, *B. simplex* K1-6, *B. simplex* K2-2, *B. simplex* K3-2, *B. simplex* K5-2, *B. simplex* K5-3, *Bacillus* sp. K4-10, *Bacillus* sp. K1-8, *B. subtilis* K3-9, *B. subtilis* K4-7, *B. subtilis* K4-11, *Halobacillus campisialis* K5-5, *Marinibacillus campisialis* K4-3) reported in this article were submitted to GenBank and assigned the accession numbers KJ161398 - KJ161424, respectively (Figure 2).



0.02

FIGURE 2
The dendrogram of isolates based on 16s rRNA genes.

TABLE 4
MICs of sediment isolates against heavy metals.

| Isolates | Heavy Metals (mM) | | | | | |
|---------------------------------------|-------------------|------|----|-----|-----|----|
| | Hg | Cd | Ni | Cr | Cu | Pb |
| <i>Marinibacillus campisalis</i> K4-3 | 0.01 | 0.6 | 4 | 0.8 | 1 | 4 |
| <i>B. simplex</i> K1-6 | 0.01 | 0.2 | 11 | 2 | 2 | 2 |
| <i>B. megaterium</i> K3-11 | 0.01 | 0.2 | 8 | 2 | 2 | 2 |
| <i>B. halmapalus</i> K4-2 | 0.01 | 0.4 | 4 | 0.8 | 0.8 | 2 |
| <i>Bacillus</i> sp. K4-10 | 0.02 | 1.0 | 8 | 2 | 2 | 6 |
| <i>B. marisflavi</i> K4-4 | 0.01 | 0.2 | 6 | 2 | 2 | 4 |
| <i>Bacillus</i> sp. K1-8 | 0.01 | 0.2 | 13 | 2 | 2 | 4 |
| <i>B. simplex</i> K3-2 | 0.01 | 0.08 | 10 | 0.8 | 2 | 4 |
| <i>B. pumilus</i> K4-5 | 0.01 | 0.1 | 10 | 2 | 2 | 6 |
| <i>B. indicus</i> K1-5 | 0.01 | 0.2 | 2 | 2 | 2 | 2 |
| <i>B. simplex</i> K2-2 | 0.01 | 0.08 | 8 | 0.8 | 2 | 2 |
| <i>B. subtilis</i> K4-11 | 0.01 | 0.04 | 8 | 2 | 2 | 4 |
| <i>B. aquimaris</i> K3-5 | 0.04 | 2.0 | 10 | 2 | 2 | 4 |
| <i>B. firmus</i> K3-12 | 0.01 | 0.04 | 2 | 2 | 2 | 2 |
| <i>B. simplex</i> K2-6 | 0.01 | 0.06 | 6 | 2 | 2 | 4 |
| <i>B. simplex</i> K5-2 | 0.01 | 0.2 | 10 | 0.8 | 2 | 2 |
| <i>B. subtilis</i> K4-7 | 0.01 | 0.2 | 13 | 2 | 2 | 4 |
| <i>B. safensis</i> K4-13 | 0.01 | 1.0 | 2 | 2 | 2 | 4 |
| <i>B. simplex</i> K1-4 | 0.01 | 0.2 | 10 | 2 | 2 | 4 |
| <i>B. simplex</i> K3-7 | 0.01 | 0.2 | 6 | 2 | 2 | 2 |
| <i>B. subtilis</i> K3-9 | 0.01 | 0.1 | 6 | 2 | 2 | 4 |
| <i>B. endophyticus</i> K4-12 | 0.01 | 0.1 | 8 | 2 | 2 | 6 |
| <i>Halobacillus campisialis</i> K5-5 | 0.01 | 0.2 | 4 | 2 | 2 | 4 |
| <i>B. simplex</i> K5-3 | 0.01 | 0.1 | 10 | 2 | 2 | 2 |
| <i>B. cereus</i> K3-4 | 0.01 | 0.4 | 10 | 0.4 | 2 | 4 |
| <i>B. baekryungensis</i> K1-7 | 0.01 | 0.2 | 8 | 2 | 2 | 2 |

Minimum inhibition concentrations of heavy metals are shown in Table 4. Sediment isolates showed higher tolerance to Ni and Pb. The highest resistance to nickel was in *Bacillus* sp. K1-8 and *B. subtilis* K4-7 isolates with 13 mM concentration. Resistance to lead was found in *Bacillus* sp. K4-10, *B. pumilus* K4-5, *B. endophyticus* K4-12 with concentration of 6 mM. The highest resistance values for copper, chrome and cadmium were

determined as 2 mM. Mercury was the most toxic metal for all of the isolates and it had the lowest inhibition concentration. Among the antibiotic resistance analyses working by the same mechanism as heavy metal resistance, the highest resistance was commonly detected to Ampicillin (Table 5).

TABLE 5
Antibiotic resistance of sediments isolated (mm).

| Isolates | Antibiotics | | | |
|---------------------------------------|---------------------------|------------------------|----------------------|----------------------|
| | Chloramphenicol (30µg) | Tetracycline (30µg) | Ampicillin (10µg) | Tobramycin (10µg) |
| <i>Marinibacillus campisalis</i> K4-3 | 24 | 22 | 16 | - |
| <i>B. simplex</i> K1-6 | 22 | 18 | 10 | 24 |
| <i>B. megaterium</i> K3-11 | 34 | 22 | 16 | 20 |
| <i>B. halmapalus</i> K4-2 | 30 | 22 | 20 | 10 |
| <i>Bacillus</i> sp. K4-10 | 20 | 8 | 14 | 13 |
| <i>B. marisflavi</i> K4-4 | 40 | 18 | 22 | 18 |
| <i>Bacillus</i> sp. K1-8 | 35 | 18 | 18 | 26 |
| <i>B. simplex</i> K3-2 | 22 | 18 | 8 | 26 |
| <i>B. pumilus</i> K4-5 | 23 | 16 | 8 | 20 |
| <i>B. indicus</i> K1-5 | 32 | 22 | 14 | 18 |
| <i>B. simplex</i> K2-2 | 16 | 18 | 10 | 22 |
| <i>B. subtilis</i> K4-11 | 18 | 20 | - | 18 |
| <i>B. aquimaris</i> K3-5 | 32 | 20 | 22 | 20 |
| <i>B. firmus</i> K3-12 | 38 | 24 | 12 | 28 |
| <i>B. simplex</i> K2-6 | 22 | 20 | - | 22 |
| <i>B. simplex</i> K5-2 | 18 | 20 | 8 | 25 |
| <i>B. subtilis</i> K4-7 | 36 | 20 | 20 | 26 |
| <i>B. safensis</i> K4-13 | - | 16 | 8 | 18 |
| <i>B. simplex</i> K1-4 | 20 | 20 | 12 | 22 |
| <i>B. simplex</i> K3-7 | 18 | 20 | 8 | 24 |
| <i>B. subtilis</i> K3-9 | 30 | - | 13 | 26 |
| <i>B. endophyticus</i> K4-12 | 16 | 21 | 18 | 28 |
| <i>Halobacillus campisialis</i> K5-5 | 38 | 26 | 30 | 22 |
| <i>B. simplex</i> K5-3 | 18 | 22 | 10 | 22 |
| <i>B. cereus</i> K3-4 | 36 | 22 | 22 | 24 |
| <i>B. baekryungensis</i> K1-7 | 32 | 20 | 13 | 19 |

When the isolates were analyzed, the species and antibiotics they were resistant to most were as follows: *B. subtilis* K4-11 and *B. simplex* K2-6 to Ampicillin, *Marinibacillus campisalis* K4-3 to Tobramycin, *B. subtilis* K3-9 to Tetracycline, and *B. safensis* K4-13 to Chloramphenicol. Most of the bacterial isolates were determined to be sensitive to Chloramphenicol. According to the similar studies, the areas taking various anthropogenic inputs have a natural reservoir role for bacterial strains with polyresistance. The most common bacteria in areas polluted with heavy metals were the members of heterotrophic *Bacillus* genus [8, 30, 31]. Zolgharnein et al. [32] determined that bacteria isolated from aquatic environments near industrial areas live in 0.5-1.0 mM metal concentrations (i.e; Zn, Cu, Pb ve Cd). In another study, it was shown that heavy metal and antibiotic resistance were related to each other [23, 30, 31]. In a study by Nithya et al. [8] in India Palk Gulf, it was determined that Co, Cd, Pb, Hg, As and Se resistant

bacteria were *B. arsenicus*, *B. pumilus*, *B. indicus*, *B. clausii* after molecular analysis and it was revealed that these sediment bacteria might be biological indicators of heavy metal contamination. Kamala-Kaanan and Lee [31] identified that *Bacillus* species isolated from sediments in Sunchan Bay (South Korea) showed maximum resistance to Mn and Ampicillin, Tetracycline, Kanamycin and Streptomycin. In a study performed in Izmit Bay in Turkey Gul-Seker and Mater [30] identified that Isolates Antibiotics Chloramphenicol (30µg) Tetracycline (30µg) Ampicillin (10µg) Tobramycin (10µg) *Marinibacillus campisalis* K4-3 24 22 16 - *B. simplex* K1-6 22 18 10 24 *B. megaterium* K3-11 34 22 16 20 *B. halmapalus* K4-2 30 22 20 10 *Bacillus* sp. K4-10 20 8 14 13 *B. marisflavi* K4-4 40 18 22 18 *Bacillus* sp. K1-8 35 18 18 26 *B. simplex* K3-2 22 18 8 26 *B. pumilus* K4-5 23 16 8 20 *B. indicus* K1-5 32 22 14 18 *B. simplex* K2-2 16 18 10 22 *B. subtilis* K4-11 18 20 - 18 *B. aquimaris* K3-5 32 20 22 20 *B. firmus* K3-12

38 24 12 28 *B. simplex* K2-6 22 20 - 22 *B. simplex* K5-2 18 20 8 25 *B. subtilis* K4-7 36 20 20 26 *B. safensis* K4-13 - 16 8 18 *B. simplex* K1-4 20 20 12 22 *B. simplex* K3-7 18 20 8 24 *B. subtilis* K3-9 30 - 13 26 *B. endophyticus* K4-12 16 21 18 28 *Halobacillus campisialis* K5-5 38 26 30 22 *B. simplex* K5-3 18 22 10 22 *B. cereus* K3-4 36 22 22 24 *B. baekryungensis* K1-7 32 20 13 19 bacteria species resistant to Cu, Cd, Cr, Chloramphenicol and Ampicillin were the members of *Acinetobacter* and *Bacillus* genus. Another study in Turkey was performed in Iskenderun Bay which was industrially polluted. In that study, metal toxicity of the isolates was determined as Cd>Cu>Cr>Pb>Mn and that they reported to have high resistance to Ampicillin, Streptomycin and Cefazolin [23]. Bacteria can adapt to the environment by developing tolerance mechanism to heavy metals due to lateral gene transfer between bacterial populations and plasmid resources [33, 34]. Aquatic environments are of interest to all stakeholders in terms of their users and their contribution to socioeconomic development. These areas are under pressure regarding coastal infrastructures, pollution, direct exploitation, biological resources and marine biodiversity [35]. Therefore, biological accumulation of heavy metals in aquatic environments and their bioavailability potential are becoming a matter of concern globally [8, 31]. The identification of species with polyresistance to especially heavy metals and antibiotics may indicate that these coastal areas may have problems, which may pose a risk for humans and animals. Thus, further investigations are needed.

CONCLUSION

When the average threshold values were compared to Mediterranean background levels, Hg, Pb and Cu concentrations were high in sediments in the Harbor area stations. Besides, Cr and Ni levels were higher than these levels in all of the stations. According to the enrichment index, Pb, Cr, Ni enrichments were observed in all of the stations. However, Hg, Cu and Zn enrichment was determined in the Harbor area stations. According to this information, Pb, Cr and Ni pollution in all of the Bay area and Hg, Cu and Zn pollution in the Harbor area were anthropogenic. In present study, the members of *Bacillus* genus were shown to be dominant among the bacteria isolated from Izmir Bay sediments. When we consider Pb, Cr and Ni enrichment, each isolate was resistant at different concentrations due to their heavy metal resistance but it was determined that generally resistance was as Ni>Pb>Cu>Cr>Cd>Hg. It was determined that

the most toxic metal for bacteria was Hg. In present study, it was observed that the highest resistance was to Ampicillin as in most of the studies considering heavy metal and antibiotic resistance mechanisms are similar. These results showed that the identified bacteria are able to survive in sediments polluted by heavy metals. While most of the bacteria are sensitive to even low concentrations of metals under specific conditions, they can adapt to their environment quickly and develop resistance to metals. For this reason, it is considered that these bacteria may be suitable indicators for the identification of heavy metal potential toxicity in coastal areas polluted with metal and they can be also designed for bioremediation applications by further investigations.

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ERRATUM

Unfortunately, we had published an incorrect version of the authors' manuscript previously in FEB Vol. 25 - 1/2016 (pages 55-66).

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RESPONSE OF ANTIOXIDANT DEFENSE SYSTEM TO OSMOTIC STRESS IN MAIZE SEEDLINGS

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ABSTRACT

In order to evaluate the effects of osmotic stress on fresh weight, H₂O₂, MDA, total phenolics, total soluble proteins and response of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) were mentioned. A factorial experiment was carried out under laboratory conditions with two maize inbred lines (MO₁₇ and B₇₃) and two osmotic stress levels induced by PEG (control and -6 MPa). Electrophoretic analyses were performed for SOD, POX and CAT in leaves of maize seedlings using 8% slab polyacrylamide gels. For SOD, POX and CAT, three, two and one isoforms were observed, respectively. On the basis of percent of inhibition in fresh weight under drought stress MO₁₇ was ranked as tolerant (47% inhibition) and B₇₃ drought sensitive (more than 50% inhibition). Drought stress enhanced antioxidant enzymes activity and total phenolics in maize seedlings. MO₁₇ also exhibited low levels H₂O₂, MDA and total soluble proteins, compared to B₇₃. In addition, MO₁₇ showed higher enzymatic activity of SOD₁, SOD₂, SOD₃, POX₁, POX₂, CAT and total phenolics leaves than B₇₃. These results indicate that MO₁₇ inbred line tolerance to drought stress during seedling is associated with enhanced activity of antioxidant enzymes. This study provides a mechanism for drought tolerance in maize seedlings.

KEY WORDS:

Antioxidant enzyme, Drought, Maize, Phenolics.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important crops. Various environmental stresses, especially drought adversely affect the growth and productivity of this crop. The limitation in the availability of water induces osmotic stress [1]. Under drought stress, photosynthesis decreases due to stomatal limitation when light energy absorption

exceeds its capacity for utilization [2]. The excess light energy, which is neither consumed in photosynthesis nor dissipated as fluorescence or heat, will be improperly transferred to oxygen or neighboring molecules, creating reactive oxygen species (ROS), such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (·OH), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids [3]. To prevent and alleviate oxidative damage caused by ROS, plants have evolved an antioxidant defense system for scavenging and detoxifying ROS. The system can be categorized as a combination of an enzymatic system, which includes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and a nonenzymatic system comprising ascorbate, glutathione, tocopherol, polyphenol and anthocyanin [3]. A time course measure of SOD APX and GR activities under a mild water stress imposed by a PEG treatment (-0.7 MPa) on maize detached leaves also showed a significant increase in these enzyme activities [4]. Polyethylene glycol (PEG) compounds have been used to simulate water stress effect in plants [5]. PEG has higher molecular weight considered to cause blockage of the pathway of water movement, reducing water absorption and causing desiccation of the plant [6]. It is envisaged from the findings PEG solution can be frequently used in the laboratory for screening drought tolerant genotypes at early stage. Therefore, the present study aimed to determine the effect of PEG-induced osmotic stress on fresh weight, MDA, H₂O₂, total phenolics and enzymatic antioxidant systems in maize seedlings, to evaluate the oxidative stress and response of the protective system to drought stress.

MATERIALS AND METHODS

Plant material and experimental conditions. The experiment was conducted in factorial form, using a completely randomized design with four replications. Two inbred lines (MO₁₇ and B₇₃) were evaluated under laboratory

conditions. Seeds of maize were surface sterilized with 0.01% HgCl_2 solution for three minutes, followed by washing several times by distilled water. Ten seeds of each inbred lines then were placed on the moist Whatman germination papers in Petri dishes and were germinated using distilled water for 3 days under control conditions (light/dark regime of 16/8 h at 25/20 °C, relative humidity of 60-70%, Light intensity during the daytime was $350 \mu\text{mol m}^{-2} \text{s}^{-1}$). After 3 days, osmotic stress was imposed by application of PEG-6000 (polyethylene glycol) for 5 days. Using the Michel Kaufmann equation, 171 g of PEG-6000 was dissolved in 200 ml of distilled water and total volume was raised up to one liter to produce solutions of -0.6 (MPa) osmotic potential [7].

Hydrogen peroxide (H_2O_2). Hydrogen peroxide levels were determined using previously described method by Velikova et al. [8]. Briefly, leaf tissues (500 mg) were homogenized in ice bath with 5 ml 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000g for 15 min and 0.5 ml of the supernatant was added to 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M KI. The absorbance of supernatant was read at 390 nm.

Malondialdehyde (MDA). The level of lipid peroxidation in leaf samples was determined in terms of thiobarbituric acid-reactive substances (TBARS) concentration as described by Carmak and Horst [9] was used. Fresh leaf material (500 mg) was homogenized in 3 ml 1.0% (w/v) TCA solution. The homogenate was centrifuged at 15,000g for 20 min and 0.5 ml of the supernatant was added to 1 ml 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for 30 min, and the reaction stopped by placing the reaction tubes in an ice bath. Then the samples were centrifuged at 10,000g for 10 min, and the absorbance was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated from the extinction coefficient $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Total phenolics. Fresh leaf tissue (50 mg) was homogenized with 80% acetone and centrifuged at 10,000g for 10 min. One-hundred microlitres of the supernatant were diluted with 2 mL of water and 1 mL of Folin-Ciocalteu's phenol reagent and shaken vigorously. Then 5 mL of 20% sodium carbonate solution was added and the volume was made up to 10 mL with distilled water. The contents were mixed thoroughly and the absorbance was recorded at 750nm [10]. The results were expressed as mg/g of fresh leaf.

Native polyacrylamide gel electrophoresis. The activities of SOD, POX and CAT were determined in native PAGE (Polyacrylamide gel electrophoresis). The crude extract of fresh and healthy shoots were prepared with separate mortars and pestles in a Tris-HCl extraction buffer pH 7.5 (Tris 50 mM, sucrose 5%, ascorbic acid 50 mM, sodium metabisulfite 20 mM, PEG 2% and 2-mercaptoethanol 0.1%) before use with a ratio of 0.1 gr μl^{-1} (W: V) and centrifuged (Model EBA 12R) at 4 °C and 10 000 rpm for 10 minutes [11]. Enzyme extracts were immediately absorbed onto 3×5 mm wicks cut from Whatman 3 mm filter paper and loaded onto 7.5% horizontal slab polyacrylamide gels ($0.6 \times 15 \times 12$ cm), prepared by Poulik buffer [12] using TBE (Tris-Borate-EDTA) electrode buffer (pH 8.8). Electrophoresis was carried out at 4 °C for 3 hours (constant current of 30 mA, and voltage of about 180 V). For each genotype, analysis was repeated three times, each time from bulked material of at least five seedlings. After electrophoresis, two slices of slab gel were prepared. The staining protocol for SOD was performed according to Soltis and Soltis [12], POX and CAT according to Olson and Varner [13]. The gels were fixed and scanned immediately after staining. An image analysis program (MCID Analysis Evaluation 0.7) was used to measure $D \times A$ (optical density \times area) parameter for each isozyme band to evaluate the enzymatic activity. The protein content of the enzyme extracts were determined by Bradford [14] method using bovine serum albumin (BSA) as a standard.

Statistical analysis. Data was analyzed by using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). The assumptions of variance analysis were tested by ensuring that the residuals were random and homogenous, with a normal distribution. For treatment showing a main effect, means (\pm SE) compared by Duncan test. $P \leq 0.05$ was considered as significant differences between treatments.

RESULTS AND DISCUSSION

The fresh weight of maize was drastically impaired under drought stress in both maize inbred lines. However, this impairment was lower in B₇₃ as compared to MO₁₇, while maximum fresh weight was recorded in plants raised under well-watered conditions (Table 1).

TABLE 1.
Mean (\pm SE) data in fresh weight, H₂O₂, MDA, total phenolics and total soluble proteins of two maize inbred lines in control and drought conditions.

| Inbred | Fresh weight (g) | | H ₂ O ₂ (μ mol/g fr. wt.) | | MDA (nmol/g fr. wt.) | | Total phenolics (mg/g fr. wt.) | | Total soluble proteins (μ g/plant fresh wt.) | |
|--------|------------------|---------------|--|---------------|----------------------|----------------|--------------------------------|---------------|---|---------------|
| | Control | Drought | Control | Drought | Control | Drought | Control | Drought | Control | Drought |
| MO17 | 6.8 \pm 0.2 | 3.6 \pm 0.4 | 7.9 \pm 0.3 | 8.1 \pm 0.1 | 17.6 \pm 0.7 | 18.0 \pm 0.6 | 7.7 \pm 0.3 | 8.4 \pm 0.2 | 1.7 \pm 0.4 | 1.5 \pm 0.1 |
| B73 | 4.4 \pm 0.1 | 2.1 \pm 0.1 | 8.6 \pm 0.2 | 9.1 \pm 0.5 | 18.2 \pm 0.5 | 20.1 \pm 0.5 | 7.0 \pm 0.2 | 7.5 \pm 0.1 | 1.9 \pm 0.1 | 2.6 \pm 0.2 |

The difference among the inbred lines with respect to fresh weight was the drought stress, percent fresh weight inhibition at this level was used to rank the maize inbred lines for drought tolerance. Therefore, MO₁₇ was ranked as tolerant (47% inhibition) and B₇₃ as drought sensitive in response to drought stress (more than 50% inhibition) in fresh weight. From the results of the present study, it is obvious increasing supply of drought caused a marked reduction in the growth of two maize inbred lines. Drought-induced inhibition in growth may have disturbed various physiological and biochemical processes at the cellular, tissue or at the whole plant level. The decline in plant growth due to drought stress is associated with a number of physiological and biochemical processes governing plant growth. Thus, the genetic variation for drought tolerance in the maize inbred lines observed in the present study might have been due to variation in photosynthesis, nutrient imbalance, accumulation of compatible solutes, enzyme activities, etc., which in turn can affect crop growth [15].

Tendency in alters of H₂O₂ were similar in both inbred lines. H₂O₂ increased with increasing lengths of water stress, but H₂O₂ content was lower in the MO₁₇ than in B₇₃ inbred (Table 1). H₂O₂ content in leave tissue of sensitive genotype of maize, as compared to tolerant ones under drought stress induced by polyethylene glycol reported [16]. Higher H₂O₂ content in the leaves of drought sensitive maize seedlings under drought stress as compared to drought tolerant maize seedlings has been observed [17].

MDA level increased with water stress (Table 1). Compared to MO₁₇ seedlings, B₇₃ seedlings had a higher MDA content during water deficit stress. The content of MDA is often used as an indicator for lipid peroxidation in plant tissues, resulting from an oxidative stress induced by different abiotic stresses. Recently, an increase in MDA content under drought stress has been reported in leaves of drought sensitive genotype of maize at seedling stage, whereas no change is observed in tolerant genotypes [18].

Total phenolics increased in the leaves of MO₁₇ and B₇₃ under drought stress (Table 1). Of

various secondary metabolites, phenolics are more important to abiotic stress tolerance than the others [19]. For example, enhanced synthesis of soluble phenolics directly correlated with salt and heat tolerance of sugarcane [20]. Total soluble proteins increased in the leaves of B₇₃, whereas decreased in MO₁₇ under drought stress (Table 1).

The specific SOD activity was increased in the shoots of maize seedlings under stress conditions. Increase in enzyme activity coincided with a variable increment in the individual isoform expression. Three isoforms of SOD were detected in the leaves of two maize inbred lines (Fig. 1).

SOD isoforms activities were significantly increased in both inbred lines under stress conditions. At the water deficit stress, MO₁₇ was the highest and B₇₃ the lowest in three SOD isoforms activities of maize (Fig.4). SOD is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress. It is well established various environmental stresses often lead to the increased generation of ROS, where, SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS [21]. SODs are classified by their metal cofactors into three known types: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD), which is localized in different cellular compartments [22]. Pan et al. [23] studied the effect of salt and drought stress on *Glycyrrhizauralensis* and found significantly increased SOD activity but an additional Mn-SOD isoenzyme was detected under only salt stress. Increase in SOD isoforms activity following drought stress was noted in wheat [24]. Wang and Li [25] studied the effect water stress on the activities of total leaf SOD and chloroplast SOD in *Trifolium repens* and reported significant higher increase in SOD activity under water stress. Maintaining the SOD status under drought stress might protect plants from oxidative injury and possibly would not favour accumulation of O₂⁻. A higher level of SOD in drought tolerant maize inbreds, as compared to drought intolerant ones has been reported previously [26].

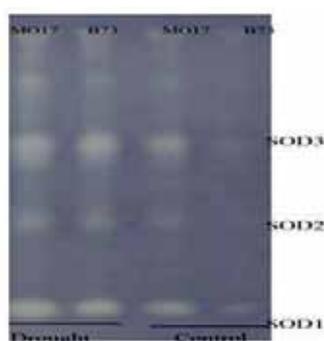


FIGURE 1
Example of isoform pattern and relative activity of SOD in the leaves of two maize seedlings for control and drought stress conditions.

Two isoforms were detected in the leaves of maize seedlings with POX₁ and POX₂ being the major ones (Fig. 2).

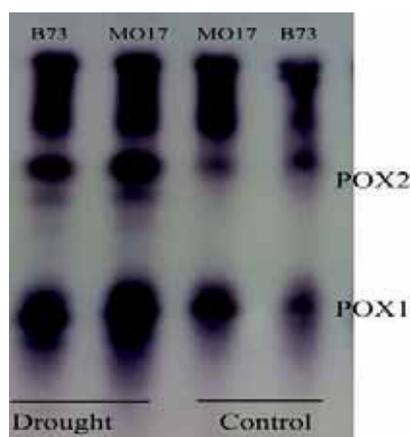


FIGURE 2
Example of isoform pattern and relative activity of POX in the leaves of two maize seedlings for control and drought stress conditions.

The highest enzymatic activity increment was belonged to MO₁₇ under drought stress (Fig. 4).

POX and CAT are two important H₂O₂ scavenging antioxidant enzymes functioning in different sub cellular compartments [27]. Increase in POX activity in leaves of drought tolerant and susceptible wheat cultivars has also been reported earlier under water deficit stress. Sairam and Saxena[28] reported that tolerant genotypes, with highest POX activity, had the lowest lipid peroxidation and highest membrane stability under water stress at different stages, while the

susceptible genotypes exhibited the lowest antioxidant enzyme activity, membrane stability and the highest lipid peroxidation. An increase in POX activity in drought tolerant as well as susceptible maize genotypes at seedling stage under 72 h drought stress has been reported by Chugh et al. [18]. In a review applied at seedling stage of wheat cultivars, antioxidant enzymes activities were increased with the decrease of osmotic potential in both tolerant and sensitive cultivars. The tolerant cultivar exhibited a higher antioxidant activity compared to the sensitive one [29].

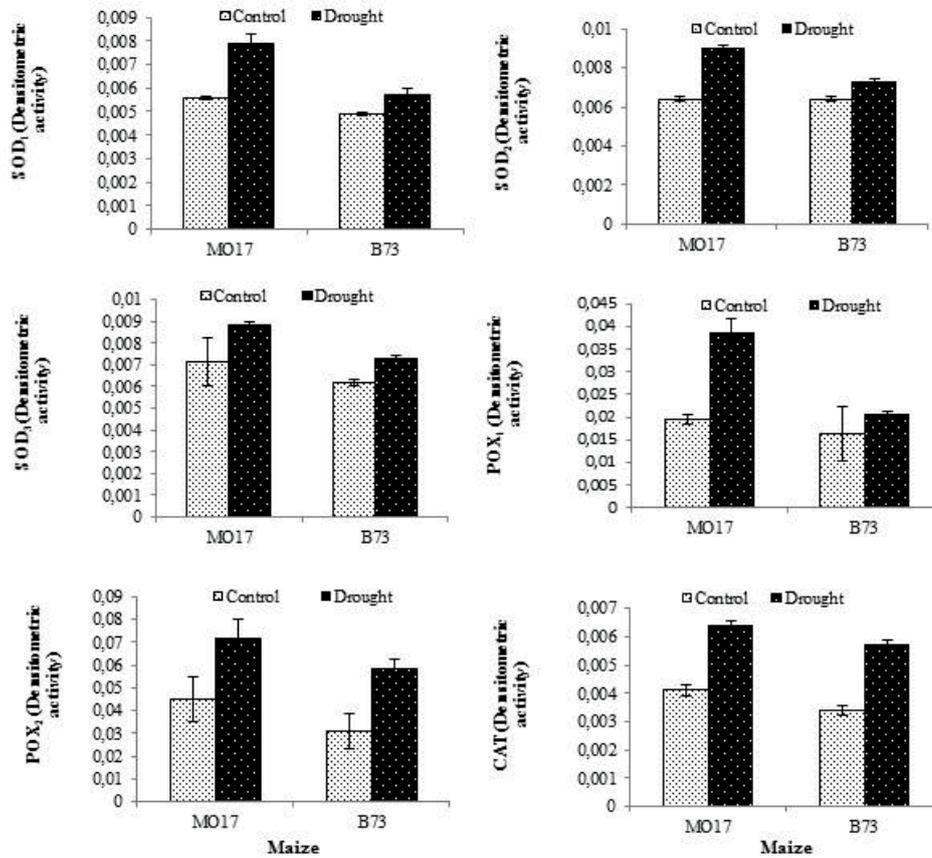


FIGURE 4

SOD1, SOD2, SOD3, POX1, POX2 and CAT (\pm S.E) of two maize seedlings under control and drought conditions.

A single band of CAT was observed in the leaves of maize seedlings upon native PAGE separation. In severe stress condition, CAT showed

higher activity in comparison to drought stress and control condition (Fig.3).

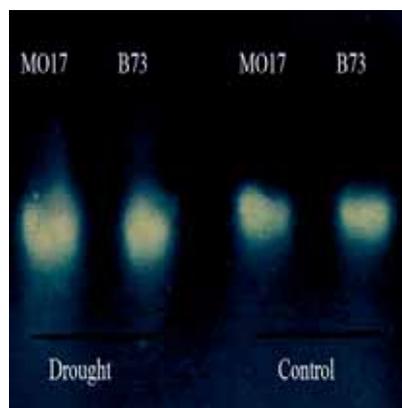


FIGURE 3

Example of isoform pattern and relative activity of CAT in the leaves of two maize seedlings for control and drought stress conditions.

The highest increment CAT activity was observed in MO17 under drought stress (Fig.4). Reports on CAT activity under stress condition are heterogeneous. CAT activity has been shown to increase in maize [30], wheat [30] and also to remain unchanged or even decrease under water stress in sunflower [31]. Luna et al. [30] reported leaf H₂O₂ content increased even though total CAT activity doubled in wheat seedling under severe stress conditions. Our results are consistent with works reporting the increased CAT activity in response to osmotic stress in maize seedling.

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SINGLE LAND USE CHANGES IMPACT ON HYDROLOGICAL RESPONSE BASED ON SWAT MODEL AND GREY RELATIONAL ANALYSIS

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ABSTRACT

The Soil and Water Assessment Tool (SWAT, 2009) model and Grey Relational Analysis were used to research hydrological response affected by land use changes for long periods in Qingzhang River Basin. The results showed a general agreement between simulated and observed monthly discharge rates throughout the calibration period. Relative error was 1.92%, Coefficient of determination and Nash–Sutcliffe efficiency were 0.90 and 0.88 respectively. For validation period, relative error, Coefficient of determination and Nash–Sutcliffe efficiency were -4.79%, 0.79 and 0.78, respectively. Grey relational grades between annual average water yields and transformation areas from FRSD to FRS2, PAST, AGRR and URBN were 0.41, 0.48, 0.35 and 0.87, respectively which indicated grey relational grade could effectively reflect the influence of one-single land use transformation on discharge. Discharges were increased in different degrees with four scenarios, with the largest increase occurred when transforming into URBN. A slightly increase occurred when transforming into FRS2 and PAST. Increase was the most modest when FRSD transforming into AGRR. Slightly increase occurred when the land use transformation from AGRR to PAST and FRS2, while significantly increase showed when other land use types transforming to URBN.

KEYWORDS:

SWAT model; Grey Relational Analysis; hydrological responses; land use; Qingzhang River Basin

INTRODUCTION

Land use and land cover changes (LUCC) plays a significant role in influencing the hydrological responses at the land surface via its effect on transpiration, interception, and evaporation from soil and crop[1-3]. Population

growth, urbanization and the policy of Reform and Openness have a strong influence on basin hydrological processes and water balance. These changes lead to ecological problems such as land degradations [4, 5]. Several previous studies have focused on hydrological response to land use in a variety of watersheds[6, 7]. Feng Zhou et al. demonstrated that the expansion of urban areas had a slight influence on the simulated annual streamflow and evapotranspiration in the Yangtze River Delta [8]. Li Jia et al. proposed to increase the area of forestland and grassland for preventing surface runoff from decreasing [9]. However, it is less rigorous to research the influence of one single land use on discharge. Many research have demonstrated that the transformation from one land use type into other types can produce different results [10, 11]. Quantitatively evaluation of single land use changes impact on hydrological response is still a controversial issue.

Soil and water assessment tools (SWAT) is a large scale distributed hydrologic model, was mainly used to simulate the influence of land management practices on hydrological responses in large rural watersheds [12]. Over the past decades, SWAT has been used successfully to simulate the response of hydrological responses to environmental or LUCC, at a variety of scales, which mainly because of the advantages as wide adaptability, good application and high simulation efficiency [13-16]. Based on calibrated SWAT model, we can get results of discharge affected by slight land use changes. Cooperate with mathematical methods, it will be an appropriate approach in researching hydrological response to land use changes. Grey theory is a decision-making approach under conditions of uncertainty, and has been found to be superior to comparable methods in the mathematical analysis of systems with incomplete information [17]. Although we find several examples of grey relational models applications in the research literature, it still has an uncertain applicability in quantifying land use changes impact on hydrological response[18, 19].

The objectives of this research were to (1) calibrate the model in Qingzhang River Basin for researching the applicability of the model in complex semiarid region, (2) modify the traditional grey relational analysis model to research single land use changes impact on hydrological response, and (3) calculate grey relational grade between annual average water yields and land use transformation areas.

MATERIALS AND METHODS

Study area. The study area is located in east of Shanxi province, China, with an area of 3800 km² (Fig.1). This watershed belongs to semiarid continental monsoon climate area of temperate zone, and has 7.4 °C mean annual temperature. The average annual precipitation is 554 mm, with more than 70% falling in the period from June to September. Annual frostless seasons range from 110d to 160d. Altitude ranges from 605 m to 2147 m, mainly of mountain area. The flow in the river is the highest during flood seasons and very low or zero during dry seasons. The whole basin just has one outlet, Liujiashuang hydrologic gauge (36°45'N, 113°31'E) which has integrated monitoring data.

Swat model description. Soil and Water Assessment Tool (SWAT) is a river basin scale model developed to predict the impact of land management practices on water, sediment and agricultural chemical yields in large complex watersheds over long periods of time [20-24]. Based on three digital maps, digital elevation model (DEM), land use map and soil map, SWAT model subdivides watershed into sub-basins and multiple hydrological response units (HRUs) consisting of areas with homogeneous soils, land use and slope [25-28]. Water balance is the basic driving force of SWAT model and the basic equation is as follows (all units in mm):

$$sw_t = sw_0 + \sum_{i=1}^t (R_d - Q_{surf} - E_a - W_s - Q_w) \quad (1)$$

where sw_t is the soil water content after t days, sw_0 is the initial soil water content, R_d , Q_{surf} , E_a , W_s , Q_w are precipitation, surface runoff, evapotranspiration, water entering vadose zone, and return flow respectively on day i .

As precipitation descends, water will be intercepted and held in the vegetation canopy or fall to the soil surface, and then redistributed through water management on the ground. Flow generation from each hydrological response unit (HRU) in a sub-basin is summed and the resulting loads are routed through channels to the watershed outlet [22]. This increases accuracy and gives a much better physical description of the water balance.

Model set-up. In this study, we applied ArcSWAT version 2009 in the ArcGIS (version 9.3) environment. The Digital elevation map (DEM, Fig.1) with a resolution of 30 m × 30 m was obtained from International Scientific & Technical Data Mirror Site, Computer Network Information Center, Chinese Academy of Sciences. (<http://datamirror.csdb.cn>)

Soil data with key physical and chemical properties such as depth of horizon, percentage of sand, silt, clay, organic carbon, bulk density, saturated hydraulic conductivity and water content at different tension values for each horizon were obtained from Harmonized World Soil Database (HWSD) made by Food and Agriculture Organization of the United Nations (FAO) and International Institute for Applied Systems Analysis (IIASA) at a scale of 1:1000000. Soil unit symbol used the FAO-90 classification system and was classified into 8 types (Fig.3). In which, 46.89% of the soil classified as Calcaric Cambisols (Table 1).

The land use map (1:250000) was obtained from Data Sharing Infrastructure of Earth System Science (<http://www.geodata.cn>) (Fig.2). As the major land-use type, grassland (PAST) accounted for 47.07% of the total land area, followed by shrubwood (FRS2) and agricultural land (AGRR), with the area percentage of 18.74% and 17.78% respectively. Land-use classification circumstance was shown in Table 1.

Meteorological data, daily values of precipitation, maximum and minimum temperature, solar radiation, relative humidity and wind speed, which obtained from four local gauging stations, were input into SWAT model. To verify the model suitability, simulated discharge results should be calibrated by monitoring data. Because of the missing flow data in 2005, the period March–December 1999 served as the warm up period for the model, calibration period was performed for the years 2000 to 2004, and validation period was 2006 to 2010.

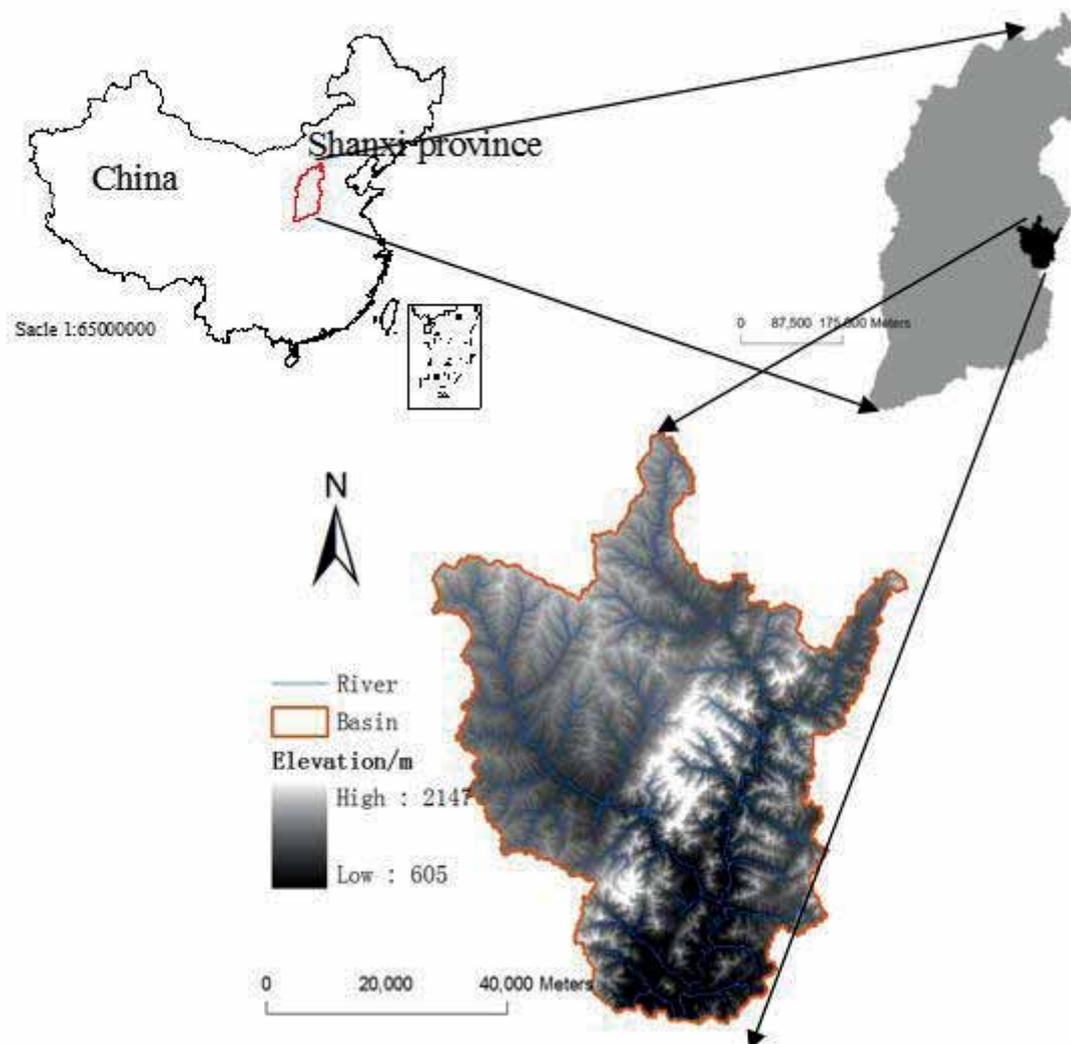


FIGURE 1
Location of the study area.

TABLE 1
Land use classes and Soil type classes in study area.

| Land use | | | Soil | | |
|----------|--------------|--------------|------|--------------------|--------------|
| Code | Full name | Percentage/% | Code | Full name | Percentage/% |
| FRSD | forestland | 12.56 | CF | Calcaric Fluvisols | 6.17 |
| FRS2 | Shrubwood | 18.74 | EC | Eutric Cambisols | 2.38 |
| PAST | grassland | 47.07 | CC | Calcaric Cambisols | 46.89 |
| AGRR | agricultural | 17.78 | EL | Eutric Leptosols | 3.74 |
| URBN | residential | 3.4 | RL | Rendzic Leptosols | 12.67 |
| WATR | Water | 0.45 | CL | Calcic Luvisols | 6.89 |
| | | | ER | Eutric Regosols | 1.42 |
| | | | CR | Calcari Cregosols | 19.86 |

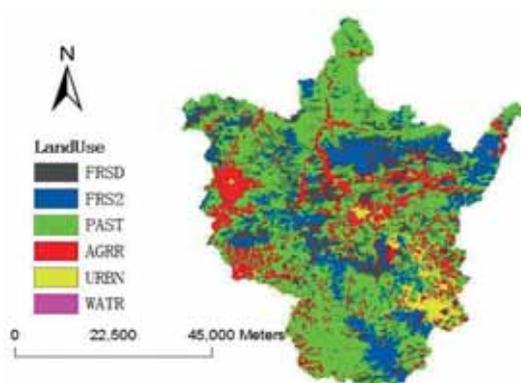


FIGURE 2
Major land uses in study area

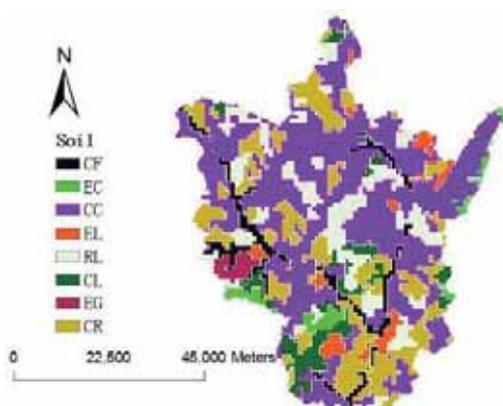


FIGURE 3
Major soil types in study area

Land use changes assessment. Hydrological process cannot be response only for one-single land use. Discharge would decrease with the transformation from one land use type to another playing a relatively more important role in water conservation, or present inverse variation in another

case. In order to research hydrological responses to land use changes, in this study, FRSD, FRS2, PAST, AGRR and URBN were recorded as L1, L2, L3, L4 and L5, respectively. The matrix was established as follows.

TABLE 2
The matrix of transformation area

| Land use | L1 | L2 | L3 | L4 | L5 |
|----------|-----|-----|-----|-----|-----|
| L1 | — | Δ21 | Δ31 | Δ41 | Δ51 |
| L2 | Δ12 | — | Δ32 | Δ42 | Δ52 |
| L3 | Δ13 | Δ23 | — | Δ43 | Δ53 |
| L4 | Δ14 | Δ24 | Δ34 | — | Δ54 |
| L5 | Δ15 | Δ25 | Δ35 | Δ45 | — |

Where Δ_{ij} represents the transformation area from L_i to L_j ($i, j=1,2,3,4,5$). The final area after

transformed will be calculated as follows:

$$L'x = Lx - \sum_{j=1}^5 \Delta x_j + \sum_{i=1}^5 \Delta i_x \quad (x = 1,2,3,4,5) \quad (2)$$

Based on multiple simulated results with calibrated SWAT model, Grey Relational Analysis is used for quantitative researching the impact of single land use changes on hydrological responses. Grey Relational Analysis is part of Grey System Theory and has the basic idea of estimating the correlation degree of two data curves [17, 18, 29]. The grey relational coefficient is calculated based on normalized data for representing the correlation between two sets of data. The mean of grey relational coefficient is taken for calculating grey relational grade which represents the overall performance of the process. In this method, ranking of grey relational grade is selected for optimization rather than real values. The higher grade is taken as optimal correlation.

RESULTS

Model Calibration and Validation. As it is not feasible to include all parameters being calibrated in SWAT model, sensitivity analyses were performed during calibration phase with LH-OAT method proposed by Morris [30]. The most sensitive 11 parameters for discharge values were considered as critical calibration. Final results will

be accepted when simulated data act well with actual data. Relative error, coefficient of determination and Nash–Sutcliffe efficiency were selected to quantify the achieved levels of calibration and to evaluate the overall performance of the model [22]. Table 3 has a list of the most sensitive parameters and their final values after calibration.

Runoff simulation results, Fig. 4 a presented the comparison of the simulated and measured monthly discharges of Liujiazhuang gauge over calibration (2000~2004) period. Visual inspection of Fig. 5a showed a general agreement between simulated and observed monthly discharge rates throughout the calibration period. Relative error was 1.92%, Coefficient of determination and Nash–Sutcliffe efficiency were 0.90 and 0.88 respectively. Fig. 4 b compares the simulated flow rates to the observed rates of Liujiazhuang gauge for the time period 2006 through 2010, the validation period. The model simulations correlate reasonably well with the observed flow rates. Relative error, Coefficient of determination and Nash–Sutcliffe efficiency were -4.79%, 0.79 and 0.78, respectively, which indicated that the model performed very well in this study area. Based on these statistics, the model performance can be used for simulating the runoff process under different land uses. The measured and simulated annual average water yields were 73.34 million m³ and 71.05 million m³.

TABLE 3
Basic parameter settings and variation ranges

| Parameter | Definition | Min. value | Max. value | Calibrated value | File |
|-----------|--|------------|------------|------------------|------|
| CN2 | SCS curve number | 35 | 98 | 55~86 | .mgt |
| SOL_AWC | Available water capacity of the soil layer | 0 | 1 | 0.147~0.175 | .sol |
| SOL_K | Saturated hydraulic conductivity | 0 | 1000 | 8.89~19.23 | .sol |
| ESCO | Soil evaporation compensation factor | 0 | 1 | 0.75 | .bsn |
| EPCO | Plant water uptake compensation factor | 0 | 1 | 0.97 | .bsn |
| GW_DELAY | Groundwater delay | 0 | 500 | 50 | .gw |
| GW_REVAP | Groundwater revap coefficient | 0.02 | 0.2 | 0.07 | .gw |
| RCHRG_DP | Deep aquifer percolation factor | 0 | 1 | 0.21 | .gw |
| ALPHA_BF | Baseflow alpha factor | 0 | 1 | 0.5 | .gw |
| CH_N2 | Manning's "n" value for main channel | 0.01 | 0.5 | 0.04 | .rte |
| SURLAG | Surface runoff lag time | 0 | 10 | 1.5 | .bsn |

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DISCUSSION

Grey Relational Analysis deficiency. Grey relational analysis provides a useful tool to deal with the problems of limited and superficial ruleless data processing [29]. At present, several kinds of grey relational models, such as Deng’s correlation degree, T’s correlation degree, Absolute correlation degree and Slope correlation degree, have been put forward [31, 32]. S.Wang et al. analyzed the

influence of operational parameters on the combustion performance with Deng’s correlation degree [29]. Sun Yugang analyzed the relationship of investment and innovative technology production in Jiangsu province with improved T’s correlation degree and Slope correlation degree [33]. However, these models are not suitable for researching the impact of land use changes on hydrological process due to two drawbacks.

Firstly, these models focus more on the similarity level of two data curves than the influence between reference sequence and comparability sequence. T’s correlation degree and Slope correlation degree have a higher grey relational grade when comparability sequence has closer slope or increase value with reference sequence [33]. While in actual application, the comparability sequence shows higher correlation degree which has relatively larger volatility with reference sequence.

Secondly, these models cannot calculate negative correlation coefficient. For general models, grey relational grade between two data sequences was supposed to be negative when they showed inverse change direction. However, grey relational grade is confined within the range of 0 and 1 [29, 34].

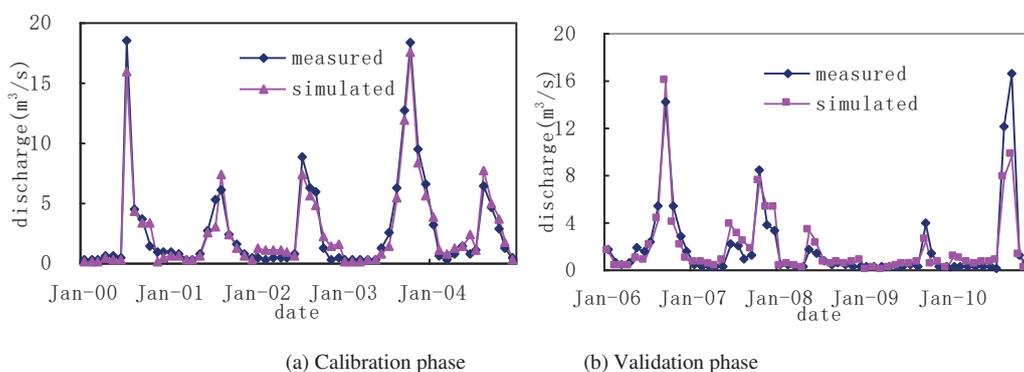


FIGURE 4
Comparison of measured and simulated discharge for the study area during calibration period (2000~2004) and validation period (2006~2010)

Grey Relational Analysis modification. In present research, we made a slightly different from general models which concentrate mainly on the influence between reference sequence and comparability sequence. The reference sequence is

$$X_0(k) = \{x_0(1), x_0(2), \dots, x_0(n)\} \tag{3}$$

And comparability sequence is

$$X_i(k) = \{x_i(1), x_i(2), \dots, x_i(n)\} \tag{4}$$

$(i = 1, 2, 3, \dots, m)$

In grey relation analysis, the data pre-processing is the first stride accomplished to



normalize the random grey data. The original sequence is normalized through:

$$y_i(k) = \frac{x_i(k)}{\frac{1}{n} \sum_{k=1}^n |x_i(k)|} \quad (k = 0, 1, 2, 3, \dots, n) \quad (5)$$

$$\xi(k) = \begin{cases} \operatorname{sgn}(\Delta y_0(k) \cdot \Delta y_i(k)) \frac{|\Delta y_i(k)|}{\sqrt{\Delta y_0(k)^2 + \Delta y_i(k)^2}} & \Delta y_0(k) \cdot \Delta y_i(k) \neq 0 \\ 0 & \Delta y_0(k) \cdot \Delta y_i(k) = 0 \end{cases} \quad (6)$$

$$\Delta y_i = y_i(k) - y_i(k-1) \quad (7)$$

$$\operatorname{sgn}(\Delta y_0(k) \cdot \Delta y_i(k)) = \begin{cases} 1, & \Delta y_0(k) \cdot \Delta y_i(k) > 0 \\ -1, & \Delta y_0(k) \cdot \Delta y_i(k) < 0 \end{cases} \quad (8)$$

$\xi(k)$ is the distinguishing or identification coefficient whose value is confined within the range of -1 and 1.

Once the grey relation coefficients are determined, usually the simple average values of the grey relation coefficients unfurl the grey relational grade. The grey relational grade is expressed by:

$$\gamma(X_0, X_i) = \frac{1}{n} \sum_{k=1}^n \xi(k) \quad (9)$$

Function $\operatorname{sgn}(\Delta y_0(k) \cdot \Delta y_i(k))$ was introduced to reflect positive or negative correlation. The grey relational coefficient is greater than zero when $\Delta y_0(k) \cdot \Delta y_i(k) > 0$, which indicates sequence X_0 and X_i change in the same direction from $k-1$ to k . While it will be less than zero when $\Delta y_0(k) \cdot \Delta y_i(k) < 0$, which indicates X_0 and X_i change in the opposite direction from $k-1$ to k . γ is confined within the range of -1 and 1. X_0 and X_i show negative correlation when $-1 \leq \gamma < 0$. The higher $|\gamma|$ values, the more significantly X_i decreases with the increase of X_0 and the higher degree of negative correlation. X_0 and X_i show positive correlation when $0 < \gamma \leq 1$, and have no correlation when γ is equal to zero.

Where $x_i(k)$ denotes the original sequence, $y_i(k)$ is known as comparability sequence which is had after pre-processing. The grey relational coefficient is expressed by:

Comparison of FRSD transformation results and grey relational grades. Previous studies have researched hydrological responses to land use with many methods. Liu Lanlan analyzed the land use changes impact on runoff and rainfall infiltration in Shanghai city with calibrated SCS model [35]. Wang [29] and Bai *et al* [36] calculated the runoff contribution coefficient of each land use with SWAT model and HSPF model respectively, which didn't consider the changes between different types.

We took the transformation from FRSD to other types of land uses for instance to validate the effectiveness of equations for grey relational grade. The area of FRSD was 477 km² according to proportion of land uses distribution. Based on calibrated SWAT model, discharge processes were obtained under different land uses and used for calculating annual average water yields. The relationships between annual average water yields and transformation areas are shown in Fig.5.

The simulation result showed that discharges were increased in different degrees with four scenarios, with the largest increase occurred when transforming into URBN. Dazhi *et al*[2] and Bai *et al*[36] indicated that FRSD can reduce surface runoff because of water and soil conservation. In view of this, FRSD in Qingzhang River basin played an effective role in water conservation. Meanwhile, transformation from FRSD to URBN had a significantly influence on the hydrological environment. A slightly increase occurred when transforming into FRS2 and PAST because of

smaller evaporation in FRS2 and PAST compared with FRSD. Besides, the mainly reason for this phenomenon was due to the low cover degree of FRS2 and PAST in Shanxi province which mostly located in sloping fields on hilly regions. It was difficult to have better effect to retain slope runoff. Increase in transforming into AGRR was the most

modest. Grey relational grades between annual average water yields and transformation areas from FRSD to FRS2, PAST, AGRR and URBN were 0.41, 0.48, 0.35 and 0.87, respectively. Thus it can be seen that, grey relational grade could effectively reflect the influence of one-single land use transformation on discharge.

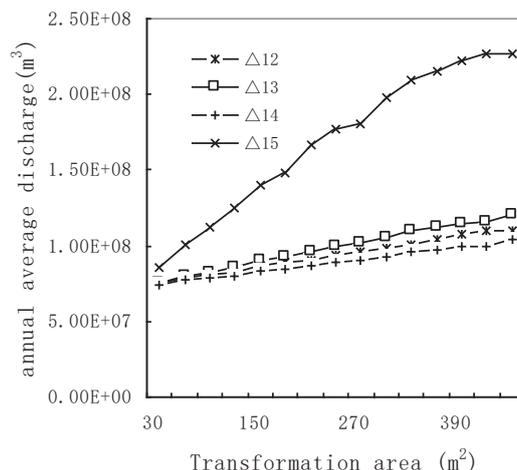


FIGURE 5
The relationships between annual average water yields and transformation areas

Grey relational analysis results. Table 4 presented grey relational grade between annual average water yields and transformation areas. Slightly increase occurred when the land use transformation from AGRR to PAST and FRS2. Many previous studies showed PAST and FRS2 had an effect on water conservation as well, and suggest to return grain plots to grassland for conserving water [2, 37]. However, in Qingzhang River basin, in addition to poor water conversation of PAST, quantity of irrigation water in AGRR was also a primary factor. Traditional constant flooding was the main irrigation measure which was inefficiency in water using and lacking of water saving measures. Although part of irrigation water will return to river channel finally, agricultural activities, such as plough the earth and reclaim virgin land, facilitated water infiltration process to a large extent and restrained the increase of conversion from precipitation to runoff. In view of this, irrigation water on AGRR in Qingzhang River basin had a serious effect on surface runoff.

Since FRSD and AGRR have the effect of reducing surface runoff compared with PAST and FRS2 based on different reasons, for preserving the ecological environment in study area, we do not suggest returning grain plots to grassland, and furthermore, we suggest returning grain plots to forestland with taking water-saving irrigation measures on AGRR.

Significantly increase showed when other land use types transforming to URBN which indicated that URBN had a great contribution to the discharge increase. Feng Zhou et al.[8] and Luo et al.[37] also showed that the similar change characteristics especially in flood season. Construction of buildings, parking lots and paved roads increased the impervious cover in a watershed and reduced infiltration. With development, the hydraulic efficiency of flow was increased through artificial channels, curbing and storm drainage and collection systems. The net effect of these changes was an increase in the volume and velocity of runoff.

TABLE 4
Grey relational analysis results of annual average water yields and transformation areas

| Land use | L1 | L2 | L3 | L4 | L5 |
|----------|------|-------|-------|-------|-------|
| L1 | — | -0.39 | -0.51 | -0.37 | -0.85 |
| L2 | 0.41 | — | -0.10 | 0.08 | -0.78 |
| L3 | 0.48 | 0.09 | — | 0.20 | -0.77 |
| L4 | 0.35 | -0.08 | -0.17 | — | -0.84 |
| L5 | 0.87 | 0.80 | 0.78 | 0.82 | — |

CONCLUSIONS

SWAT model was simulated in Qingzhang River Basin which achieved good simulations of flow for long hydrological periods. The results showed a general agreement between simulated and observed monthly discharge rates throughout the calibration period. Relative error was 1.92%, coefficient of determination and Nash–Sutcliffe efficiency were 0.90 and 0.88 respectively. For validation period, the model simulations correlate reasonably well with the observed flow rates. Relative error, Coefficient of determination and Nash–Sutcliffe efficiency were -4.79%, 0.79 and 0.78, respectively, which indicated that the model performed very well in this study area.

Grey relational grades between annual average water yields and transformation areas from FRSD to FRS2, PAST, AGRR and URBN were 0.41, 0.48, 0.35 and 0.87, respectively which indicated modified grey relational model could effectively reflect the influence of one-single land use transformation on discharge.

Discharges were increased in different degrees with four scenarios, with the largest increase occurred when transforming into URBN which indicated that FRSD in study area played an effective role in water conservation. A slightly increase occurred when transforming into FRS2 and PAST, and increase was the most modest when FRSD transforming into AGRR. Slightly increase occurred when the land use transformation from AGRR to PAST and FRS2 which showed different results with many previous studies mainly due to poor water conservation of PAST and quantity of irrigation water in AGRR. Since FRSD and AGRR have the effect of reducing surface runoff compared with PAST and FRS2 based on different reasons, for preserving the ecological environment in study area, we do not suggest returning grain plots to grassland, and furthermore, we suggest returning grain plots to forestland with taking water-saving irrigation measures on AGRR. Significantly increase showed when other land use types

transforming to URBN which indicated that URBN had a great contribution to the discharge increase.

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MONITORING SPATIOTEMPORAL DYNAMICS OF INDOOR RADON CONCENTRATIONS IN THE BUILT ENVIRONMENT OF A UNIVERSITY CAMPUS

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ABSTRACTS

This study aims to model spatiotemporal variability of indoor radon (^{222}Rn) concentrations measured for one year from May 2012 to May 2013 in the built environment of Abant Izzet Baysal University. There exist a few studies about data-driven modeling of spatiotemporal dynamics of indoor radon and their validation. Mean indoor radon concentration varied spatially between $14 \pm 8.5 \text{ Bq/m}^3$ and $28.5 \pm 17.5 \text{ Bq/m}^3$ and on a monthly basis between $37.3 \pm 21.6 \text{ Bq/m}^3$ in September and $13.1 \pm 7.7 \text{ Bq/m}^3$ in April, and on a seasonal basis between $23.4 \pm 18.4 \text{ Bq/m}^3$ for the summer period of June to September and $13.3 \pm 7.9 \text{ Bq/m}^3$ for the spring period of April to May. The best-fit multiple non-linear regression (MNL) model developed in this study elucidated 57.9% (R^2_{adj}) of the spatiotemporal variability, with a cross-validation-derived predictive power of 57.1% (R^2_{cv}). The two-way interactions among the temporal predictors of hour and month, air temperature, relative humidity, and location were most influential in predicting indoor radon levels. Parsimonious versus data-hungry empirical non-black-box models appear to be of great practical importance to the quantification, monitoring, and mapping of short- and long-term local, regional, or global spatiotemporal dynamics of indoor and outdoor radon concentrations.

KEYWORDS:

indoor radon, indoor air quality, air quality monitoring, empirical modeling, spatiotemporal dynamics

INTRODUCTION

Radon (^{222}Rn) and its decay products (polonium-218, lead-214, bismuth-214, and polonium-214) are the main sources of the indoor environmental radioactivity and the main determinants of indoor environmental quality and health. Radon progeny quickly diffuses from soils and rocks to the air with the worldwide average

annual effective dose of 1.2 mSv for indoor radon exposure [1]. The first indoor radon measurements were performed in 1950s by Hultquist for 300 buildings and four different cities of Sweden [2]. In subsequent measurements, indoor radon level was found to be highly correlated with types of dwellings, and building materials [3-7]. Therefore, a considerable amount of radon can accumulate in buildings, especially, those with poor ventilation [8], and International Agency for Research on Cancer [9] reported inhalation of radon in high concentrations to be carcinogenic. There has been a study about the mutagenetic effects of the exposure to Radon-222 and its progeny in laboratory mice by [10] which showed in mice groups exposed to more than 700 kBq/h/m^3 of Radon-222, the micronuclei frequency was significantly higher than that observed before exposure.

Faculty members, students, and staff in university buildings are exposed to both short-term and long-term indoor pollutants. However, there exist a few studies that focus on indoor radon concentrations in university buildings, modeling of their spatiotemporal controls in a parsimonious way, and model validation. For example, Hasan [11] reported exposure of workers in Hebron University to indoor radon concentrations. Atik et al. [12] for the first time applied artificial neural networks to account for variation in indoor radon concentration in university buildings as a function of air temperature, location, floor level, and aspect. The objective of the present study was to model spatiotemporal dynamics of indoor radon concentration in the five buildings of Abant Izzet Baysal University (AIBU) based on one-year data of air temperature, relative humidity, air pressure, and their interaction terms.

MATERIALS AND METHODS

Sampling. Radon measurements were performed in cafeterias of five Faculty buildings of AIBU about 900 m above sea level in Bolu (Turkey) between May of 2012 and 2013. The

Faculty buildings randomly selected at the campus area for radon measurements were (1) the Faculty of Arts and Science (FAS), (2) the Faculty of Economics and Administrative Sciences (FEAS), (3) the Faculty of Education (FE), (4) the School of Physical Education and Sports (SPES), and (5) the School of Foreign Languages (SFL). Cafeterias of

the buildings were chosen as the most representative places to carry out radon measurements because undergraduate students spend most of their spare times there and they all are located in the basement except for SPES whose cafeteria is on its second floor (Fig. 1).



FIGURE 1

Five Faculty buildings randomly selected in the campus area of Abant İzzet Baysal University (Bolu, Turkey): FAS: the Faculty of Arts and Science; FEAS: the Faculty of Economics and Administrative Sciences; FE: the Faculty of Education; SPES: the School of Physical Education and Sports; and SFL: the School of Foreign Languages.

A calibrated AlphaGUARD P30 ionization detector as a portable radon monitor (Saphymo GmbH, Germany) was used for active measurements at a 10-min time interval during daytime for each month of the study period. In addition, AlphaGUARD registers such ancillary variables as ambient temperature (T_a), relative humidity (RH), atmospheric pressure (P_a), and local time. The sampling strategy employed included monitoring over a year-long period, with each sample representing a 10-minute average. A total of 1996 samples distributed among the five sites randomly selected (1% of the year's time monitored at each location, with average of 66 h when compared to 8760 h per year) were taken so as to represent average exposure conditions during occupancy. Such considerations for the detector as standing position, height from the floor, and distance from ventilation of the measurement area were held constant during the measurements.

Data Analysis. Statistical analyses were performed using Minitab 17.1 (Minitab Inc. State College, PA, USA). Anderson-Darling (AD) statistic was used to capture the optimal statistical distribution of the measured variables, with the smallest AD value being the closest fit to the data. Pearson's correlation matrix was employed to

detect significant linear relationships between indoor radon concentrations and ancillary data. Tukey's multiple comparisons following one-way analysis of variance (ANOVA) were used to capture significant mean differences in T_a , RH, P_a , and radon concentration spatially (among the five locations) and temporally (among the 12 months). Best-fit multiple non-linear regression (MNL) models were built using a stepwise procedure with alpha-to-enter and -to-remove values set to 0.5. The categorical predictor of location was incorporated in the MNL models as a dummy variable which was coded as follows: 1 = FAS (as the reference level); 2 = FEAS; 3 = FE; 4 = SPES; and 5 = SFL. Autocorrelation and multicollinearity in the MNL models were measured using Durbin-Watson statistics and variance inflation factors (VIF), respectively. Goodness-of-fit and cross-validation-derived predictive power of the best-fit MNL models were measured using adjusted coefficient of determination (R^2_{adj}) and R^2_{CV} , respectively.

RESULTS AND DISCUSSIONS

Consistent with the fact that the distribution of indoor radon concentration follows a lognormal

distribution [13], 3-parameter lognormal distribution best fitted our indoor radon data (Table 1). Indoor radon concentration across the five Faculty buildings varied from a minimum of 0 Bq/m³ in FE from June to August, SPES in July and September, and SFL in April, July and October to a

maximum of 85 Bq/m³ in FAS in December (Table 1). Mean indoor radon concentration ranged from 14 ± 8.5 Bq/m³ in SFL to 28.5 ± 17.5 Bq/m³ in FAS (Table 2). Maximum indoor radon concentrations were 60.0 Bq/m³ in FEAS, 54.0 Bq/m³ in FE, 61.0 Bq/m³ in SPES and 52.0 Bq/m³ in SFL.

TABLE 1
Descriptive statistics of radon concentration (²²²Rn) and ancillary measurements (*n* = 1996).

| Variable | ²²² Rn (Bq/m ³) | <i>T</i> _a (°C) | <i>P</i> _a (mbar) | RH (%) |
|--------------|---|--------------------------------------|---------------------------------|------------------------|
| Mean | 19.9 | 22.9 | 918.2 | 34.9 |
| SD | 13.5 | 2.1 | 4.6 | 11.6 |
| CV | 67.5 | 9.1 | 0.5 | 33.2 |
| Minimum | 0 | 12.6 | 903.6 | 12.7 |
| Median | 17 | 23.1 | 917.8 | 36.1 |
| Maximum | 85 | 28.4 | 928.9 | 60.5 |
| Mode | 9.0 | 25.1 | 914.4 | 43.0 |
| | (<i>n</i> = 177) | (<i>n</i> = 66) | (<i>n</i> = 36) | (<i>n</i> = 40) |
| Skewness | 1.53 | -0.34 | -0.13 | -0.12 |
| Kurtosis | 3.15 | 0.05 | 0.36 | -0.98 |
| Distribution | 3-parameter lognormal (AD ¹ = 2.9) | 3-parameter Weibull (AD = 2.0) | Loglogistic (AD = 11.3) | Weibull (AD = 14.5) |
| Location | 3.11 | | 6.82 | |
| Scale | 0.49 | 12.96 | 0.002 | 38.93 |
| Threshold | -5.52 | 10.88 | | |
| Shape | | 6.63 | | 3.42 |

¹AD: Anderson-Darling statistic

TABLE 2
Tukey's multiple comparisons test for mean indoor radon concentration and ancillary data in response to changes in location (*P* ≤ 0.001).

| Building | <i>n</i> | RH (%) | <i>P</i> _a (mbar) | <i>T</i> _a (°C) | ²²² Rn (Bq/m ³) |
|----------|----------|------------------------|---------------------------------|-------------------------------|---|
| FAS | 508 | 37.1±7.9 ^{ab} | 917.9±3.7 ^b | 24.2±1.2 ^a | 28.5±17.5 ^a |
| FEAS | 379 | 35.5±10.0 ^b | 916.8±5.3 ^c | 22.4±2.5 ^c | 21.2±11.1 ^b |
| FE | 373 | 38.6±8.6 ^a | 920.2±5.4 ^a | 22.1±1.9 ^{cd} | 16.9±9.8 ^c |
| SPES | 372 | 30.2±13.7 ^c | 915.5±2.0 ^d | 23.8±1.7 ^b | 16.0±10.1 ^{cd} |
| SFL | 364 | 32.1±15.2 ^c | 920.9±3.8 ^a | 21.9±2.1 ^d | 14.0±8.5 ^d |

The mean indoor radon concentration of 19.9 ± 13.5 Bq/m³ for the five buildings was below the worldwide geometric mean values of 37.0 ± 2.2 Bq/m³ (unweighted) and 30.0 ± 2.3 Bq/m³ (population weighted) reported for indoor radon concentrations by UNSCEAR [14]. The maximum indoor radon concentrations measured in the built environment of AIBU were below the reference level of 100 Bq/m³ set by WHO [15] to minimize health risks due to indoor radon exposure. By measuring indoor radon concentrations in 7293

dwelling in 153 residential units of the total 81 provinces of Turkey, Celebi et al. [8] estimated the geometric mean to be 57 ± 2.3 Bq/m³ with the range of 1 to 1400 Bq/m³. However, there exists considerable variability both within and between countries. For example, Canoba et al. [16] reported mean indoor radon concentrations for the following six Latin countries of Argentina (37.0 ± 9.4 Bq/m³; *n* = 2034), Brazil (81.3 ± 4.5 Bq/m³; *n* = 320), Ecuador (94.3 ± 17.2 Bq/m³; *n* = 61), Mexico (67.9 ± 34.6 Bq/m³; *n* = 4630), Peru (32.3 ± 2.4 Bq/m³; *n*

= 168), and Venezuela (52.5 ± 24.7 Bq/m³; $n = 143$). UNSCEAR [17] reported nominal geometric mean indoor radon levels to range from < 10 Bq/m³ in Egypt and Cuba to > 100 Bq/m³ in a number of European countries and even to > 600 Bq/m³ in some parts of Iran.

Mean indoor radon concentration of the university buildings reached the maximum (37.3 ± 21.6 Bq/m³) in September ($n = 196$) and the minimum (13.1 ± 7.7 Bq/m³) in April ($n = 190$). Mean indoor radon concentration was highest (23.4 ± 18.4 Bq/m³) for the summer period of June to September ($n = 576$) and lowest (13.3 ± 7.9 Bq/m³) for the spring period of April to May ($n = 332$). Median indoor radon concentration was highest

(20.0 Bq/m³) for the autumn period of October to November ($n = 423$) and lowest (11.0 Bq/m³) for the spring period.

On average, the FAS building had the maximum indoor radon concentration and temperature, whereas the SFL building had the minimum indoor radon concentration, temperature and RH and the maximum P_a (Table 2) ($P \leq 0.001$). On average, the winter period showed the minimum RH, P_a and T_a , while the minimum indoor radon concentration was observed in the spring period. Unlike the winter and spring periods, the maximum values of indoor RH, T_a and radon concentration were observed in the summer period (Table 3) ($P \leq 0.001$).

TABLE 3
Tukey's multiple comparisons test for mean indoor radon concentration and ancillary data in response to changes in month ($P \leq 0.001$).

| Month | n | RH (%) | P_a (mbar) | T_a (°C) | ²²² Rn (Bq/m ³) |
|-----------|-----|------------------------|-------------------------|-------------------------|--|
| January | 154 | 21.3±7.1 ^h | 919.5±6.0 ^b | 22.0±2.5 ^g | 19.3±9.0 ^{bc} |
| February | 101 | 30.2±4.6 ^c | 916.6±1.5 ^{de} | 21.9±1.6 ^g | 21.7±10.6 ^b |
| March | 245 | 25.4±8.4 ^{fg} | 912.8±4.6 ^f | 22.4±1.9 ^{fg} | 16.2±8.8 ^{cd} |
| April | 190 | 26.6±6.4 ^f | 920.9±5.7 ^a | 22.8±2.4 ^{ef} | 13.2±7.7 ^d |
| May | 142 | 42.6±4.6 ^d | 917.9±1.7 ^{cd} | 22.9±2.0 ^{def} | 13.7±8.2 ^d |
| June | 130 | 45.2±6.6 ^{bc} | 916.3±1.6 ^e | 23.8±1.2 ^{bc} | 14.9±9.9 ^{cd} |
| July | 131 | 48.1±5.9 ^a | 916.5±1.8 ^{de} | 25.1±1.5 ^a | 14.9±11.2 ^{cd} |
| August | 119 | 41.9±5.9 ^d | 916.3±4.6 ^c | 23.7±1.6 ^{bcd} | 19.3±11.7 ^{bc} |
| September | 196 | 43.0±2.7 ^{cd} | 919.6±2.4 ^b | 23.1±0.8 ^{cde} | 37.4±21.6 ^a |
| October | 262 | 46.7±5.7 ^{ab} | 921.1±3.6 ^a | 22.1±2.4 ^g | 21.6±12.0 ^b |
| November | 161 | 24.0±3.8 ^g | 918.3±2.7 ^{bc} | 24.2±1.8 ^b | 20.3±11.0 ^b |
| December | 165 | 26.0±6.4 ^{fg} | 921.5±3.3 ^a | 22.8±2.1 ^{ef} | 23.0±10.8 ^b |

Indoor radon concentrations showed significant positive correlations with local time ($r = 0.54$), month ($r = 0.22$), RH ($r = 0.15$), and P_a ($r = 0.09$) and a significant negative correlation with T_a ($r = 0.54$) ($P < 0.05$). However, the stepwise procedure based on the significance level of 0.05 for the removal and addition of predictors, and their interaction and polynomial terms through a specified order of 3 pointed out to a MNLR model as the best-fit one in accounting for and predicting variation in spatiotemporal dynamics of indoor radon concentration. Thus, three- and two-way interaction terms, and quadratic and cubic terms of the predictors were found to elucidate spatiotemporal dynamics of indoor radon concentration better than linear relationships as measured by Pearson's correlation matrix. The best-fit MNLR model found in this study explained and predicted 57.9% (R^2_{adj}) and 57.1% (R^2_{CV}) of variation in spatiotemporal dynamics of indoor

radon concentration, respectively (Table 4). Although extensive surveys have been reported to characterize indoor radon concentrations in a variety of settings, there are only a few highly predictive empirical models reported using the parsimonious factors that mechanistically govern indoor air concentrations. For example, Bochicchio et al. [18] elucidated 26% of the total variation in indoor radon concentration measured in 334 primary schools of 13 municipalities of three districts in Southern Serbia using the best-fit multiple linear regression (MLR) model as a function of municipality (three categories), village/town (two categories), floor (four categories), school size, and intended use of the room. Using Kernel regression, Kropat et al. [19] accounted for 28% of the variations of indoor radon concentration as a function of building type, foundation type, year of construction, detector type, geographical coordinates, altitude, temperature, and

lithology based on 238,769 measurements in 148,458 houses in Switzerland. Andersen et al. [20] reported a better predictive MLR model for indoor radon in Danish houses than MLR models specified for other countries. The best-fit MLR model developed by Andersen et al. [20] using nine explanatory variables had an R^2_{adj} of 40% and an R^2 of 45% based on a comparison between predicted values and an independent dataset of measured indoor radon ($n = 788$).

Two-way interaction terms make up 74% of the 19 predictors used in the best-fit MNLR model, with the remaining being three-way interaction (16%) and cubic (10%) terms. The three predictors that exerted the most positive influence on the rate of change in indoor radon concentration were two-way interactions between hour and month, hour and RH, and hour² and T_a , respectively. Likewise, the three predictors that caused the highest rate of negative change in indoor radon concentration were two-way interactions between hour² and month, T_a and month, and hour and month². The two-way interactions of location were significant with the temporal predictors of hour and month, T_a , and RH.

According to the two-way interaction of location by hour, indoor radon concentration of FAS was the highest and differed most (82%) from that of FEAS and least (38.5%) from that of FE. According to the interaction between location and RH, indoor radon concentration of FAS was the highest again; however, differed most (0.4%) from that of FE and least (0.09%) from that of FEAS. Coefficients associated with the location by T_a interaction indicated that indoor radon concentration of FAS was the lowest and differed most (1.6%) from that of FEAS and least (0.9%)

from that of SPES. The interaction of the temporal predictors (hour*location) showed that indoor radon concentration of FEAS was the highest, that of FAS differed most (0.8%) from that of SFL and least (0.2%) from that of FE.

CONCLUSIONS

One-year indoor radon measurements were taken in the five buildings of the university campus. Spatiotemporal dynamics of indoor radon concentrations were modeled and validated using the best-fit MNLR model. Non-linear relationships among the temporal predictors, RH, T_a , and location, in particular, their two-way interactions emulate spatiotemporal dynamics of indoor radon concentration in the built environment of the university campus. All the mean indoor radon concentrations across the AIBU buildings were found below the global geometric mean indoor radon concentration of 30 Bq/m³ (population weighted), and the maximum indoor radon concentrations were below the reference level of 100 Bq/m³ determined to minimize health risks due to indoor radon exposure. The modeling approach illustrated in this study can be extended to the quantifications of short- and long-term local, regional, or global spatiotemporal dynamics of indoor and outdoor radon concentrations. To better understand controls over and improve indoor air quality for a particular built environment, a stochastic component such as Markov Chain and Monte Carlo algorithms should be coupled to non-block-box, data-driven and deterministic models such as MLR and MNLR models.

TABLE 4
Multiple non-linear regression (MNLR) model of spatiotemporal dynamics of indoor radon concentration (Bq/m³) with the FAS building as the reference level ($R^2_{adj} = 57.95\%$; $R^2_{CV} = 57.13\%$; D-W = 1.5; VIF > 10; SE = 10.6 Bq/m³; $n = 1996$; $P \leq 0.001$).

| Term | Coefficient | SE | T-value | P |
|----------------|-------------|-------|---------|--------|
| Intercept | 13.3 | 2 | 5.8 | 0.001 |
| hour*RH | 20.3 | 3 | 7.6 | 0.001 |
| hour*month | 27.1 | 4 | 6.5 | 0.001 |
| Ta*RH | 0.053 | 0.01 | 7.2 | 0.001 |
| Ta*month | -0.751 | 0.09 | -8.0 | 0.001 |
| hour*location | | | | |
| FEAS | -82.0 | 8 | -10.3 | 0.001 |
| FE | -38.5 | 8 | -5.0 | 0.001 |
| SPES | -44.0 | 8 | -5.6 | 0.001 |
| SFL | -60.0 | 8 | -7.8 | 0.001 |
| Ta*location | | | | |
| FEAS | 1.674 | 0.26 | 6.5 | 0.001 |
| FE | 1.361 | 0.26 | 5.3 | 0.001 |
| SPES | 0.918 | 0.18 | 5.0 | 0.001 |
| SFL | 1.391 | 0.19 | 7.3 | 0.001 |
| RH*location | | | | |
| FEAS | -0.094 | 0.09 | -1.1 | > 0.05 |
| FE | -0.446 | 0.09 | -4.9 | 0.001 |
| SPES | -0.137 | 0.07 | -2.0 | 0.04 |
| SFL | -0.141 | 0.07 | -2.0 | 0.04 |
| month*location | | | | |
| FEAS | 0.643 | 0.23 | 2.8 | 0.006 |
| FE | -0.241 | 0.24 | -1.0 | > 0.05 |
| SPES | -0.300 | 0.2 | -1.5 | > 0.05 |
| SFL | -0.871 | 0.21 | -4.2 | 0.001 |
| hour3 | -0.490 | 0.07 | -6.6 | 0.001 |
| month3 | -0.023 | 0.01 | -3.4 | 0.001 |
| hour2*Ta | 1.492 | 0.12 | 12.2 | 0.001 |
| hour2*RH | 0.263 | 0.04 | 6.0 | 0.001 |
| hour2*month | -4.171 | 0.43 | -9.7 | 0.001 |
| hour*Ta*RH | -0.143 | 0.02 | -7.0 | 0.001 |
| hour*Ta*month | -0.655 | 0.12 | -5.5 | 0.001 |
| hour*Pa*RH | -0.021 | 0.003 | -7.3 | 0.001 |
| hour*month2 | -0.691 | 0.26 | -2.6 | 0.008 |
| Ta2*month | 0.011 | 0.002 | 4.9 | 0.001 |
| Ta*month2 | 0.049 | 0.01 | 6.7 | 0.001 |

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COMPARISON OF O₃ AND KMnO₄ IN THE MICROFILTRATION MEMBRANE SYSTEM OF SURFACE WATER

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ABSTRACT

The aim of the study is to explore the effects of O₃ and KMnO₄ on organic matter and microfiltration membrane performance. The results showed that pre-oxidation has a great effect on organic matter of raw water and membrane performance. Compared with KMnO₄, ozone dramatically changes the structure of organic matters. During 3-5 kDa and near 0.2 kDa molecular weight (MW) distribution of raw water, 3-5 kDa MW organic matter markedly decreased by ozone and changed slightly near 0.2 kDa MW. Ozone markedly transfers the hydrophobic fraction to the hydrophilic fraction. However, MW and the hydrophobicity/hydrophilicity change slightly due to the weak oxidation of KMnO₄. The membrane filtration results showed that pre-oxidation can improve membrane flux. It seems that there was an optimal dosage for membrane flux with ozone and KMnO₄. Enhancement of the membrane flux by ozone oxidation was far better than that by KMnO₄, which can be confirmed by membrane resistance results and the scanning electron microscope images of membrane-filtrated water samples by ozone and KMnO₄ oxidation, respectively. Membrane fouling resistance by ozone oxidation was lower than that by KMnO₄, and matter deposit onto the membrane surface by ozone oxidation was larger and looser than that by KMnO₄ oxidation.

KEYWORDS:

Surface water; Ozone; Potassium permanganate; Microfiltration; Membrane flux

INTRODUCTION

Low-pressure membranes such as microfiltration (MF) and ultrafiltration (UF) have become an accepted process in drinking water production in recent years (Meier-Haack et al. 2003; Ma et al. 1998; Lipp et al. 2002; Mozia et al. 2004; Xia et al. 2004). However, one of the important issues of membrane application in drinking water is membrane fouling, which results

in membrane flux decrease and poor performance. Some research has indicated that natural organic matter (NOM) plays a major role in the fouling membrane of drinking water (Huang et al. 2007; Lee et al. 2001; Kennedy et al. 2008). To reduce organic matter-fouled membrane, peroxidation may be an option (Geluwe et al. 2011; Schlichter et al. 2004; Song et al. 2004; Xu et al. 2014).

Ozone and potassium permanganate are widely used as oxidants in water treatment. Ozone is a powerful oxidant and reacts with double bonds, and aromatic rings quickly exist in NOM. Some studies showed that membrane fouling could be reduced by ozone (Kim et al. 2008; Park et al. 2002; Geluwe et al. 2011). Schlichter et al found that membrane fouling for all membranes could be greatly reduced by adding ozone during the MF and UF of the humic acid solutions (Schlichter et al. 2003). You et al maintained the residual ozone concentration of approximately 4.02 mg/L in the influent flow, and a permeate flux of 90% was maintained. The SEM observations showed that much of the fouling that had clogged the membrane was effectively removed by ozonation (You et al. 2007). The researcher analyzed permeates to estimate the molecular weight of resulting humic acid oxidation products with the HPLC technique. The results showed that the hybrid process of membrane microfiltration-ozonation resulted in a substantial reduction of the membrane fouling rate for all examined concentrations (Szymanska et al. 2014). Unlike the strong oxidation of ozone, potassium permanganate has some advantages, such as a low price and fewer by-products in the water treatment process despite relatively weak oxidation. Researchers have used potassium permanganate in peroxidation to reduce and mitigate membrane fouling. Lin et al found significant improvements to the efficiency of NOM removal during the KMnO₄/UF process, and KMnO₄ lowered the total membrane resistance in comparison with results without KMnO₄ (Lin et al. 2012). For potassium permanganate, there is an optimal dosage of 0.4 mg/L KMnO₄ in terms of both the trans-membrane pressure and the product water quality. The cake layer showed that KMnO₄ oxidized the macromolecules of hydrophobic NOM to lower-

molecular-weight hydrophilic organic matter, which mitigated the membrane fouling caused by the organic matter (Lin et al. 2013). Katsoyiannis et al found oxidized iron and manganese onto recirculated suspended solids which flocculated in the pipe reactor, thus eliminating the need for mechanical cleaning of the MF membrane, while keeping the Trans-Membrane Pressure (TMP) constantly low (Katsoyiannis et al. 2013).

In this study, ozone and potassium permanganate peroxidation were used to MF membrane process of surface water. The aim of this research was to investigate the effects of ozone and potassium permanganate peroxidation on NOM and membrane performance, especially on MW and the hydrophobicity/hydrophilicity of organic matter, membrane flux and membrane resistance.

EXPERIMENTAL METHODS

Pre-oxidation. Ozone was produced by an ozone generator (Sankang, China) with pure oxygen. Ozone concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 mg/L were added into the raw water. Raw water was taken from the Huangpu River, which is one of drinking water sources for the city of Shanghai, China. Contact time of the ozone was 10 min, and then pure N₂ aerated residual ozone in order to prevent the ozone from destroying the membrane.

Jar tests were conducted to apply potassium permanganate for the pre-oxidation of raw water. Special potassium permanganate doses of 0.3, 0.5, 0.8, 1.0, 1.5 or 2.0 mg/L were added into 1 L of raw water. Potassium permanganate was added to the water sample and stirred for 30 min with rapid mixing at 200 rpm, respectively.

Microfiltration procedure. The experiment was carried out using a polyvinylidene fluoride (PVDF) hollow fiber MF membrane with an average pore size of 0.1 μm and 75 cm² surface areas (Toray, Japan). The membrane process used dead-end filtration and the out-in operation mode. The MF experiment set-up consists of a N₂ pressure cylinder, feed water tank and membrane module. The water sample in the feed water tank was pressed by N₂ into the membrane module. The filtration finished until 800 mL of water sample was filtrated, and then chemical cleaning respectively using 1% oxalic acid and 5 g/L sodium hypochlorite for 2 hours circular clean was carried out to reduce membrane fouling.

Analytical method. Molecular weight (MW) analysis was conducted by a gel permeation

chromatography (GPC) system (Shimadzu, Japan) with G2500PWXL column (TSK, Japan). 0.05 mol/L sodium sulfate as the moving phase was pumped with a flow rate of 0.5 mL/min. The MW distribution results were analyzed using the response (volt) data with the elapsed time.

The resin method was used to separate the DOM fraction following the procedure described by Carroll et al (Carroll et al. 2000). Adsorbent resin (Supelite DAX-8, Amberlite XAD-4) was used to separate the organic substances into the following three groups: the hydrophobic (HPO) fraction, the transphilic (TPI) fraction and the hydrophilic (HPI) fraction.

The MF membrane resistances included intrinsic resistance R_m , adsorption resistance R_a , surface cake layer resistance R_c and concentration polarization resistance R_i . The sum of R_a , R_c and R_i was defined as the fouling resistance R_n . The relationships between membrane fluxes and membrane resistances were calculated by the following equation:

$$J = \frac{\Delta P}{\mu(R_m + R_c + R_i + R_a)} \quad (1)$$

Where J is the membrane flux (m³/m²s); ΔP is the trans-membrane pressure (Pa); μ is the water viscosity coefficient (Pa·s); R_m is the intrinsic resistance (m⁻¹); R_c is the surface cake layer resistance; R_i is the concentration polarization resistance and R_a is the adsorption resistance. The plat PVDF MF membrane was used to identify membrane resistances. The procedures are similar to Wang's and as follows (Wang et al. 2007):

- 1) Pure water was used to filter the membrane and got membrane flux. Then, the R_m was calculated;
- 2) The above-used membrane was soaked in the water sample for 24 h, and then pure water flux was measured, and the $R_m + R_a$ was calculated;
- 3) the membrane was again soaked in the water sample for 24 h, the sample water flux was measured, and the $R_m + R_a + R_i + R_c$ was calculated;
- 4) the pure water flux of the above-used membrane was measured, and $R_m + R_a + R_c$ was calculated;
- And 5) the material attached on the membrane surface was then washed, and the pure water flux was measured. $R_m + R_a$ was calculated.

Filtrates were analyzed for DOC and ultraviolet absorbance at 254 nm (UV₂₅₄). The DOC of filtered samples (0.45 μm) was then analyzed in a TOC analyzer (Shimadzu TOC-VCPN). UV absorbance was measured in a UV spectrophotometer (HACH DR5000). An image of the SEM of membrane was conducted in ESEM (PHILIPS XL30).

RESULTS AND DISCUSSION

Effect of pre-oxidation on DOC and UV₂₅₄ removal. Removal rates of DOC and UV₂₅₄ with different ozone and potassium permanganate doses are shown in Fig. 1 a) and b), respectively. From the figure, UV₂₅₄ removal was markedly different between ozone and potassium permanganate. The removal of UV₂₅₄ by ozone is far more than that by potassium permanganate. The removal of UV₂₅₄ was 42% with 2.0 mg/L ozone and only 12.7% UV₂₅₄ removal by 2.0 mg/L potassium permanganate. Generally, UV₂₅₄ represents compounds with aromatic hydrocarbons, double bonds or carbonyls. Ozone possesses powerful oxidation, which easily decomposes compounds with double bonds or the aromatic rings present in NOM molecules. Compared with ozone, UV₂₅₄

removal by potassium permanganate was worse due to the relatively weak oxidation of potassium permanganate.

Although high UV₂₅₄ removal was obtained with ozone, DOC removal by ozone was limited. When the ozone dosage reaches 2.0 mg/L, only the DOC removal of 8.9% was obtained. It seems that ozone efficiently removes unsaturated bonds, but it has a minor degree of DOC mineralization. DOC removal by potassium permanganate is similar to DOC removal by ozone. DOC removal increased from 1.2% to 7.2% with the potassium permanganate dosage changing from 0.3 mg/L to 2.0 mg/L. DOC removal increases slightly during low potassium permanganate doses, such as less than 1.0 mg/L. When the potassium permanganate dosage increases to 1.5 mg/L, the DOC removal efficiency raises to 6.9%.

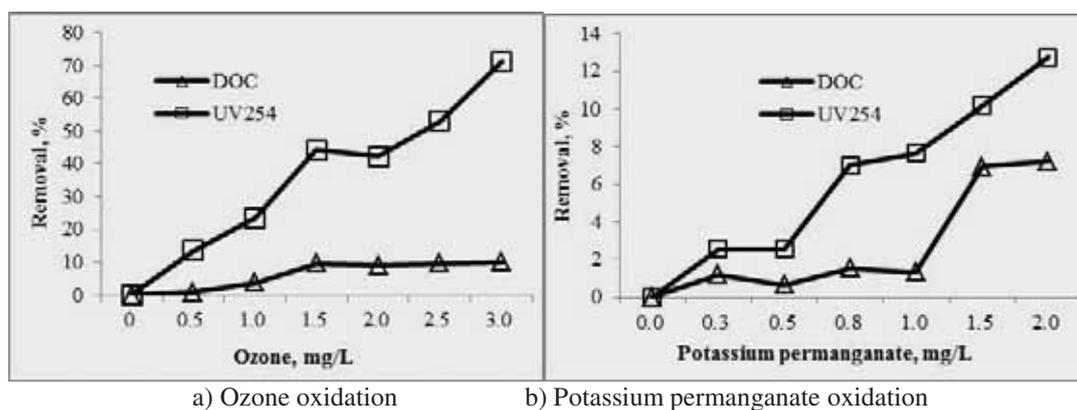


FIGURE 1
Effect of pre-oxidation on DOC and UV₂₅₄ removal

Effect of pre-oxidation on molecular weight distribution. Fig. 2 shows the effect of ozone and potassium permanganate oxidation on the organic matter molecular weight distribution. Significant differences can be seen between ozone and potassium permanganate. The influence of ozone on the molecular weight is greater than that of potassium permanganate. The decrease in organic matter of approximately 3-5 kDa molecular weights by ozone oxidation is obvious. Compared with

ozone, potassium permanganate has almost no effect on organic matter molecular weight. The likely reason is that the oxidation of potassium permanganate is weaker than that of ozone. As the above study (refer to Fig. 1) has shown, potassium permanganate removes less organic matter than does ozone. For organics near 0.2 kDa, ozone and potassium permanganate had no influence.

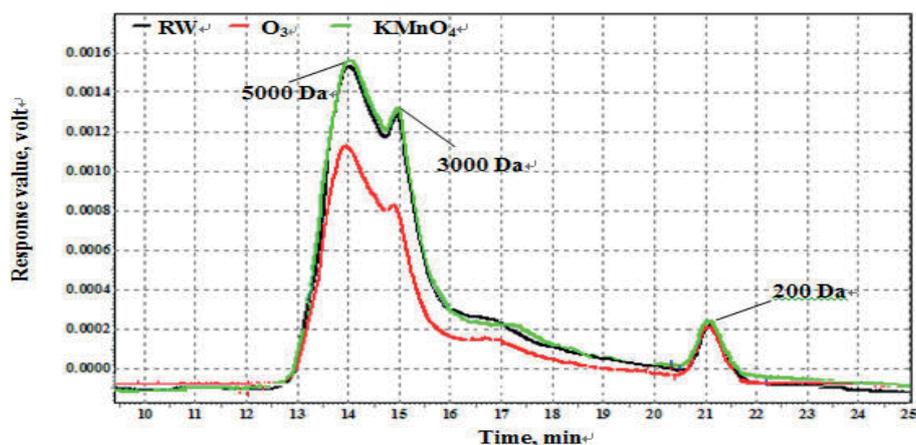


FIGURE 2

Effect of pre-oxidation on organics molecular weight distribution (RW- Raw water)

Effect of pre-oxidation on organic hydrophobicity/hydrophilicity. In this study, organic matter was separated into HPO fraction, TPI fraction and HPI fraction. Changes in the organic hydrophobicity/hydrophilicity by peroxidation are shown in Fig. 3. From the figure, there is more HPI organic matter than HPO matter in raw water. It can be seen that the great difference in the HPO and HPI fraction was obtained with ozone and potassium permanganate oxidation. From Fig. 3 a), ozone markedly reduced HPO and TPI fraction, especial HPO fraction. In contrast, HPI fraction concentration with ozone oxidation was higher than that of raw water. This result suggests that ozone oxidation transforms HPO substance to HPI substance. Compared with ozone, potassium permanganate reduced the HPO and HPI fraction slightly.

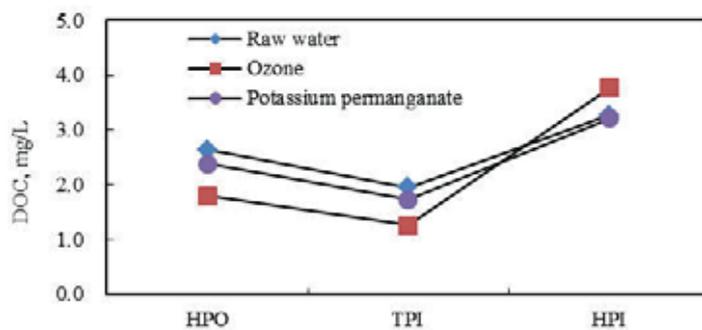
Fig. 3 b), c) and d) showed the MW distribution of different organic fraction with ozone and potassium permanganate oxidation. It can be seen that ozone and potassium permanganate (especial ozone) were prone to reducing the HPO fraction. For the TPI fraction, the value of potassium permanganate oxidation was higher than that of raw water, which is likely given that potassium permanganate transforms HPO to TPI. From Fig. 3 d), the HPI fraction changed markedly after ozone and potassium permanganate oxidation.

When ozone and potassium permanganate were added, the 5 kDa MW HPI organic matter increased. Furthermore, the HPI fraction of ozone was higher than that of potassium permanganate, which is similar to the HPI fraction shown in Fig. 3 a). The reason for this change is likely that ozone oxidation converted the HPO substance to a HPI substance easily and with less NOM mineralization, which is similar to the DOC removal by ozone oxidation shown in Fig. 1. Compared with ozone, the relatively weak oxidation of potassium permanganate transformed less HPO substance to HPI matter.

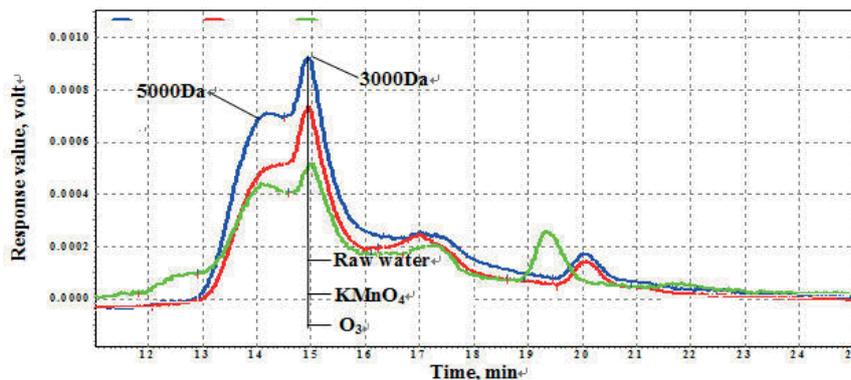
Effect of pre-oxidation on membrane permeability. In this study, the membrane fouling decline rate (Φ) was used to determine the influence of ozone and potassium permanganate oxidation on membrane permeability. The calculation of Φ is as follows:

$$\Phi = \frac{(J_C - J_R)}{(100 - J_R)} \times 100 \quad (2)$$

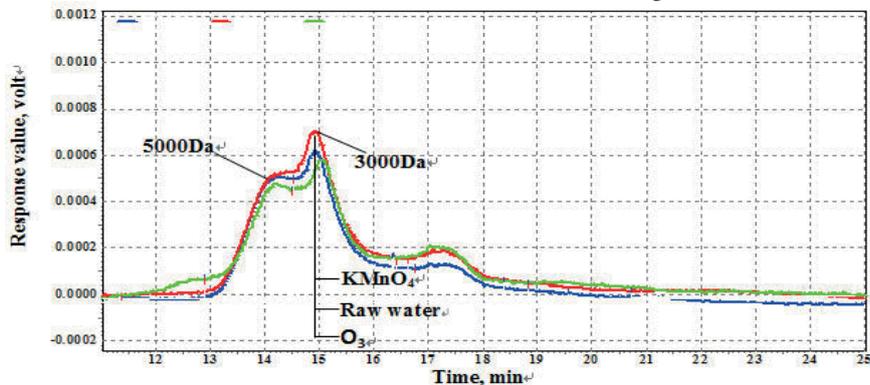
Where Φ is membrane fouling decline rate (%); J_R is final membrane flux of raw water (%); J_C is final membrane flux of oxidized water samples (%).



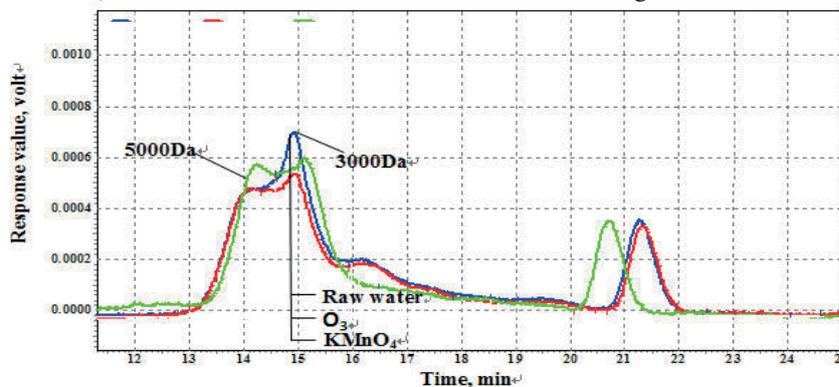
a) Effect of different oxidants on hydrophobicity/ hydrophilicity of organic matter



b) Effect of different oxidants on HPO fraction of organic matter



c) Effect of different oxidants on TPI fraction of organic matter



d) Effect of different oxidants on HPI fraction of organic matter

FIGURE 3
Effect of different oxidants on hydrophilicity/hydrophobicity of organic matter

Fig. 4 shows the effect of ozone and potassium permanganate oxidation on membrane fouling decline rate. As seen from Fig. 4, the Φ by ozone oxidation was obviously better than that by potassium permanganate. Ozone markedly improves Φ . During the range of 0.5-3.0 mg/L ozone dosage, a maximum Φ of 22.7% was obtained. When a low ozone concentration (0.5 mg/L and 1.0 mg/L) was added, the Φ increased slightly. Then, the Φ was further increased with the increasing ozone concentration. However, it was not true that a higher ozone concentration meant a better Φ . There was an optimal ozone dose of 1.5

mg/L that maximized the Φ during a 0.5-3.0 mg/L ozone dosage. This result is similar to that from the study of Wang et al (Wang et al. 2007).

Compared with ozone, potassium permanganate improves Φ slightly. The optimum dose of 1.5 mg/L potassium permanganate obtained a maximum Φ of 8.5%. The low potassium permanganate (less than 1.0 mg/L) has almost no effect on the enhancement of Φ . The Φ of 8.2% was obtained with a potassium permanganate dosage of 2.0 mg/L. It seems that an optimum dosage exists for potassium permanganate in improving Φ .

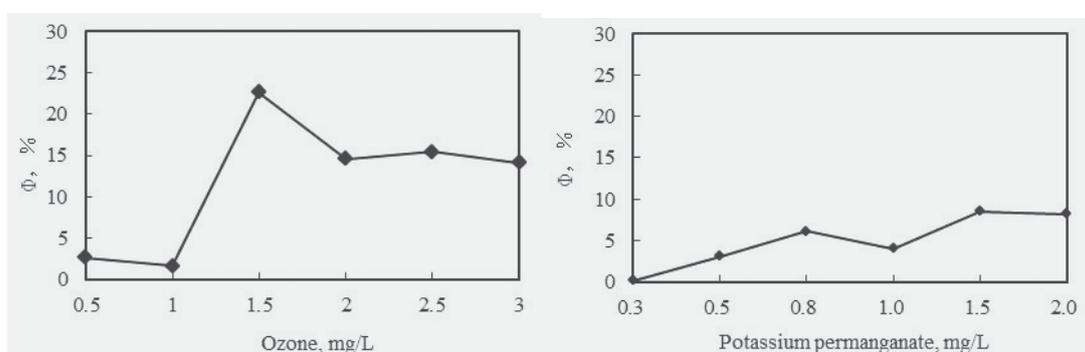


FIGURE 4
Membrane fouling decline rate variation in different oxidant concentrations

Discussion. NOM has an important effect on membrane fouling during membrane drinking water treatment. In this study, pre-oxidation has a great effect on organic matter and then influences the membrane performance. In the above study, ozone and potassium permanganate brought different influences to the NOM. On the whole, ozone removes organic matter better than potassium permanganate. Ozone oxidizes high molecular weight organics to low molecular weight organics and transfers the HPO fraction to the HPI fraction. However, potassium permanganate influences organic matter slightly. In the results, ozone improved the membrane flux far better than potassium permanganate.

To further determine the effect of pre-oxidation on membrane performance, the membrane resistance was measured. As is shown in Fig. 5, there are great differences in the membrane

resistance with ozone and potassium permanganate oxidation. During adsorption resistance R_a , surface cake layer resistance R_c and concentration polarization resistance R_i , R_c and R_i greatly influence fouling resistance R_n , and R_a has less effect on R_n . In the ozone dose range of 0.5-3.0 mg/L, there was a minimum fouling resistance of $1.1 \cdot 10^{12} \text{ m}^{-1}$ with the 1.5 mg/L ozone dose, which is consistent with a maximum Φ with 1.5 mg/L ozone concentration. It is obvious that the lower the membrane fouling resistance, the higher the membrane flux. In contrast, potassium permanganate reduces membrane resistance slightly. When potassium permanganate was added at 1.5 mg/L, a membrane fouling resistance of $2.72 \cdot 10^{12} \text{ m}^{-1}$ was obtained, which is far higher than that of ozone. This result also verifies that ozone improves the membrane flux far better than potassium permanganate.

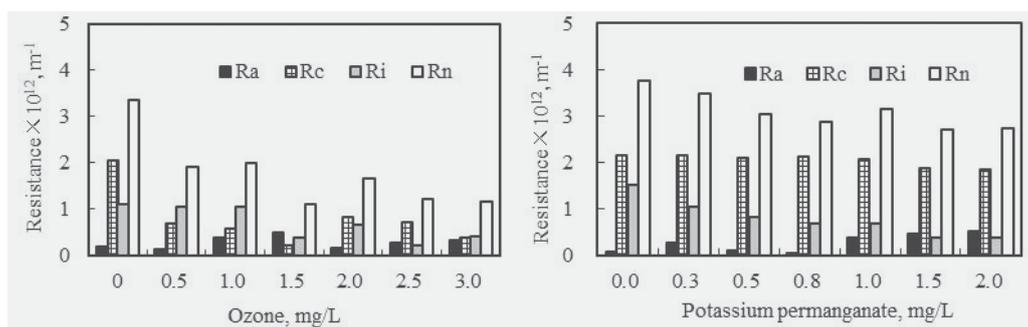


FIGURE 5
Effect of different oxidants on membrane resistances

We used a scanning electron microscope (SEM) to determine the effect of pre-oxidation on matter change and membrane filtration. The SEM images of membrane-filtered oxidized raw water are shown in Fig. 6. Large, loose materials were deposited onto the membrane surface when ozone was added into raw water. It is obvious that ozone changes the molecule structure and then changes the joint characteristics between organic matter and the

suspended substance, resulting in matter depositing loosely on the membrane surface. This is likely the reason that the membrane fouling resistance decreased after ozone oxidation. When potassium permanganate was added, the scatter characteristics of the matter deposited on the membrane surface changed slightly due to a reduced effect of potassium permanganate on the organic matter.

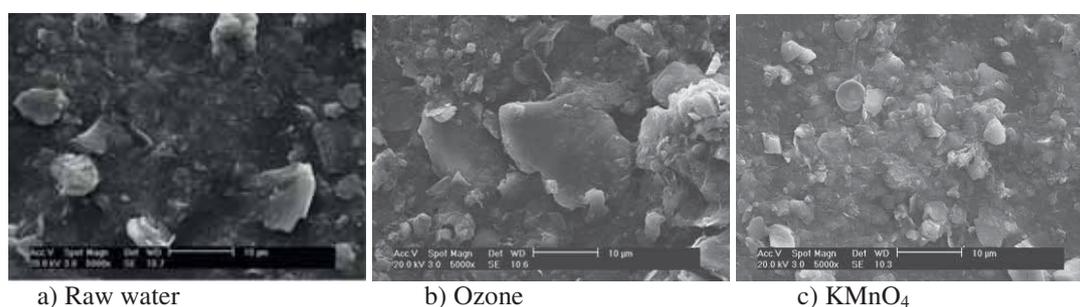


FIGURE 6
SEM of membrane surface after different oxidants and filtration ($\times 5000$)

CONCLUSIONS

Ozone and potassium permanganate are frequently used as oxidants in water treatment. The oxidation of ozone is far higher than that of potassium permanganate. In this study, there are great differences in the NOM and membrane performance between ozone and potassium permanganate. Ozone oxidation offers higher UV_{254} removal than does potassium permanganate. However, fewer DOC removals are obtained regardless of ozone or potassium permanganate. Ozone transfers high MW to low MW and hydrophobic fractions to hydrophilic fractions. Potassium permanganate converts MW organics slightly and has fewer effects on

hydrophobicity/hydrophilicity due to its weak oxidation.

Ozone improves membrane flux better than potassium permanganate. It seems that an optimum dose of ozone and potassium permanganate exists for enhancing membrane flux, which can be confirmed by the effects of ozone and potassium permanganate on membrane resistance. Large, loose matter on the membrane surface reduced the membrane resistance and improved the membrane flux after ozone oxidation. Small, compact matter deposits on the membrane surface after potassium permanganate oxidation reduced the membrane resistance slightly.

ACKNOWLEDGEMENT

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POLLUTION LEVELS AND SPATIAL DISTRIBUTION OF HEAVY METALS IN HOUSE DUST FROM AN INDUSTRIAL AREA IN XI'AN, CENTRAL CHINA

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ABSTRACT

Industrial activities are thought to contribute large amounts of heavy metals to the environment, posing risks to human and ecosystem health. To investigate heavy-metal pollution in a heavy industrial area in Xi'an, house dust samples were collected from households in sixteen residential quarters, and their <63 µm fractions were analyzed for heavy metals. The results show that Cr, Mn, Co and V in dust are basically unpolluted; whereas Ni, Cu, Zn and Pb are moderately to strongly polluted, mainly caused by industrial emissions in this area. Spatial analysis suggests a relatively high spatial variability of the four polluted metals in house dust, implying that area-source and point-source pollution may occur simultaneously. High pollution levels of heavy metals occur mainly in the southwest, presumably resulting from the influence of emissions of a coal-fired power generation plant. The pollution load index of these eight metals suggests a moderately polluted level in most house dust, implying that heavy-metal pollution related to industrial activities in this area is an important environmental problem and thus should be controlled.

KEYWORDS:

House dust, Heavy metal, Industrial activities, Enrichment factor, Geo-accumulation index, Pollution load index.

INTRODUCTION

Industrial activities, such as metallurgy, foundry, electroplating, coking, and cement manufacturing, etc., as well as related fossil fuel burning, can emit large amounts of particulates and various organic and inorganic pollutants into the environment [1-3]. Among various pollutants heavy metals are a representative kind. They can bioaccumulate in vital organs in human body and can lead to human damage when accumulated to a toxic concentration level [2, 4, 5]. Heavy metals adhered in dust particles, which can be introduced into the human body via direct inhalation, ingestion and dermal contact absorption, have long been recognized as a major source of heavy metals for human beings, especially in areas with heavy atmospheric particulate pollution [6-9].

The western urban industrial area in Xi'an city, where hundreds of heavily polluting units, such as machinery factories, pharmaceutical factories, chemical plants, coal-fired power generation plants, cement plants etc., are clustered (Fig. 1c), is considered to be one of the most heavily polluted areas in this city [10, 11], especially for atmospheric particulate pollution. Although the atmospheric environment has been severely polluted in this area, there are still several hundred thousand people living there. Therefore, understanding the pollution characteristics of heavy metals in atmospheric dust in this area is of great importance for protecting health of the general population and for establishing reasonable pollution control measures.

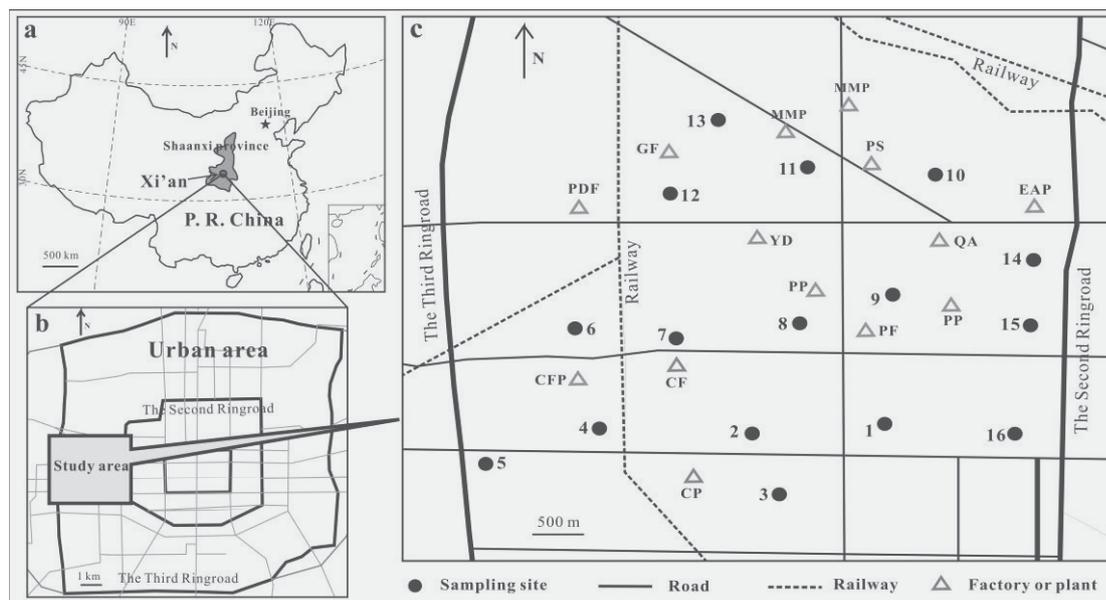


FIGURE 1

Location of the study area (a and b) and sampling sites (c) of house dust in the Xi'an western industrial area. Representative polluting units in the study area are also shown. It should be note that there are also hundreds of other relatively small polluting units distributed in the study area besides these shown in the figure. PDF, GF, MMP, PS, EAP, YD, QA, PP, PF, CFP, CF and CP in the figure represent printing and dyeing factory, gear factory, metallurgy and machinery plant, passenger station, electric appliance plant, Yuandong co., LTD, Qing'an co., LTD, printing plant, pharmaceutical factory, coal-fired power generation plant, crane factory, and chemical plant, respectively.

To understand heavy-metal pollution in atmospheric dust, especially the dust which has relatively close relationship with human health, in the Xi'an western industrial area, (1) house dust (indoor dust), which is of special importance for human health because people spend at least one-third of their time at home every day [12-17], was collected from households in representative residential quarters in this area, and (2) only particles smaller than $63\ \mu\text{m}$ were used for heavy-metal analysis, because these particles have relatively high concentrations of pollutants and can be easily introduced into human body relative to coarse particles [12, 18, 19]. The aims of this study are (1) to give information on levels of representative heavy metals, (2) to assessment their integrated pollution status using a pollution load index, and (3) to find out areas where heavy metals show relatively high pollution status and to preliminarily deduce the major sources of heavy metals in house dust in these areas.

Study area. Xi'an ($33^{\circ}39' - 34^{\circ}45' \text{ N}$, $107^{\circ}40' - 109^{\circ}49' \text{ E}$) (Fig. 1a), located in central China, is the capital of Shaanxi province and one of the most important industrial cities in China. The total urban area of Xi'an city is approximately $3,580\ \text{km}^2$, and it has a population of 6 470,000 in 2009 in the urban

area [20]. The city is located in a semi-arid zone, where the climate is a typical temperate continental semi-humid climate, with an annual average temperature of $\sim 13.0\ ^{\circ}\text{C}$ and an annual rainfall of $\sim 570\ \text{mm}$ [21]. The maximum rainfall takes place during the summer monsoon (July-August) period. The predominant wind direction is northeasterly in winter and autumn and is southwesterly in summer and spring [20]. Because the city is surrounded by mountains, inversion weather occurs frequently, and this weather often prevent atmospheric particulate matter from migrating, which has an adverse influence on the local atmospheric environment [10]. The adverse weather conditions in this area, along with rapid development of the economy, fast increase in private cars and other factors, make Xi'an often to be heavily polluted with atmospheric particulate matter, especially during the heating season in recent years [20, 22, 23].

The study area in this work, the Xi'an western industrial area, is situated at the western urban area of Xi'an, which is generally outside the Second Ringroad and inside the Third Ringroad (Fig. 1b), covering a flat area of approximately $20\text{-}30\ \text{km}^2$. This area is considered to be the heavy industrial base of the Xi'an city, where more than two hundred heavily polluting units cluster, such as the printing and

dyeing factory (PDF), gear factory (GF), metallurgy and machinery plant (MMP), passenger station (PS), electric appliance plant (EAP), Yuandong co., LTD (YD), Qing'an co., LTD (QA), printing plant (PP), pharmaceutical factory (PF), coal-fired power generation plant (CPF), crane factory (CF), and chemical plant (CP) shown in the Fig. 1c. Since 2007, the Xi'an environmental protection administration has started to pay attention to the serious environmental pollution in this area and to take measures to control industrial pollution, but the pollution status of the environment, especially the atmospheric suspended particulate pollution, is still serious today.

MATERIALS AND METHODS

DUST SAMPLING AND ANALYSIS

This sampling campaign was carried out in April and May of 2013. The sampling design was approximately grid based. In each sampling site, at least two households were chosen for dust collection. House dust samples were primarily collected from windowsills, lamp covers, ceiling fans, cabinet roofs, and others that had not cleaned for a long time, from private households in sixteen relatively large residential quarters within the Xi'an western industrial area (Fig. 1c). Dust was not collected from sites adjacent to site-specific pollution sources, e.g., construction sites, gasoline stations, and others [24]. Dust samples were collected by using a small brush and a clean plastic dustpan [7, 10, 25, 26]. During dust sampling, care was taken to avoid other obvious extraneous matter and to reduce the disturbance to fine particles. All the dust samples were stored in coded self-sealing polyethylene bags, labeled, and then taken back to the laboratory for subsequent sample preparation and analysis.

After taken back to the laboratory, the dust samples were dried in an oven at 50 °C, and impurities were picked out; then the dust was sieved through a 250 mesh (~63 μm) sieve. For each house dust, only < 63 μm particles were used to measure heavy metals, given that these particles have a relatively close relationship with human health [12, 18, 19].

After being oven-dried at 105 °C for about two hours, approximately 0.25 g of each dust sample was weighed using a 0.1 mg-precision electronic balance. The dust samples were then hot-digested with HClO₄, HF and HNO₃ in Teflon beakers on a hot plate. After the samples were fully digested, the acid was evaporated to dryness and the residue was re-dissolved with hydrogen nitrate and added with deionized water to a constant volume of 25 ml.

All dust samples were analyzed for heavy-metal

(Cr, Mn, Co, Ni, Cu, Zn, Pb and V) concentrations, as well as other major elements, by an Inductively Coupled Plasma Optical Emission Spectrometry (Thermo Fisher Scientific, iCAP 6300). Blanks and duplicate determinations were performed with the same treatments to ensure quality and accuracy. The recovery rates for these elements relative to the standard reference material (GSB 04-1767-2004) were satisfactory and ranged between 92% and 105%. Replicate analyses suggested that the precision of these analyses was approximately <5% relative standard deviation at a 95% confidence level.

ASSESSMENT METHOD FOR HEAVY-METAL POLLUTION

Enrichment factor. Enrichment factor (EF) is often used to assess the enrichment degrees of metals in soil, sediment and dust, and the EF can also provide insight to help differentiate an anthropogenic source from a natural origin [10, 26-28], which can be calculated as follows:

$$EF = (X_n/R)_{\text{dust}} / (X_n/R)_{\text{background}} \quad (1)$$

where X_n is the concentration of metal n and R is the concentration of reference element in dust. In this study, Al is used as the reference element [10, 26, 27, 29], and local (Shaanxi Province) natural soil [30] is used as the background material.

Based on the EF values, the enrichment degrees of metals can be divided into five classes [10, 25, 31] as follows: (1) $EF \leq 2$, deficiency to minimal enrichment; (2) $2 < EF \leq 5$, moderate enrichment; (3) $5 < EF \leq 20$, high enrichment; (4) $20 < EF \leq 40$, very high enrichment; and (5) $EF > 40$, extremely high enrichment.

Geo-accumulation index.

The geo-accumulation index (I_{geo}) introduced by Müller [32] is widely used to assess the pollution levels of heavy metals in soil, sediment and dust [33-36]. This index is defined by the following equation:

$$I_{\text{geo}} = \log_2[X_n / (1.5X_{\text{nb}})] \quad (2)$$

where X_n represents the concentration of metal n in dust and X_{nb} is the concentration of metal n in background material. In this study, local (Shaanxi Province) natural soil [30] is used as the background material.

According to Müller [32], the I_{geo} can be divided into seven classes as follows: (1) $I_{\text{geo}} \leq 0$, practically unpolluted; (2) $0 < I_{\text{geo}} \leq 1$, unpolluted to moderately polluted; (3) $1 < I_{\text{geo}} \leq 2$, moderately polluted; (4) $2 < I_{\text{geo}} \leq 3$, moderately to heavily polluted; (5) $3 < I_{\text{geo}} \leq 4$, heavily polluted; (6) $4 < I_{\text{geo}} \leq 5$, heavily to extremely polluted; and (7) $I_{\text{geo}} > 5$, extremely polluted.

Pollution load index. To assess the integrated

pollution status of heavy metals in house dust, a pollution load index (PLI) of heavy metals in dust is employed in this study. The PLI of heavy metals in a dust sample can be calculated by using the following formula [26, 37]:

$$PLI = \sqrt[n]{PI_1 \times PI_2 \times PI_3 \cdots \times PI_n} \quad (3)$$

where PI represents the pollution index of each metal. It is the ratio of the metal concentration in house dust to the concentration of the corresponding metal in the background material, which is calculated as follows:

$$PI_i = C_i / B_i \quad (4)$$

where C_i is the measured concentration of metal i in the house dust sample and B_i is the background value of metal i in Shaanxi natural soil [30].

Generally, the PLI can be divided into the following seven classes:

- (1) $PLI = 0$, background concentration;
- (2) $0 < PLI \leq 1$, unpolluted;
- (3) $1 < PLI \leq 2$, unpolluted to moderately polluted;
- (4) $2 < PLI \leq 3$, moderately polluted;
- (5) $3 < PLI \leq 4$, moderately to heavily polluted;
- (6) $4 < PLI \leq 5$, heavily polluted; and
- (7) $PLI > 5$, very heavily polluted.

RESULTS AND DISCUSSION

Concentrations of heavy metals in house dust.

The descriptive statistical results for concentrations of heavy metals (Cr, Mn, Co, Ni, Cu, Zn, Pb and V) in house dust, along with heavy-metal concentrations in campus dust [38], in street dust [10, 39] and in topsoil [11, 40] from the Xi'an urban area, in natural soil from Shaanxi province [30] and in house dust from other typical cities over the world, are listed in

Table 1.

As shown Table 1, the concentrations of Cr, Mn, Co, Ni, Cu, Zn, Pb and V in house dust are in the ranges of 74–143, 392–549, 11–53, 30–1,367, 69–280, 385–1,026, 93–266 and 44–63 mg/kg, with averages of 95, 453, 17, 157, 101, 621, 148 and 52 mg/kg, respectively. Relatively large coefficients of variation (CV) are found for all the heavy metals except Cr, Mn and V, indicating large variations in the concentrations of Co, Ni, Cu, Zn and Pb in house dust, which may be caused by the fact that these metals in dust have been influenced for different degrees by human activities at different sites in the Xi'an western industrial area.

Compared with the background values of these metals in natural soil, the concentrations of Cr, Mn, Co, Ni, Cu, Zn, Pb and V in house dust are 1.2–2.3 (1.5), 0.7–1.0 (0.8), 1.0–5.0 (1.6), 1.0–47.1 (5.5), 3.3–13.3 (4.7), 5.6–14.9 (8.9), 4.4–12.7 (6.9) and 0.7–1.0 (0.8) times higher than their corresponding background values in the Shaanxi natural soil [30] (Table 1), respectively. The mean concentrations of these eight heavy metals in house dust, divided by their corresponding background values in Shaanxi soil, decrease in the order of $Zn > Pb > Ni > Cu > Co > Cr > Mn = V$. With the exception of Cr, Mn, Co and V, the mean concentrations of other heavy metals in house dust are significantly higher than the background values of these metals in Shaanxi natural soil, particularly that of Cu, Zn and Pb which are 5–9 times higher, implying that these metals in house dust from the Xi'an western industrial area may be heavily influenced by human activities.

Compared with campus dust [38], street dust [10, 39] and topsoil [11, 40] in the Xi'an urban area, it can be found that the concentrations of Ni, Cu and Zn are often significantly higher in the house dust in the Xi'an western industrial area (Table 1). Nickel, Cu and Zn are representative metals emitted by industrial activities such as metallurgy, machining, coal combustion and so on [2, 3, 10]; hence, this comparison indicates that the significant enrichment of these three metals in house dust may be dominantly influenced by industrial activities in the Xi'an western industrial area.

TABLE 1
Descriptive statistical results for concentrations of heavy metals
(Cr, Mn, Co, Ni, Cu, Zn, Pb and V) in house dust in the Xi'an urban-industrial area.
Besides, heavy metal concentrations in campus dust, in street dust and in topsoil from the Xi'an urban
area, in natural soil from Shaanxi province and in house dust from other typical cities over the world are
also listed.

| City | Area | Type | Item | Cr | Mn | Co | Ni | Cu | Zn | Pb | V | Data source | | |
|--------------------|---------------------------|--------------------|--|---------|-------|-------|---------|-------|---------|-------|-------|-------------|------|------|
| | | | | mg/kg | | | | | | | | | | |
| Xi'an | The industrial area | House dust | Min | 74.1 | 392.1 | 11.3 | 29.6 | 69.0 | 385.0 | 92.9 | 43.8 | This work | | |
| | | | Max | 142.6 | 549.2 | 52.8 | 1,367.1 | 279.8 | 1,026.4 | 266.0 | 62.6 | | | |
| | | | Mean | 94.6 | 452.9 | 17.0 | 157.5 | 100.7 | 621.1 | 148.4 | 52.3 | | | |
| | | | Median | 91.5 | 455.0 | 14.0 | 75.0 | 87.5 | 582.0 | 134.5 | 52.0 | | | |
| | | | SD ^a | 17.1 | 42.8 | 9.8 | 323.7 | 49.2 | 174.5 | 52.0 | 4.6 | | | |
| | | | CV ^b (%) | 18.2 | 9.5 | 58.2 | 205.6 | 48.9 | 28.1 | 35.0 | 8.8 | | | |
| Xi'an | Urban area | Campus dust | Mean | 154.2 | 546.2 | 39.6 | 32.2 | 62.1 | 390.7 | 151.6 | 687.0 | [38] | | |
| | | | Street dust (1998-2001) | Mean | 167.3 | 687.0 | – | – | 95.0 | 421.5 | 230.5 | – | [10] | |
| | Urban area | Street dust (2009) | Mean | 96 | 501 | 10 | 29 | 74 | 301 | 104 | – | [39] | | |
| | | | Inside Second Ringroad Surrounding the industrial area | Topsoil | Mean | 81.1 | 671.5 | 19.3 | 34.5 | 54.3 | 186.2 | 59.7 | – | [40] |
| | | | | Topsoil | Mean | 76.5 | – | 15.5 | 29.9 | 34.8 | 134.9 | 61.4 | 84.8 | [11] |
| Shaanxi | Shaanxi Province | Natural soil | Mean | 62.5 | 557.0 | 10.6 | 28.8 | 21.4 | 69.4 | 21.4 | 66.9 | [30] | | |
| Guangzhou | Urban area | House dust | Mean | 188.0 | 321.2 | – | 94.9 | 68.4 | 344.2 | 699.1 | – | [41] | | |
| South China | An e-waste recycling area | House dust | Mean | – | – | – | 142 | 1712 | 2,250 | 1,467 | – | [44] | | |
| Japan | Japan | House dust | Mean | 67.8 | 226.0 | 4.7 | 59.6 | 304.0 | 920.0 | 57.9 | 24.7 | [45] | | |
| Birmingham | Urban area | House dust | Mean | – | 416.0 | – | 44.8 | 344.0 | 774.0 | 90.5 | – | [29] | | |
| Sydney | Urban area | House dust | Mean | 83.6 | 76.1 | – | 27.2 | 147.0 | 657.0 | 389.0 | – | [43] | | |
| Selangor, Malaysia | Urban area | Dust in preschools | Mean | 11.9 | – | – | – | – | 144.9 | 253.5 | – | [42] | | |

^a Standard deviation.

^b Coefficient of variation (CV = SD/Mean × 100).

– No analysis.

Compared with house dust from other representative cities over the world, the concentrations of Ni, Cr and Mn in house dust in the Xi'an western industrial area are generally higher than those in other cities; Cu and Zn are higher than those from the urban area in Guangzhou [41] and in Selangor Malaysia [42], close to that from the urban area in Sydney [43], but lower than that from an e-waste recycling area in South China [44], from Japanese households [45] and from the urban area in

Birmingham [29]; while the concentration of Pb are higher than that from Japanese households [45] and from the urban area in Birmingham [29], but are lower than that from an e-waste recycling area in South China [44] and from the urban area in Guangzhou [41], in Sydney [43] and in Selangor [42] (Table 1). These comparisons suggest a relatively moderate pollution status for heavy metals in house dust from the Xi'an western industrial area among representative cities over the world.

Pollution levels of heavy metals in house dust. The EF and I_{geo} values of heavy metals in house dust are shown in box-plots in Fig. 2. In addition,

percentages (%) of EF and I_{geo} in each class for heavy metals in house dust from the Xi'an western industrial area are presented in Table 2.

TABLE 2
Percentages (%) of the EF and I_{geo} in each class for heavy metals in house dust from the Xi'an western industrial area.

| Item | Class | Value | Quality | Percentage (%) | | | | | | | | | |
|-----------|-------|----------------------|-----------------------------------|----------------|-----|------|------|------|------|------|----|--|-----|
| | | | | Cr | Mn | Co | Ni | Cu | Zn | Pb | V | | |
| EF | 1 | $EF \leq 2$ | Deficiency to minimal enrichment | 50 | 100 | 62.5 | 6.3 | | | | | | 100 |
| | 2 | $2 < EF \leq 5$ | Moderate enrichment | 50 | | 31.3 | 75 | 25 | | | | | |
| | 3 | $5 < EF \leq 20$ | High enrichment | | | 6.3 | 12.5 | 75 | 93.8 | 100 | | | |
| | 4 | $20 < EF \leq 40$ | Very high enrichment | | | | | | | 6.3 | | | |
| | 5 | $EF > 40$ | Extremely high enrichment | | | | | 6.3 | | | | | |
| I_{geo} | 1 | $I_{geo} \leq 0$ | Practically unpolluted | 50 | 100 | 62.5 | 6.3 | | | | | | 100 |
| | 2 | $0 < I_{geo} \leq 1$ | Unpolluted to moderately polluted | 50 | | 31.3 | 68.8 | | | | | | |
| | 3 | $1 < I_{geo} \leq 2$ | Moderately polluted | | | 6.3 | 18.8 | 93.8 | 6.3 | 37.5 | | | |
| | 4 | $2 < I_{geo} \leq 3$ | Moderately to heavily polluted | | | | | | | 81.3 | 50 | | |
| | 5 | $3 < I_{geo} \leq 4$ | Heavily polluted | | | | | 6.3 | 12.5 | 12.5 | | | |
| | 6 | $4 < I_{geo} \leq 5$ | Heavily to extremely polluted | | | | 6.3 | | | | | | |
| | 7 | $I_{geo} > 5$ | Extremely polluted | | | | | | | | | | |

The EF values of Cr, Mn, Co, Ni, Cu, Zn, Pb and V are in the ranges of 1.7–2.6, 1.0–1.3, 1.5–6.4, 1.6–61.3, 4.4–16.9, 7.6–20.5, 5.9–16.5 and 0.9–1.1, with averages of 2.0, 1.1, 2.1, 7.3, 6.3, 11.9, 9.2 and 1.0 (Fig. 2a). The I_{geo} values of Cr, Mn, Co, Ni, Cu, Zn, Pb and V are in the ranges of -0.3–0.6, -1.1–0.6, -0.5–1.7, -0.5–5.0, 1.1–3.1, 1.9–3.3, 1.5–3.1 and -1.2–0.7, with averages of 0.0, -0.9, 0.1, 1.9, 1.6, 2.6, 2.2 and -0.9 (Fig. 2b). Both the mean EF and the mean I_{geo} values of these heavy metals decrease in the following order: Zn > Pb > Ni > Cu > Co > Cr > Mn \geq V.

With respect to Zn and Pb, both their EF and I_{geo} values are the highest, suggesting that they are the most heavily polluted among all the heavy metals. Nearly all the EF values for Zn and Pb in dust are higher than 5, and 62.5% of EF values of Zn and 31.3% of EF values of Pb are even higher than 10. The mean I_{geo} values of Zn and Pb in dust are

between 2.2 and 2.6, with 93.8% of I_{geo} values for Zn and 62.5% for I_{geo} values of Pb higher than 2 (Table 2). These results suggest that Zn and Pb are moderately to strongly polluted, according to the EF and I_{geo} categories in Table 2, in house dust collected from the Xi'an western industrial area, implying that these two metals are heavily influenced by human activities and mostly originate from anthropogenic sources.

For Ni and Cu, their EF and I_{geo} values in most house dust samples are between 3 and 6 and between 0 and 2, respectively (Fig. 2ab), except a house dust collected at Site 4 that has both extremely high EF and I_{geo} values for Ni and Cu (the reason is discussed in the next part in 4.3). These results suggest a moderate pollution level of Ni and Cu in most of the house dust, indicating that these two metals originate from both anthropogenic and natural sources.

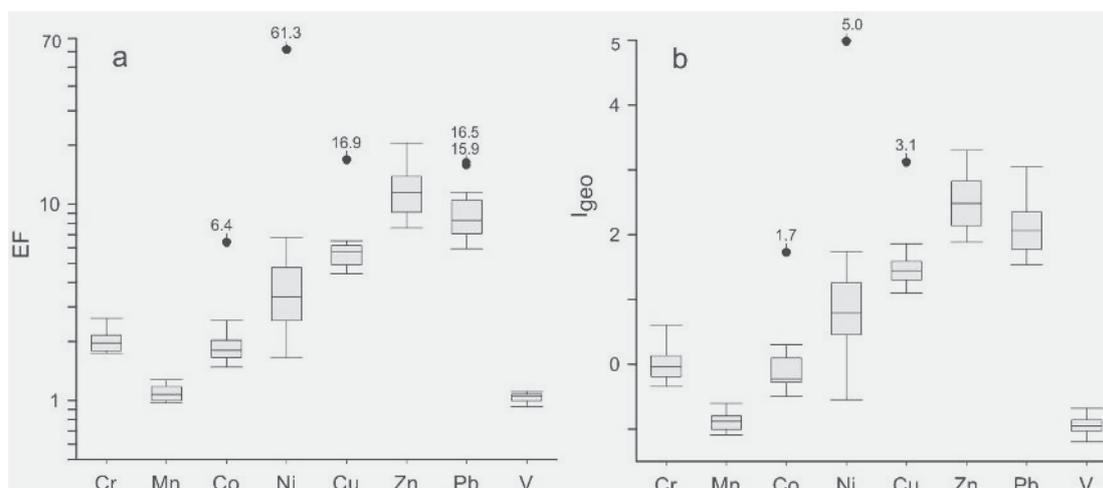


FIGURE 2

Box-plots of enrichment factors (EF) and geo-accumulation indexes (I_{geo}) for heavy metals in house dust from the Xi'an western industrial area. The y-axis is logarithmic in the figure a. The horizontal line inside the box represents the median value of each metal; the top and the bottom borders of the box mark the 25th and 75th percentiles, respectively; the horizontal lines outside the box, called whisker, mark the values that extend 1.5 times the width of the box; points outside the whisker are called outliers which are more than 3 times the width of the box.

For Cr and Co, their EF and I_{geo} values vary insignificantly in different dust samples and are averaged approximately 2 and 0, respectively (Fig. 2ab), suggesting that these two metals in house dust are slightly polluted, and they are primarily derived from natural sources and are only slightly affected by human activities.

For Mn and V, their EF values in all dust samples are close to unity, and their I_{geo} values are all smaller than 0 (Fig. 2ab), suggesting that these two metals are practically unpolluted, i.e., they are basically derived from natural sources and are seldom influenced by human activities.

Spatial distribution of heavy-metal pollution in house dust. Kriging interpolation method is applied to generate spatial distribution maps of pollution levels and PLIs for Cr, Mn, Co, Ni, Cu, Zn, Pb and V in house dust. The overall pollution patterns of these eight metals in house dust from the Xi'an western industrial area, as indicated by I_{geo} values, are shown in Fig. 3. The colors from light to dark indicate low to high pollution levels.

With respect to the relatively heavily polluted metals of Ni, Cu, Zn and Pb in house dust, it can be seen from Fig. 3 (d, e, f and g) that the pollution levels of these metals often show relatively high spatial variabilities. Ni is slightly polluted in most house dust from the northern and the eastern part of the study area (Fig. 3d). The relatively high (moderately) pollution level of Ni in house dust occurs in the southwestern area, especially at Site 4 where Ni is extremely polluted ($I_{geo} = 5.0$). In addition to Ni, other metals such as Co, Cu and Pb in house dust in the southwestern area also show relatively higher pollution levels (Fig. 3c, e and g), which may be associated with large amounts of coal combustion in a coal-fired power generation plant (CFP) in this area [46] (Fig. 1c). The investigation of heavy metals in surface soil around another large coal-fired power plant in Xi'an city also suggests that concentrations of Cu, Pb, Zn, Co and Cr are significantly higher than their corresponding background values, probably resulting from the influence of coal combustion for energy production [17, 20].

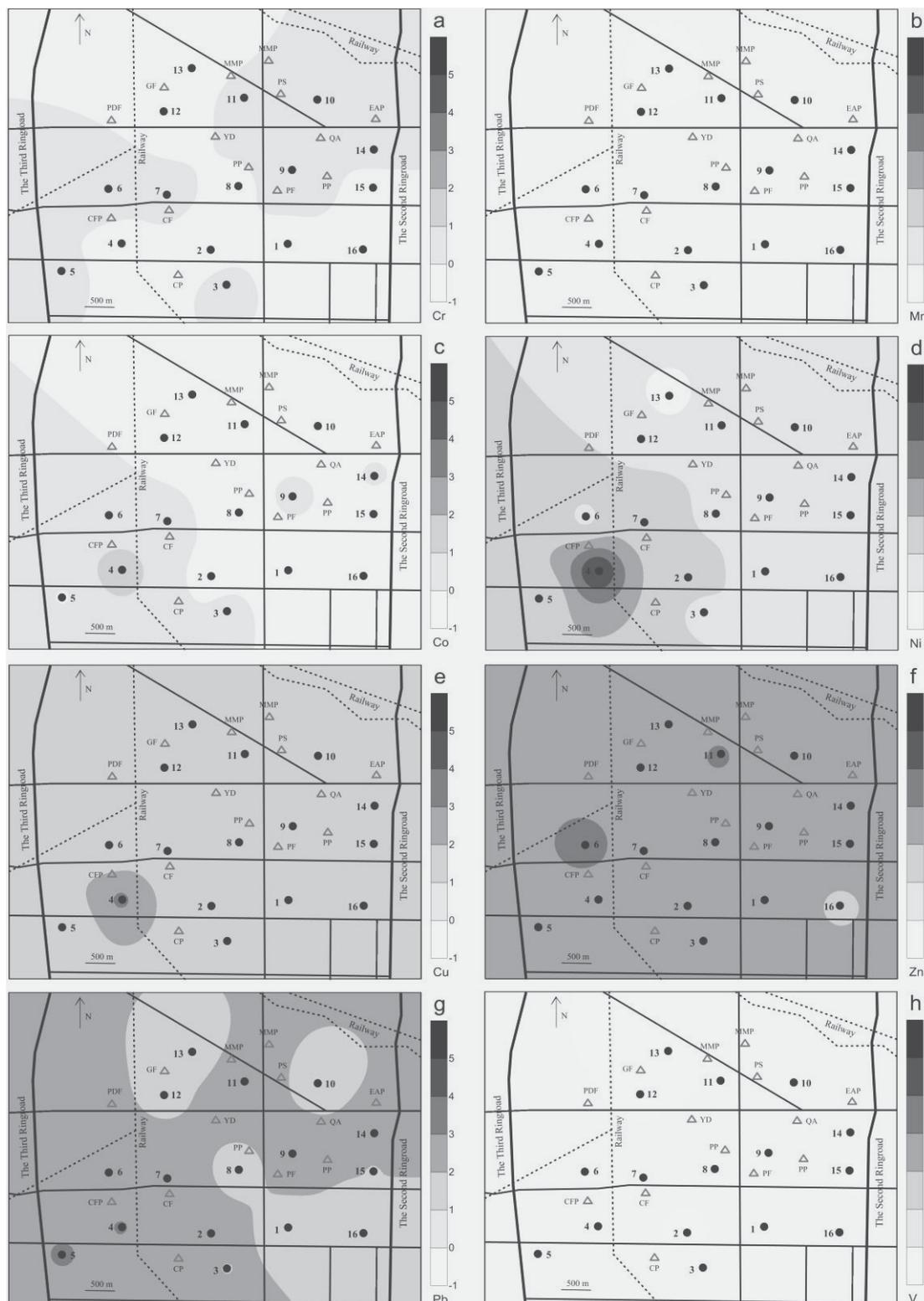


FIGURE 3
Spatial distribution of heavy-metal pollution, indicated by the geo-accumulation indexes (I_{geo}), in house dust from the Xi'an western industrial area.

Copper is moderately polluted in nearly all the house dust samples, except one at Site 4 (Fig. 3e). At Site 4 the I_{geo} of Cu in dust is as high as 3.1, indicating a strong pollution level. Zn, the most heavily polluted metal, is moderately to strongly polluted in the house dust in most of the sampling sites (Fig. 3f). Strong pollution levels of Zn in dust occur at Site 6 ($I_{geo} = 3.3$) which may be related to the occasional refuse incineration in this residential quarter that we observed during dust sampling, and at Site 11 ($I_{geo} = 3.1$) which may be related to metallurgical work [33, 47] in nearby metallurgy and machinery plants (Fig. 1c).

The pollution level of Pb in house dust is also relatively high among these eight metals. The Pb in most house dust is moderately to strongly polluted (Fig. 3g), with two peaks occurring at Site 4 and Site 5 where Pb is strongly polluted. The relatively high (strongly) pollution level of Pb in the house dust at Site 4 may be related to the influence of coal burning in the CFP, and that of Site 5 may be as a result of historical residues from gasoline combustion [8, 35, 47] related to dense traffic activities at the crossroad of the Third Ringroad. Overall, the relatively high spatial variabilities of Pb as well as Ni, Cu and Zn, which show relatively high pollution levels in house dust, suggest that area-source and point-source pollution may occur simultaneously in the sampling area.

With respect to the other four metals of Cr, Mn, Co and V with relatively low pollution levels (Fig. 3a, b, c and h), they often show relatively small spatial variabilities, especially for Mn and V. For Cr, it is unpolluted in most of the house dust. Slight pollution levels of Cr are found in house dust collected from the southwest and northeast (Fig. 3a), presumably resulting from the influence of the CFP in the southwest and of metallurgical activities and machine manufacturing in the northeast, respectively. Similar to Cr, Co is also unpolluted in most dust samples, except in dust collected from the southwest (Fig. 3c). In the southwest, the Co in most house dust is slightly polluted with a peak at Site 4 where Co is moderately polluted, which is also very likely related to the influence of the CFP as Ni, Cu and Pb. For Mn and V, they show similar pollution levels, which are practically unpolluted, in all house dust samples from

the whole study area (Fig. 3b and h), suggesting that these two metals are scarcely affected by human activities in this area.

Spatial distribution of the pollution load of heavy metals in house dust. With respect to the integrated pollution of these eight metals in the house dust, PLIs of these metals are between 1.8 and 5.1 with an average of 2.7, suggesting that a moderate integrated pollution level of these eight metals in house dust from the Xi'an western industrial area is moderately polluted, according to the PLI categories in 3.2.3. From the spatial distribution map in Fig. 4, it can be further seen that the PLIs of these metals in most house dust samples are between 2 and 3, indicating a moderate pollution level at most sampling sites. At Sites 13, 15 and 16 (Fig. 4), the PLIs of heavy metals in house dust are relatively lower, only between 1 and 2, indicating an unpolluted to moderately polluted level in house dust, and suggesting relatively good local indoor environmental conditions at these sites. A strikingly high PLI value of 5.1 occurs at Site 4 (Fig. 4), indicating a very high pollution level of these heavy metals in the house dust in this area due to the influence of the CFP. This fact suggests the significant influence of the CFP on heavy-metal pollution in the nearby atmospheric environment, which should be given special attention in the future environmental management.

It should be noted that the calculation model of PLIs in this study only refers to eight heavy metals above and does not include other important toxic elements such as Cd, As and Hg which were not detected because of limitations in experimental conditions. Hence, the real integrated pollution level of heavy metals in the house dust in this area must be even higher, if considering all toxic elements. Exposure to house dust with such high pollution levels of toxic metals must be harmful to human health, especially to children. Therefore, heavy-metal pollution in atmospheric dust is still an important environmental problem in this area and should be further controlled by creating more effective measures such as closing or relocating heavily polluting units, even though local governmental agencies have started to control since 2007.

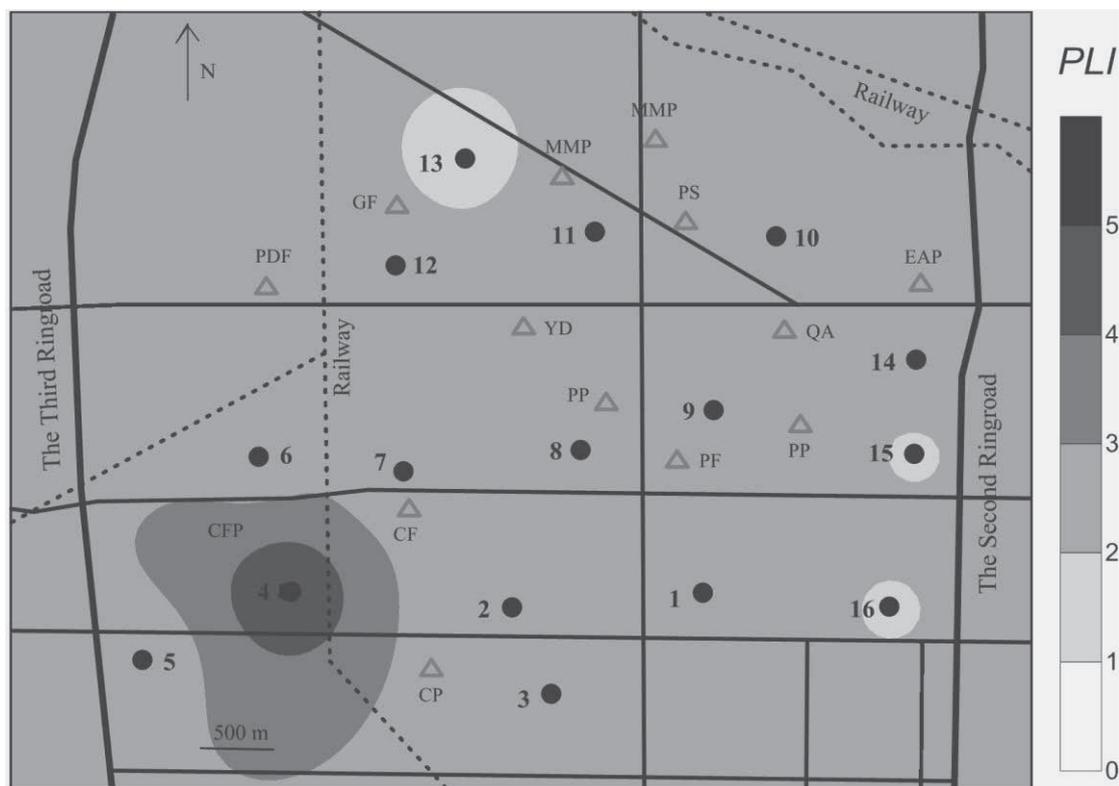


FIGURE 4

Spatial distribution of the pollution load indexes (PLI) of the eight heavy metals in house dust from the Xi'an western industrial area.

CONCLUSIONS

An investigation of representative heavy metals in house dust collected from the Xi'an western industrial area suggests that concentrations of Cr, Mn, Co and V in dust are similar to those in the background soil of Shaanxi, whereas that of Ni, Cu, Zn and Pb are often significantly elevated. Enrichments of Ni, Cu and Zn in house dust may be related to intensively industrial activities in the sampling area, indicated by the significantly higher concentrations of these three metals in the house dust compared with dust in the Xi'an urban area. Results of EF and I_{geo} suggest that the Cr, Mn, Co and V in house dust are basically unpolluted, implying that they are predominantly derived from natural sources, whereas Ni, Cu, Zn and Pb are moderately to strongly polluted, implying that they primarily originated from anthropogenic sources.

With respect to the unpolluted metals of Cr, Mn, Co and V, they often show relatively small spatial variabilities. In contrast, the polluted metals of Ni, Cu, Zn and Pb often show relatively high spatial variabilities, implying that area-source and point-source pollution may occur simultaneously in

the sampling area. The integrated pollution index of these eight metals suggests a moderately polluted level for most house dust. A high pollution load of heavy metals in house dust occurs in the southwestern part of the sampling area, presumably resulting from large amounts of coal combustion in a CFP in this area. This study indicates that the industrial activities in the Xi'an western industrial area have the potential to elevate the levels of heavy metals in environmental media, which may pose risks to human and ecosystem health and thus should be controlled as soon as possible.

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EFFECTS OF NITROGEN APPLICATION ON THE SOIL MICROBIAL ACTIVITY, ENZYME ACTIVITIES AND PROPERTIES AND THEIR RELATIONSHIPS IN A MAIZE FIELD

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ABSTRACT

To assess the effects of nitrogen (N) application on soil in maize fields and to correlate the soil microbial and enzyme activities with the soil properties, we determined the soil microbial activity, enzyme activities and soil properties under five N treatments (N0, N120, N210, N300 and N390). Results showed that the soil microbial activity, enzyme (urease and catalase) activities and soil properties (except pH) increased with the increasing N from 0 kg hm⁻² to 210 kg hm⁻², followed by a decreased trend at N above 210 kg hm⁻². The soil pH always showed a decreasing trend with the increasing N application. The positive correlation between soil properties (soil organic matter and total N) and microbial and enzyme activities indicated that good soil properties promote soil microbial growth, which interact with each other. Moreover, the significantly positive correlation of the total N, microbial activity and catalase activity indicated that optimal N conditions are beneficial for soil biological processes. Generally, the results of this study demonstrated that rational N application will increase soil physical and biological properties and respond to biogeochemical cycling in soil-maize ecosystems.

KEYWORDS:

Nitrogen; Soil microbial activity; Enzyme activity; Soil properties; Maize

INTRODUCTION

Nitrogen (N) is one of the most abundant mineral elements and is a key factor used to increase crop production [1]. With the organic fertilizer deficiency in recent years, sufficient urea for crop growth has become an essential aspect for high crop yield. Increasing N application would influence soil C/N ratio [2] and affect the soil microbial activity, thereby changing the soil

microbial properties [3, 4]. Various studies have shown that N fertilizer is an efficient management strategy in agriculture [5, 6]. The effect of N application on soil systems has been previously reported. Previous studies showed that the N supply revealed the N transformation in orchard soil [7], the effects of N application on soil nitrate leaching [8] and N management in the soil system of wheat-maize cropping [9], even total N has been one of the most main pollutants in the Haihe River of China between 2001 and 2010 [10]. However, the effects of N application on soil microbiological properties in the field under different growth times have been rarely studied.

Maize (*Zea mays* L.) is the second most important grain crops in China. Maize is mainly used as animal feed, its production is essential for food safety in China and throughout the world [11, 12]. Like in other grain crops, fertilizer application is an important measure in growing of maize grain [13] and national food security strategies (especially N fertilizer application) because of the increasing use of fertilisers for crop management. Thus, the cost of maize increases.

Microorganisms play a powerful role in soil because they drive nutrient cycling, conservation and organic matter build up [14]. Soil microbial activity is an important indicator that is increasingly used in the estimation of soil ecosystem sustainability [15, 16]. Soil enzymes come from active microorganisms and function as catalysts in soil biochemical processes. In addition, enzyme activity responds more quickly than other variables when soil environments change. Consequently, microbial and enzyme activities have been greatly recognized in the integrative assessment of soil [17]. These properties are highly sensitive to fertilisation rate [18] and changes in the soil properties [19]. Moreover, most of the research on soil microbial and enzyme activities has been based on laboratory measurements. Very few of these studies used field and growth data to study soil microbial activity by microcalorimetry.

We hypothesized the following: (1) N application would significantly affect the maize soil properties and (2) microbial activity would be associated with the enzyme activity, which is affected by N application or growth stage. In this study, we used a combination of biochemical techniques (enzyme activity) and microcalorimetry (microbial activity) to study these soil environmental factors.

MATERIALS AND METHODS

Field experiment sites. The field experiment commenced in the 2010 maize season at the Baoding field station, which is located in Hebei Province of China (3805'N, 115030'E). The study area has a temperate monsoon climate, with an annual average temperature of 12 °C and an annual precipitation of 550 mm, of which approximately 70% is from July to September. Before the experiment, the physical and chemical soil properties in surface layer (0 - 20 cm) were as follows: pH value of 8.5, soil organic matter (SOM) of 16.8 g kg⁻¹, total N of 0.9 g kg⁻¹, Olson-P of 16.6 mg kg⁻¹ and Olson-K of 99.3 mg kg⁻¹ in May 2010.

Experiment design and soil sampling. A randomized block experimental design was used with three replicates of each treatment. Each plot was 40 m² (8 m × 5 m). The experiment consisted of treatments with 0 (N0), 120 (N120), 210 (N210), 300 (N300) and 390 (N390) kg N hm⁻². The N fertilizer used was urea (46%), which was applied at seeding stage (30%), the ten-leaf stage (50%) and the silking stage (20%). All treatments included 120 kg of P₂O₅ (12%) and 120 kg of K₂O (60%) hm⁻², which were applied with basal fertilizer. Maize cultivars "Zhengdan 958" was used and planted in middle of June and harvest in early October with a planting density of 60000 plants hm⁻². Water was irrigated at 60 mm at seeding stage, after which the irrigation water was from rainfall. Other aspects of field management followed the usual practices of farmer.

Soil samples were taken from the surface layer (0 - 30 cm) in July and September 2013. Each soil sample was thoroughly mixed (three soil samples per treatment) to obtain composite samples. The soil samples were air-dried and passed through a 2 mm sieve before chemical analyses.

Soil property. Soil pH was determined using a solution of soil and distilled water (1:2.5). Soil samples were oven dried at 105 °C for 8 hour to

measure the soil moisture. Total N was measured by the semimicro-Kjeldahl method [20]. Soil organic matter (passed through a 0.15 mm sieve) was determined using K₂Cr₂O₇-H₂SO₄ titration [21]. Olsen-P was extracted with NaHCO₃ and was measured by colorimetrically method [22].

Enzyme activity. Soil urease activity was based on the colorimetric determination using 2.5 g of soil at 578 nm wavelengths measured and expressed as mg kg⁻¹ 24 h⁻¹. Soil catalase activity was based on permanganate titration method using residual H₂O₂ with KMnO₄ and was expressed in 0.002 mol L⁻¹ KMnO₄ mL g⁻¹ [23].

Microbial activity. Soil microbial activity study was using an isothermal microcalorimeter (TAM III), equipped with 12 calorimetric channels at 28 °C for some days. For the experiments each ampoule of 1.0 g soil with 5.0 mg glucose and 5.0 mg ammonium sulfate, then microcalorimetry ampoules were air-tightly sealed and introduced into the microcalorimeter, a computer is used to continuously monitor and record the metabolic power-time of soil [24].

Data analysis. Microbial activity was presented using power-time curves. Enzyme activity was presented in figures and subjected to one-way ANOVA to test for significant differences between the treatments.

The principal component analysis (PCA) was based on the soil chemical and microbial properties. The data in PCA analysis are decomposed into separate sets of scores and loadings for each of the two modes of interest (treatments and variables). All statistical analyses were performed with the SPSS 18.0 and Origin 8.0 software (significance level at P < 0.05).

RESULTS

Soil properties. The differences in the soil physicochemical properties under different N applications are shown in Table 1. The soil pH decreased with the increasing N application. The soil moisture generally increased with maize growth because of the heavy rainfall in September, when the soil moisture markedly increased from 0 kg hm⁻² to 210 kg hm⁻² of N. The total N in treatments under N120, N210 and N300 were not significantly different from each other, but were significantly higher than under the N0 and N390 treatments. The SOM was significantly increased under N210, but did not show time-related

differences. The soil Olsen-P varied within a relatively small range and fluctuated within the range 15.8 - 17.4 mg kg⁻¹. Moreover, the trends of the soil moisture, total N, SOM and Olsen-P was

similar, their values increased from N0 to N210, but decreased with higher N fertilizer rates. In particular, the pH, moisture, total N and SOM were significantly decreased under N390.

TABLE 1
Soil physicochemical properties and microcalorimetric parameters under level of N application taken in July and September.

| Treatment | pH | Moisture % | Total N g kg ⁻¹ | SOM g kg ⁻¹ | Olsen-P mg kg ⁻¹ | Q_{total} J g ⁻¹ | P_{max} μW | Biomass C mg g ⁻¹ | $k \times 10^{-3}$ min ⁻¹ | r^2 | |
|-----------|------|------------|----------------------------|------------------------|-----------------------------|-------------------------------|--------------|------------------------------|--------------------------------------|--------------|--------|
| July | N0 | 8.5±0.21a | 13.4±2.08ab | 0.63±0.12 b | 11.15±0.35c | 15.8±1.54ab | 33.7±3.38a | 296.8±29.54c | 1.70±0.01b | 3.98±0.18c | 0.9938 |
| | N120 | 8.5±0.04a | 13.9±1.15a | 0.84±0.04ab | 15.07±2.59ab | 16.1±2.67a | 32.8±3.52a | 593.1±33.96b | 3.46±0.06ab | 8.66±0.11a | 0.9967 |
| | N210 | 8.4±0.06a | 14.7±2.04a | 1.02±0.22a | 18.64±3.49a | 17.3±1.94a | 36.5±4.95a | 684.1±30.81ab | 4.01±0.13a | 9.13±0.11a | 0.9935 |
| | N300 | 8.2±0.07a | 12.2±2.10b | 0.83±0.10ab | 14.39±2.82b | 16.7±2.06a | 19.1±2.17b | 756.8±37.43a | 4.45±0.28a | 8.26±0.11ab | 0.9991 |
| | N390 | 8.0±0.10b | 11.7±1.21b | 0.75±0.06b | 13.53±1.53b | 16.4±2.68a | 33.1±4.76a | 295.7±25.19c | 1.70±0.16b | 7.86±0.09b | 0.9944 |
| September | N0 | 8.4±0.03a | 16.1±1.72bc | 0.61±0.18b | 11.01±1.29c | 15.9±2.71a | 45.1±2.86ab | 454.8±21.45d | 2.64±0.04b | 9.03±0.25c | 0.9994 |
| | N120 | 8.3±0.02a | 17.1±2.04b | 0.80±0.12a | 14.34±3.19b | 16.2±1.83a | 20.7±1.99c | 772.8±29.14c | 4.54±0.05ab | 12.00±0.33bc | 0.9976 |
| | N210 | 8.3±0.11a | 19.7±2.58a | 0.92±0.15a | 17.72±2.47a | 17.4±2.01a | 52.8±5.75a | 988.9±32.76a | 5.85±0.04a | 18.20±0.21a | 0.9996 |
| | N300 | 8.0±0.05ab | 16.6±1.91b | 0.80±0.10a | 14.14±1.41b | 16.1±1.28a | 46.7±3.08ab | 871.2±34.83b | 5.14±0.02a | 15.01±1.31ab | 0.9907 |
| | N390 | 7.9±0.13b | 15.8±2.17c | 0.71±0.05ab | 12.96±3.58b | 15.9±3.38a | 52.1±4.87a | 761.1±30.99c | 4.47±0.07ab | 13.74±0.30b | 0.9989 |

The values are the means of three replicates. Values followed by different letters differ significantly.

Soil enzyme activity. Results (Fig. 1A) showed that the soil urease activity varied from 11.35 mg kg⁻² to 27.70 mg kg⁻² per day and was lower in July than in September. The urease activity was significantly greater under N application (0-210 kg hm⁻²), but decreased when the N application exceeded 210 kg hm⁻². By contrast, only a slight increase in soil catalase (CAT) activity (Fig. 1B) was observed from July to September. In addition, no changes were evident among the N120, N210 and N300 treatments, but relatively increased

by 45.8% and 36.7% under N210 compared with N0 in July and September, respectively.

Soil microbial activity. All power-time curves, except those of N390 and N0 in July and N0 in September, presented a similar process of microbial metabolic activity, as shown in Fig. 2. The gentle curves and slow exponential phase of N390 and N0 in July and N0 in September indicated that microbial growth was lower than in the other treatments. Although the N390 treatment had more fertilizer than other N treatments, the highest microbial growth was not observed.

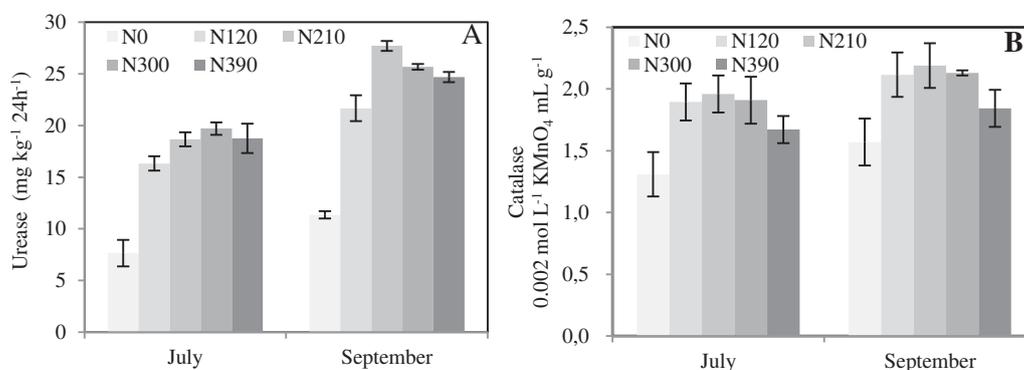


FIGURE 1
Urease (A) and catalase (B) activities from the different treatments soil in July and September. Error bars indicate standard deviations (n=3).

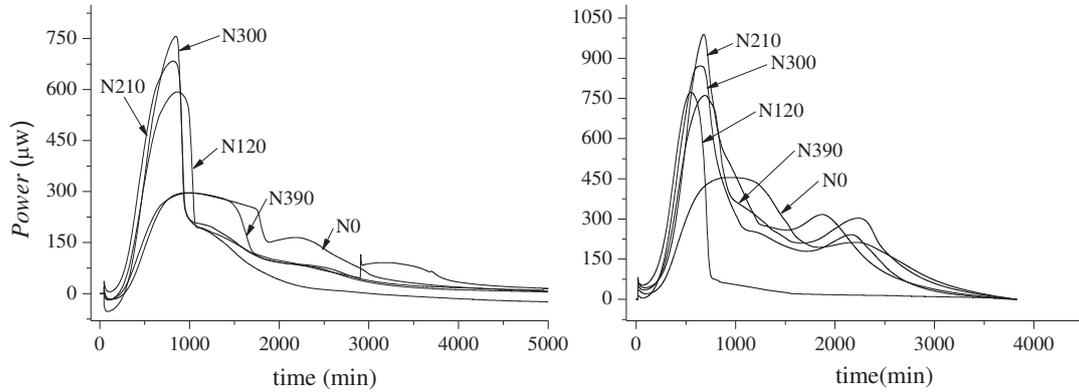


FIGURE 2
Power-time curves was recorded by microcalorimetry. A: Soil samples were collected in July, B: Soil samples were collected in September

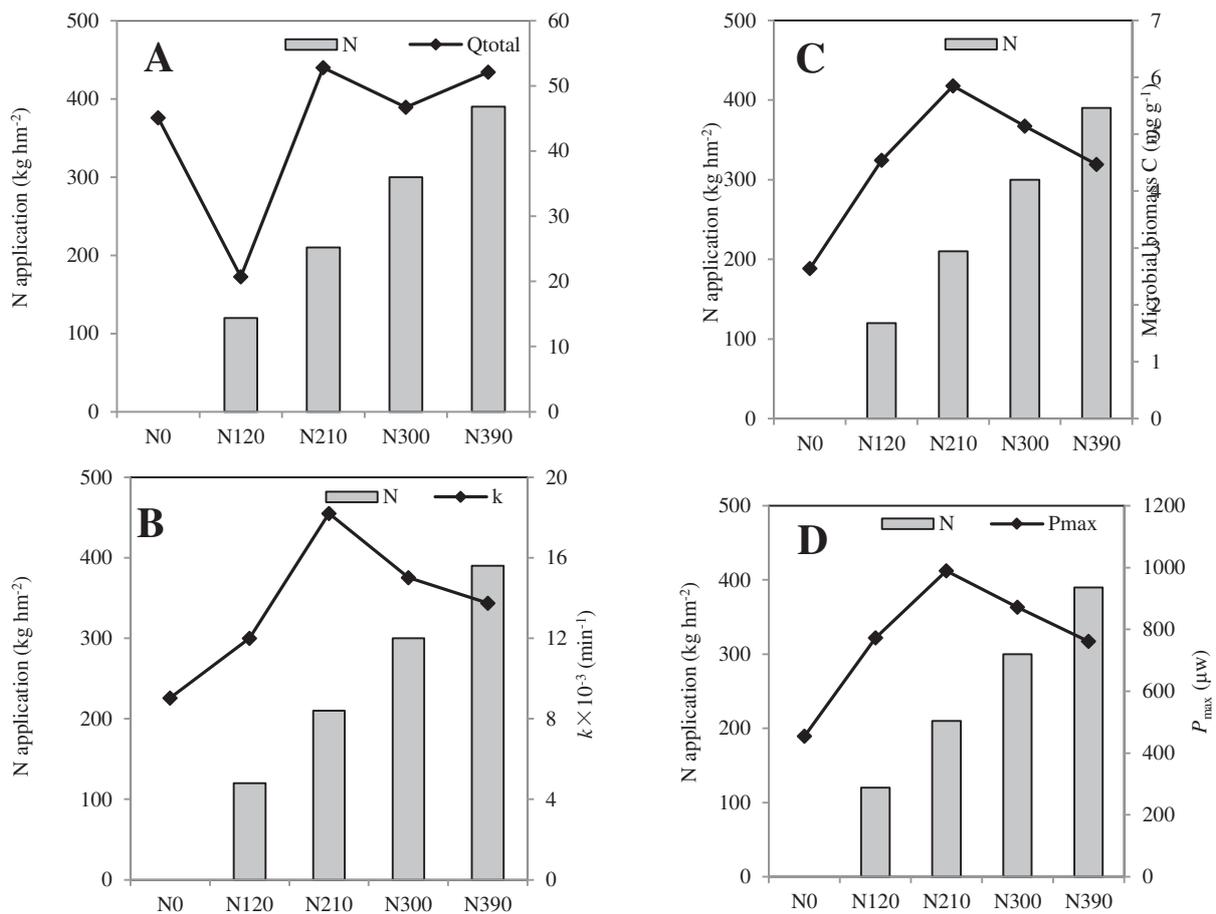


FIGURE 3
Effects of N application on (A) soil microbial total heat, (B) soil microbial growth rate constant, (C) soil microbial biomass C, (D) the power at the maximum of the peak.

From the power-time curves we calculated the Q_{total} (total heat output), P_{max} (the power at the maximum of the peak), k (microbial growth rate constant) and microbial biomass C , which are presented in Table 1. Generally, the microbial metabolism under N210 in September showed the highest value of k , P_{max} , Q_{total} and microbial biomass C . However, the highest value of k and Q_{total} in July were observed under N210, whereas the highest value of P_{max} and microbial biomass C observed under N300. Overall, these results indicated that N210 had the higher activity than the other treatments.

The relationship between the level of N application and the microcalorimetric parameters is shown in Fig. 3. The Q_{total} included the metabolic heat from all the active microorganisms. No correlation was observed between Q_{total} and N application probably because not all microorganisms in the system were counted. P_{max} , k and Q_{total} followed a similar trend, such that the microbial activity was significantly higher under N210 than under other N applications.

A significant effect of N application on soil in maize fields was revealed by PCA (Fig. 4). The PCA showed that 89.64% of the total variance in the data set. The first principal component (PCA1) explained 72.52% of the variance and the second principal component (PCA2) explained 17.12% of the variance. With increasing N application, the change trends of the scores were shown by PCA1 and PCA2 (five-pointed star). The scores for PCA1 and PCA2 both showed an increasing trend from 0 kg hm⁻² to 210 kg hm⁻² of N. Afterward, scores showed a decreasing trend from 210 kg hm⁻² to 390 kg hm⁻² of N. The factor loading of PCA (square) showed the PCA1 had high factor loading (>0.9) for microbial biomass C , P_{max} , total N, k , SOM, urease activity. Meanwhile, the PCA2 had high factor loading (>0.9) for pH, whereas microbial biomass C , P_{max} , k , Q_{total} , SOM and urease activity had negative factor loading.

All the soil physiochemical properties influenced soil microbial activity, and a close relationship was found between these properties and soil microbial activity. For example, good soil

property generally showed high microbial activity [23]. The relative coefficient between soil properties and microbial activity was strong, only Q_{total} and the soil properties showed weak r^2 values, which ranged between 0.006 and 0.382 (Table 2). All the microbial parameters had a positive correlation with soil property (moisture, total N, SOM and Olsen-P) and a negative correlation with the soil pH. In addition, P_{max} , biomass C and k were significantly associated with the total N and SOM ($P < 0.05$). The CAT activity was significantly correlated with the total N ($P < 0.05$). By contrast, the moisture content and Olsen-P of soil were not significantly associated with the soil microbial parameters.

DISCUSSION

Soil urease activity was widely reported as an important indicator of N cycling and supply [26]. Increasing its activity would effectively reduce N deposition [27]. In the present study, the urease activity rapidly responded to change resulting from the addition of N fertilizer. Sufficient N increased the enzyme activity, but excess N did not stimulate activity. This trend suggests that excess N may be leach [28]. Additionally, the negative relationship between urease activity and soil pH (Table 2) can be explained by this result because the soil urease activity is decreased by N fertilizer-induced acidification [29].

CAT is present in all aerobic microorganisms. Cells can catalyze hydrogen peroxide into molecular oxygen and water, thereby protecting organisms from damage caused by reactive oxygen species. The effect of soil contamination [30] and Bt-rice [31] on CAT activity has been reported. In our study, the significant stimulatory effect of N application on CAT activity was observed with maximum activity under N210 treatment. High N application may have enhanced nitrification and denitrification [32], and the anaerobic environment benefited from the reduced nitrates. The denitrifying bacteria released molecular N₂ and N₂O [33], thus inhibiting the CAT activity. In addition, CAT may be less sensitive than urease to the changes caused by soil environmental stress.

TABLE 2
Relationship between soil physiochemical properties and soil biological parameters in September.

| | Q_{total} | P_{max} | Biomass | | | Urease | Catalase |
|----------|-------------|---------------|---------------|---------------|--------|---------------|----------|
| | | | C | k | | | |
| pH | -0.372 | -0.340 | -0.338 | -0.317 | -0.557 | -0.159 | |
| Moisture | 0.120 | 0.684 | 0.686 | 0.736 | 0.502 | 0.652 | |
| Total N | 0.006 | 0.947* | 0.947* | 0.891* | 0.838 | 0.944* | |
| SOM | 0.105 | 0.916* | 0.917* | 0.906* | 0.801 | 0.868 | |
| Olsen-P | 0.235 | 0.701 | 0.703 | 0.782 | 0.540 | 0.619 | |

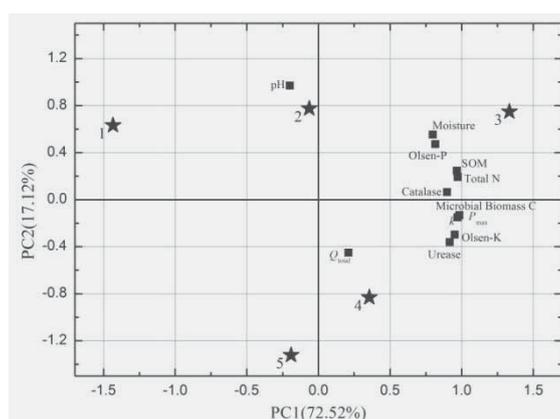


FIGURE 4
Principal Component Analysis (PCA) analysis of soil property, microcalorimetry parameter and enzyme activity under different N application in September. The numbers are as follows: 1 (N0), 2 (N120), 3 (N210), 4 (N300), 5 (N390). The symbols are as follows: the scores of PCA1 and PCA2 (five-pointed star), factor loadings (squares).

Microcalorimetry has been successfully used by various researchers to evaluate microbial metabolism [34, 35]. The power-time curves in Fig. 2 showed that the soil microbial activity was influenced by N application. To explain this effect better, the microcalorimetric parameters provided more detail information (Table 1, Fig. 3). Q_{total} is highest in N210 and lowest in N120, thereby suggesting that Q_{total} was associated with the microbial community and species, as well as the soil nutrition. All these factors were significantly affected by the fertilizer [36], thus, N210 had the highest Q_{total} . For N120, the amount of N applied to soil would cause competition between the soil microorganisms and crops, thereby causing the degradation of N nutrients in the soil and the release of less Q_{total} . Given the reduced competition of microbes and crop, N0 did not have the lowest Q_{total} even though N had zero N applied. P_{max} , k and microbial biomass C are probably the main parameters that can be used to evaluate the soil microbial activity [37]. N210 showed higher P_{max} , k and microbial biomass C value, and we obtained a significant positive

correlation among the total N, SOM, P_{max} , k and microbial biomass C. This trend is in agreement with the results of other studies [38]. The abovementioned results also proved that microcalorimetry is a simple and powerful method for assessing the soil microbial activity under different levels of N application.

Nitrogen fertilizer management is an important measure for improving soil properties. Several studies proved that the optimum N application can influence soil properties and can support the soil microbial and enzyme activities by increasing the available nutrients and ultimately improving the cyclic processes of soil ecosystem [39]. The data for SOM, P_{max} and k suggested that N is a limiting factor for soil microbes in maize fields. The total N and SOM were significantly associated with soil microbial activity because of their complex interaction. The soil chemical properties and organic matter content are strongly affected by the microbial community composition [40].

The positive influence of the improved soil properties and the negative influence of the pH

were consistent with a previous study of [41] which suggested that the soil pH had apparent control on the microbial activity, enzyme kinetics and microbial community because soil pH strongly influences the denaturation of enzyme active centers and enzyme ionisation [42].

Our results showed that the microbial and enzyme activities were higher in September than July. This trend might be related to the sampling time in our study. However, soil moisture did not affect the microbial and enzyme activity (Table 2). By contrast, other studies confirmed that moisture was an important factor that affected enzyme activity [43, 44]. In addition, soil moisture was positively correlated with the soil microbial community characteristics [45], because when N fertilizer applied to soil with more water, the soil inorganic N and dissolved carbon would increase, and the ratio of C:N would decrease [46]. Similarly, Olsen-P was also not correlated with the microbial and enzyme activities. However, Zheng [47] previously confirmed that available P was a limiting factor for microbial activity. Therefore, we suspect that the soil microbial and enzyme activities may have been caused by responses to variations of the soil environmental situation.

CONCLUSIONS

This study demonstrated that the effects of N application on the soil microbial and physicochemical properties in maize fields. With increasing N application, the soil pH decreased, whereas moisture, SOM, total N, P_{max}, k and microbial biomass C significantly increased. These soil properties showed an increasing trend initially and then a decreasing trend. The combined results suggested that N application has positive and negative effects on soil. The optimum N application increased the supply of SOM and total N, which helped improve soil properties and increase microbiological properties. However, excess N application induced low pH, with direct and indirect effects on soil properties, thereby decreasing microbial and enzyme activities. In addition, a significant correlation was observed among the total N, SOM and microbial activity, which were influenced by N application. The application of N fertilizer to soil ultimately affected the soil ecosystem processes. Therefore, reasonable N application is critical. In maize fields, the favorable N level of N application was ≤ 210 kg hm⁻².

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USING SOIL PROFILE TO ASSESS GROUNDWATER RECHARGE AND EFFECT OF LAND-USE CHANGE IN A RAIN-FED AGRICULTURAL AREA

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ABSTRACT

Soil profile has been used to estimate diffuse recharge and land-use change effects in a semiarid rain-fed agricultural area in the Chinese Loess Plateau. Measurements have included Cl, NO₃, δ²H and δ¹⁸O content in soil moisture and water chemistry and isotopes (³H, δ²H and δ¹⁸O) in groundwater. The results show that the diffuse recharge rate estimated from chloride mass balance of two soil profiles is 33-56 mm/yr beneath crops, as the main land use of the study area. The infiltration rate is 0.13 to 0.27 m/yr. Due to the limited recharge rate and thick unsaturated zone, it takes 110 to 460 years for annual precipitation to reach the water table, as confirmed by the absence of tritium (<0.3 tritium unit). The nitrate introduced by human activities is mainly distributed in the upper unsaturated zone, and the groundwater nitrate content (0.1 to 9.3 mg/L as NO₃) remains at baseline level. Groundwater Cl content and stable isotopic composition (δ²H and δ¹⁸O) is lower than that in the upper unsaturated zone, which is possibly related to recent climate change and human activities. The conversion from crops to orchard has resulted in decrease in soil moisture and increase in Cl content, suggesting decrease in deep drainage. This does not appear to be favourable to the groundwater resources, and could accelerate depletion of the soil reservoir in the Chinese Loess Plateau.

KEYWORDS:

soil profile; chloride mass balance; groundwater recharge; Chinese Loess Plateau;

INTRODUCTION

When more than two billion people in the world depend on groundwater for their daily supply [1], recharge is one of the most important parameters for a groundwater system. However, in arid and semiarid area, high evapotranspiration

results in higher uncertainty in recharge when using water mass balance to estimate recharge rate (e.g., when the uncertainty of precipitation (P) and evapotranspiration (E) is both 10%, then uncertainty of recharge (R) may exceed 150%, $P-E=R$, $(450\pm 45)-(420\pm 42)=30\pm 87$) [2]. Darcy flux measurement usually involve large errors in recharge estimation for (semi)arid areas as soil moisture content and flux is low [3]. Using the environmental tracers in the unsaturated zone (UZ) (e.g., ³H, ³⁶Cl and chloride mass balance) to estimate groundwater recharge has made great progress when considering an arid area [4]. The results obtained by the chloride mass balance (CMB) are usually consistent with the independent recharge estimates obtained from tritium peak analysis in many sites [5]. Scanlon et al. [2] made a synthesis of global groundwater recharge estimations in (semi)arid areas and pointed out that the CMB technique is now widely and generally successfully used to estimate groundwater recharge.

In China, the studies using environmental tracers are few. Available data shows that diffuse recharge is approximately 1-3 mm/yr for the Badain Jaran Desert [6-7] and Tengger Desert [8] in the Gobi desert, NW China based on the CMB, where the annual precipitation is less than 150 mm/yr. The diffuse recharge is 30-100 mm/yr in rain-fed agricultural areas based on the CMB in the Loess Plateau [9-11]. The tritium peak in soil profiles shows that the recharge is about 47-68 mm/yr and infiltration rate is 0.25-0.30 m/yr for two loess sites, where precipitation ranges from 360-550 mm/yr [12-13]. To the north of the Loess Plateau, Yin et al. [14] also use the CMB to quantify the recharge rates of groundwater in the Ordos Plateau, which varied from 5.5 to 73.9 mm/yr. However, the CMB conducted in the Ordos Plateau studies used groundwater samples. At present, few studies combine the UZ with the saturated zone together to investigate the solute distribution in the UZ and the aquifer as well as its implications on groundwater recharge [15] in the Chinese Loess Plateau. Such an investigation is important for current and future

groundwater resources management, following recent large-scale anthropogenic activities.

The other issue related to groundwater recharge is land use change (mainly vegetation change) in NW China. The great progresses in the construction of eco-environmental have been made, especially afforestation in the North, Northeast and Northwest China, and afforested area had reached 30.24 million ha. However, the relative survival rate and preservation rate is only 25% and 13%, respectively [16]. In addition, local governments want to develop orchards to help increase the income of farmers in many sites in the Loess Plateau. Previous studies show that the soil moisture has depleted after plantation [17-18]. However, it is difficult to quantitatively assess the impact of land use change on diffuse recharge rate in arid and semiarid northwest China using unconventional methods, which is mainly derived from humid area.

The goal of this study is to use multiple tracers (chloride, nitrate and stable isotopes in soil moisture; water chemistry, stable isotopes and tritium in groundwater) to 1) estimate groundwater diffuse recharge, and 2) assess the impact of land use on groundwater recharge using soil profile in a rain-fed agricultural area in the Loess Plateau.

METHODOLOGY AND MATERIALS

Methodology. The groundwater recharge was estimated using the chloride mass balance (CMB) [19-20]. Assuming that the only source of chloride is atmospheric (rainfall or dry deposition) and that there is no contribution of chloride from weathering, the surface runoff is negligible and the 1-D vertical steady-state chloride flux is tenable, the recharge rate (R) is given by:

$$R=J / C_s \quad (1)$$

where J is the atmospheric chloride deposition ($J=P \times C_p + D$, where P is precipitation, C_p is the chloride concentration in precipitation, and D is net dry deposition) and C_s is the mean Cl concentration of pore waters presenting potential recharging [5].

In rain-fed agricultural area, the higher nitrate inventories in the UZ and groundwater (if moisture from UZ enters aquifer) is mainly introduced by human activities, e.g., the overuse of fertilizer [21].

Therefore, it can be used as a recent recharge indicator.

Stable isotopes (^2H and ^{18}O) are used semiquantitatively to determine sources and mechanism of recharge. Environmental tritium, introduced into the hydrological cycle by atmospheric thermonuclear since 1950s, can be used to estimate groundwater age. At present, It is difficult to give accurate age due to relatively short half-life (12.32 a). However, the undetected tritium content can be considered as pre-modern water (before the 1950s); by contraries, water containing tritium is modern water.

Study area. The study area, Xifeng loess plain is located in the middle part of the Loess Plateau (Fig. 1). The loess depth ranges from 150 to 200 m. The silt particle content of loess accounts for a significant proportion (50-80%) [22]. The horizontal Neogene/Cretaceous mudstone and sandstone beds are covered by Quaternary loess [23]. The Quaternary loess is comprised of lower Pleistocene (Q_1), middle Pleistocene (Q_2), and upper Pleistocene (Q_3). The Q_1 Loess, with a thickness of 40-60 m, commonly crops out at the bottom of the upper and middle reaches of gullies. The hard and compacted Q_1 Loess has low permeability and is usually considered as an aquitard. The Q_2 Loess has a thickness of 120-150 m, and commonly crops out on the sides of valleys and at the heads and cliffs of gullies. The Q_2 Loess is unconsolidated and has relatively large porosity, and is considered to be a good aquifer. The Q_3 Loess has a thickness of 10-15 m, and is distributed above the Q_2 Loess, as the top soil in the area, though the Holocene (Q_4) Loess is present in some cases. In the Xifeng Loess Plain, the regional groundwater flow is from north to south. The depth of the water table mainly ranges from 30 to 60 m with an increasing trend from the centre of the plain to its surrounding. The discharge of groundwater is mainly in the form of 'suspension' gravity springs in gullies (Fig. 2) [24].

The average precipitation is 523 mm/yr and approximately 60-70% of the annual precipitation falls between July and September during the Asian monsoon. The average temperature is 8.5 °C. The main crops are winter wheat and maize (one crop per year). However, the local government wants to develop orchards to help increase the income of farmers in Xifeng.

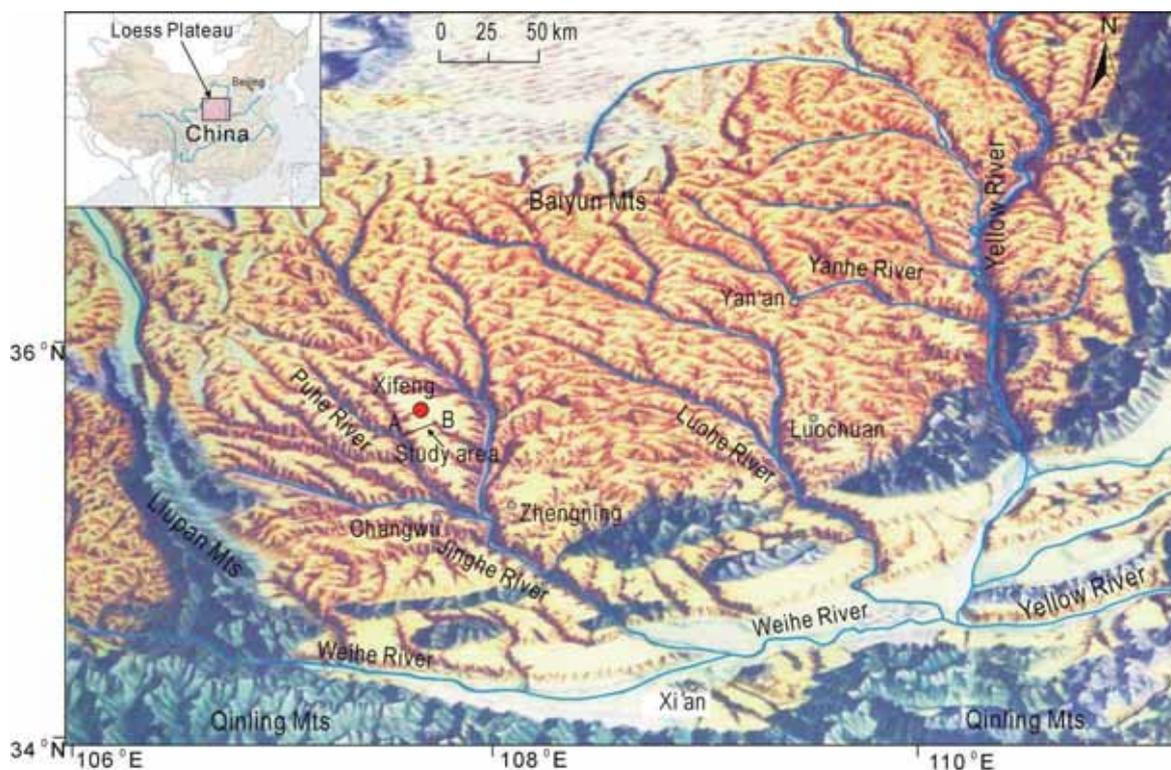


FIGURE 1

The study area (Xifeng loess plain) and topography of the Loess Plateau (modified from reference [23])

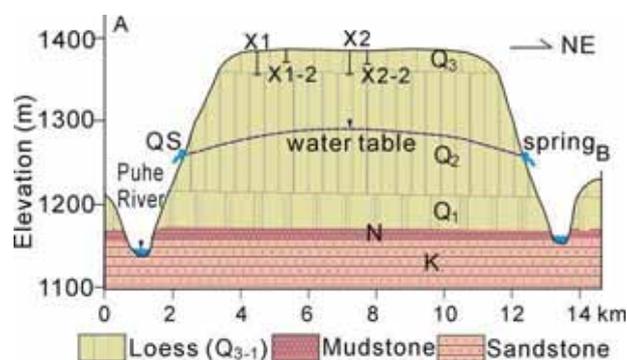


FIGURE 2

The hydrogeological profile of the Xifeng loess-plain and the soil profile location

Sampling. Soil samples were collected from four profiles in the Xifeng loess-plain (Fig. 2). Table 1 presents the characteristics of those profiles. Soil profile X1 and X1-2, with depth of 14.5 m and 6 m, is taken from crops (wheat and/or maize), which is currently the main land use, while the soil profiles X2 and X2-2 with depth of 15 m

and 6.25 m are taken from apple orchard. The soil samples were obtained using a hollow-stem hand auger. Bulk samples of 300 - 500 g were collected at an interval of 0.25 m. The samples were immediately sealed in polyethylene bags after collection. Groundwater samples and spring were also sampled.

TABLE 1
Descriptions of the unsaturated zone profiles

| Profile | Depth (m) | Land use |
|---------|-----------|---------------|
| X1 | 14.5 | wheat/maize |
| X1-2 | 6 | wheat/maize |
| X2 | 15.0 | apple orchard |
| X2-2 | 6.25 | apple orchard |

Analyses. The gravimetric moisture content (θ) for soil sample was determined by drying a minimum of 80 g of soil at 110 °C for 12 hrs. To determine the stable ion (Cl and NO_3), double-deionized water (40 ml) was added to an oven-dried sediment sample (40 g). Samples were agitated intermittently for 8 hrs. The supernatant solution was filtered through 0.45- μm filters. The solutes were then analysed using ion chromatography. The chloride and nitrate concentrations of the soil moisture were then calculated by dividing the measured concentrations by the relevant gravimetric moisture content and then multiplying by the mass ratio of the solution over the oven-dry sediment (1:1 in the study).

For stable isotopic analysis, soil moisture for profiles X1 was extracted using azeotropic distillation [25] with a reported accuracy of 2‰ for $\delta^2\text{H}$ and 0.2‰ for $\delta^{18}\text{O}$. Resulting samples were collected in glass vials, and a few grams of paraffin wax were added to the vials to remove remaining traces of toluene. Stable isotopes for groundwater

and soil moisture samples were measured at the Laboratory of Water Isotopes and Water-Rock Interaction, Institute of Geology and Geophysics, Chinese Academy of Sciences, by using Picarro L1102-I isotopic water liquid analyser. Results are reported as $\delta^2\text{H}$ and $\delta^{18}\text{O}$ ($\delta = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000$) using the Vienna Standard Mean Ocean Water (VSMOW) as the standard. The analytical precision is 1‰ for $\delta^2\text{H}$ and 0.1‰ for $\delta^{18}\text{O}$. Groundwater tritium was analysed to determine the groundwater age. The tritium contents were measured in the same laboratory through electrolytic enrichment with a tritium enrichment factor of ~ 20 and the liquid scintillation counting (Quantulus 1220) method with a detection limit of 0.3 TU (Tritium Unit). Water anions were analysed by ion chromatography (Dionex-500) and cations by inductively coupled plasma optical emission spectrometer (ICP-OES) at the Beijing Research Institute of Uranium Geology. The charge balance error for groundwater samples are within $\pm 5\%$. The results are shown in Table 2.

TABLE 2
Isotopic composition and water chemistry (mg/L) for spring and shallow groundwaters

| Sample | ^3H (TU) | $\delta^{18}\text{O}$ (‰) | $\delta^2\text{H}$ (‰) | TDS | Cl^- | SO_4^{2-} | HCO_3^- | NO_3^- | Na^+ | K^+ | Mg^{2+} | Ca^{2+} |
|--------|-------------------|---------------------------|------------------------|-----|---------------|--------------------|------------------|-----------------|---------------|--------------|------------------|------------------|
| QS | <0.3 | -9.9 | -70.0 | 308 | 8.6 | 10.3 | 340 | 7.0 | 31.3 | 1.5 | 26.0 | 53.2 |
| XG1 | <0.3 | -9.9 | -73.7 | 274 | 20.1 | 6.3 | 310 | 0.1 | 12.4 | 0.6 | 35.6 | 44.1 |
| XG2 | <0.3 | -10.0 | -71.9 | 233 | 8.4 | 5.2 | 269 | 4.3 | 17.0 | 0.8 | 26.1 | 36.7 |
| XG3 | <0.3 | -9.6 | -70.6 | 234 | 7.6 | 6.7 | 254 | 9.3 | 35.2 | 1.8 | 22.3 | 23.8 |
| XG4 | <0.3 | -9.8 | -71.3 | 308 | 7.0 | | | | | | | |

RESULTS AND DISCUSSION

Recharge to aquifer. As crops (e.g., winter wheat and/or maize) is the main land use in the study area, soil profile X1 and X1-2 beneath crops is used to estimate initial diffuse recharge. Soil moisture and chloride in the root zone (commonly less than 2 m for crops) is easily susceptible to near surface parameters, e.g., plant root uptake,

precipitation, evapotranspiration. After rainfall events, water and solutes mix in this zone, and water is either removed by evaporation or transpiration, or is transmitted below by gravity. The lower boundary of the root zone is theoretically defined as the surface below which evapotranspiration is negligible (i.e. the zero flux plane) [26]. In this study, 2 m is simply considered as the lower boundary and below 2 m as vadose zone.

The soil moisture for profile X1 ranges from 9.6% to 23.7% with an average of 17.8%, while X1-2 from 10.5% to 17.1% with an average of 14.3% (Table 3 and Fig. 3). The variation of soil moisture along depth is main due to the change in

soil particle-size [23, 24] and soil contains more clay could maintain more moisture. Although X1-2 is shallow, its soil moisture show similar trend with X1 below 2 m (Fig. 3).

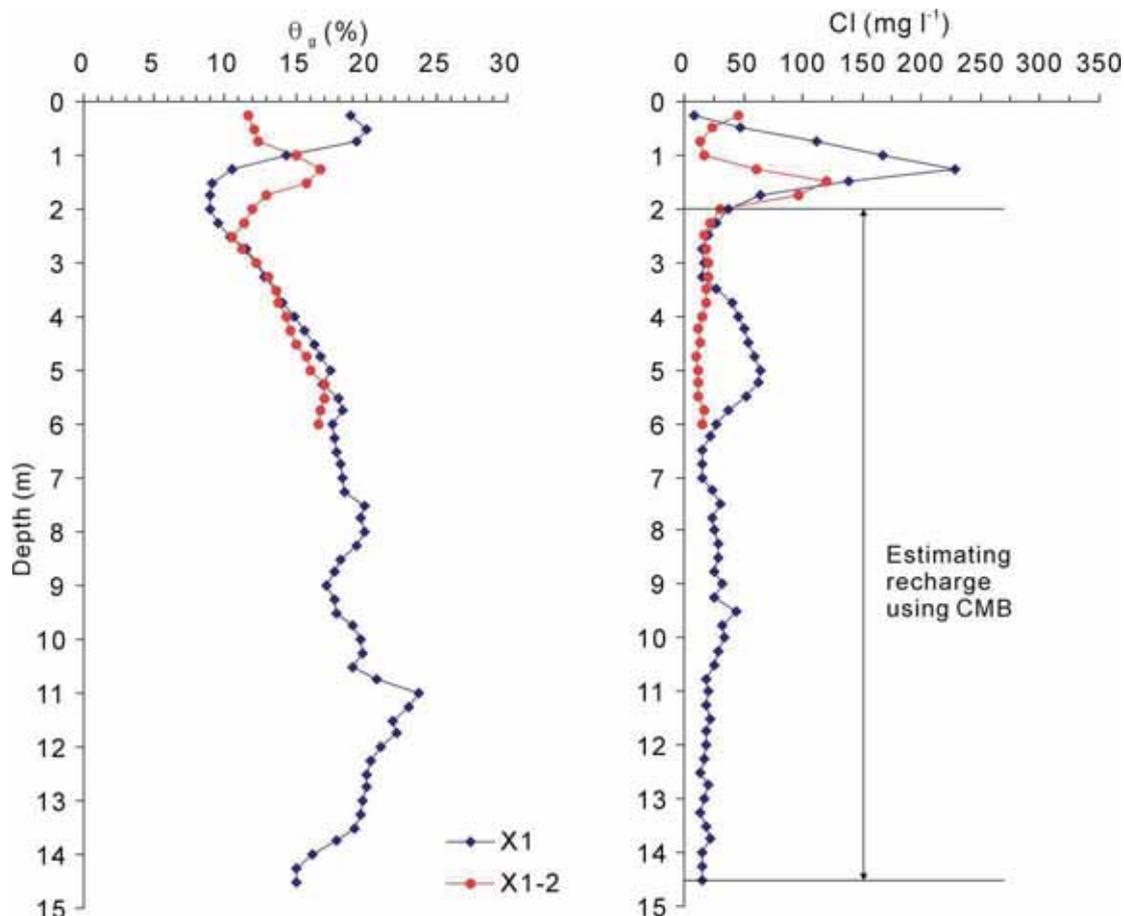


FIGURE 3
Soil moisture and Cl content of profile X1 and X1-2 beneath winter wheat / maize

TABLE 3
Estimation diffuse recharge and infiltration rate for soil profile beneath crops using the CMB

| Profile | Average Cl below 2 m (mg/L) | R (mm/yr) | R/P (%) | Average θ_g (%) | I (infiltration rate) (m/a) |
|---------|-----------------------------|-----------|---------|------------------------|-----------------------------|
| X1 | 27.3 | 33 | 6.3 | 17.8 | 0.13 |
| X1-2 | 15.8 | 56 | 10.7 | 14.3 | 0.27 |

Chloride content of soil moisture for profile X1 below 2 m ranges from 13.4 to 64.8 mg/L with an average of 27.3 mg/L, and X1-2 from 9.4 to 22.2 with an average of 15.8 mg/L. When using the CMB to estimate diffuse recharge, the chloride input flux (*J*) should be known. Due to the lack of

observation of chloride deposition in the study area, the value to 1.7 mg/L in precipitation was used. This value is derived from the volume-weighted average chloride concentration in rainfall for a rural monitoring station in the vicinity of Xi'an [27], and is similar to available observation data, e.g., 1.7

mg/L in Lanzhou, 1.5-2.8 mg/L in Shiyang river basin, 1.8 mg/L in Xi'an [8, 28-30]. The wet chloride deposition can therefore be estimated by $1.7 \text{ mg/L} \times 523 \text{ mm/yr} = 889 \text{ mg/(m}^2\text{.yr)}$. In arid areas, a component of the chloride input can also be derived from dry deposition as dust. Due to the uncertainty of dry deposition, it can be negligible for recharge estimation [31-32] which is acceptable provided that the long-term dry aerosol flux is near steady state. In addition, the chloride content in precipitation ranges of 1.7 mg/L overlap with the value of 1.2 to 2.4 mg/L of chloride content in bulk precipitation (including dry deposition) in the loess-covered areas in China. Then, the recharge rate can be estimated using the CMB. Table 3 shows that the recharge rate ranges from 33 to 56 mm/yr, corresponding 6.3% to 10.7% of annual precipitation. Although the values has some differences, the *R/P* value overlaps 6.7% obtained from water mass balance in the study area [24]. It also overlaps the values of 12-13% obtained from tritium peak [12-13] and 11-18% from the CMB [10] in other loess sites.

The infiltration rate (*I*) can also be estimated from the recharge rate (*R*) and soil moisture content ($I=R/\theta_v$, $\theta_v = w\theta_g$, where *w* is soil dry bulk density and value of 1.45 g/cm^3 is used in this study). The estimated *I* ranges from 0.17 to 0.27 m/yr (Table 3). These are also reasonable values and overlap with the values of 0.25-0.30 m/yr obtained from tritium peak [12-13] in other loess sites with annual precipitation ranging from 360 to 550 mm/yr.

Groundwater characteristics and age. The time for annual precipitation to reach the water table can be obtained from soil moisture infiltration rate and the depth of water table. The regional water table depth mainly ranges from 30 to 60 m. The age of moisture at water table derived from diffuse recharge can be estimated by the depth of water table (i.e., thickness of the UZ) divided by infiltration rate (0.13-0.27 m/yr) (Table 3), which range from 110 to 460 years. Due to the relatively low precipitation (523 mm/yr, semiarid area) and therefore limited recharge rate (33-56 mm/yr) and the thick UZ, it needs hundreds of year for annual precipitation to reach water table. However, it does not imply any recharge and it is just piston flow to push older moisture to reach the water table.

The spring (sample QS) and groundwater (sample XG1 – XG4) is tritium free (<0.3 TU), suggesting that the precipitation since 1950s has never recharged present groundwater. This is consistent with the aforementioned CMB estimation.

Water quality of spring and groundwater is pretty good. The total dissolved solids (TDS) ranges from 233 to 308 mg/L (Table 2). The major anion is HCO_3^- while cation is Ca-Mg with HCO_3^- -Ca-Mg type of water chemistry (Fig. 4). The nitrate, an indicator of modern human activities (overuse of fertilizers), is as low as <10 mg/L (as NO_3^- , 0.1- 9.3 mg/L) (Table 2). While groundwater nitrate baseline is commonly less than 13 mg/L in most temperate regions covered by grassland [33], the groundwater nitrate in the study area seems to remain at baseline level. While overuse of fertilizers in China mainly begun in 1980s [34], the long infiltration time (110-460 years) result in low nitrate content in groundwater.

Groundwater Cl concentration ranges from 7.0 to 8.6 mg/L, except for XG1 of 20.1 mg/L, while Cl content in soil moisture below 2 m beneath crops range from 9.4 to 64.8 mg/L with an average of 24.7 mg/L for the two soil profile (X1 and X1-2). The Cl content in soil moisture is larger than that in groundwater. Meanwhile, the stable isotopic composition show similar characteristics between groundwater and soil moisture. The stable isotopic composition for soil moisture from profile X1 from 1 to 14.5 m is shown in Fig. 5. $\delta^{18}\text{O}$ concentration ranges from -9.2‰ (3 m) to -7.2‰ (5 m), while $\delta^2\text{H}$ from -70.2‰ to -54.1‰. The slope of the regression line for the profile X1 is 6.8 ($R^2=0.94$), slightly greater than evaporation slope of commonly 4-6 for open water (Barnes and Allison, 1988) and the regression line is slightly sub-parallel to the Global Meteoric Water Line (GMWL, $\delta^2\text{H}=8\delta^{18}\text{O}+10$) [35]. This is mainly attributed to similar amounts of evaporation to precipitation from different rainfall events having different isotopic compositions. However, the groundwater $\delta^{18}\text{O}$ concentration ranges from -10.0‰ to -9.6‰, while $\delta^2\text{H}$ from -73.7‰ to -70.0‰ and stable isotopic composition is more depleted than that of soil moisture.

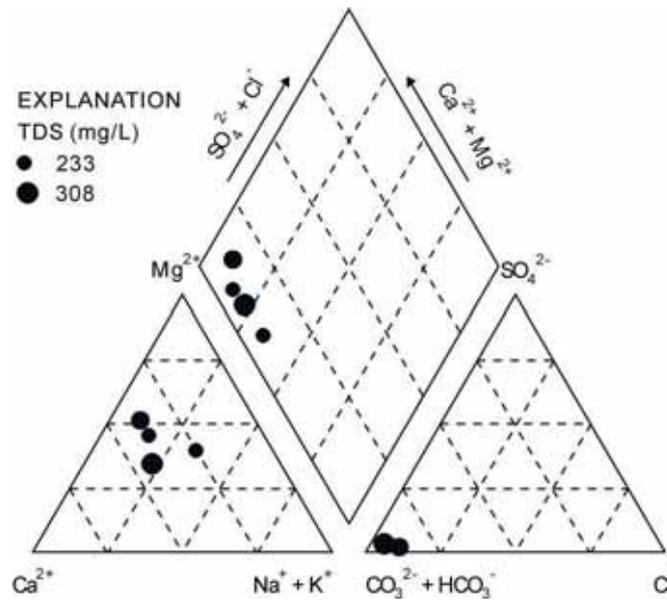


FIGURE 4
The piper diagram for groundwater and spring

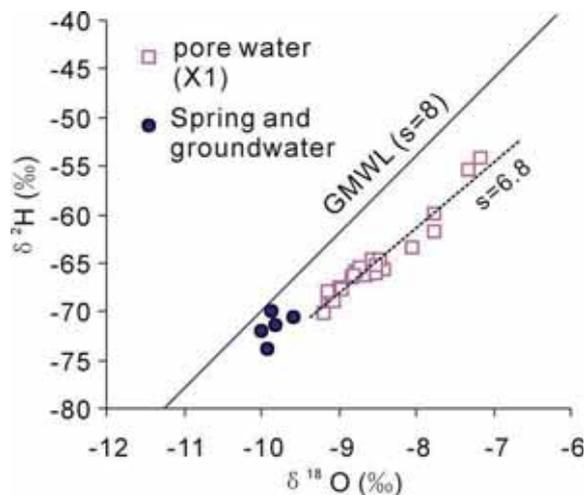


FIGURE 5
The isotopic composition of soil moisture and groundwater

Several assumptions could interpret that groundwater Cl content and stable isotopic composition is lower than that in the upper UZ.

1) Recent climate change and human activities. In the past 50 years, the climate of study area trends to being arid [36] and evaporation is also increasing [37]. Meanwhile, due to use of fertilizers, the yield of crop has been increasing. This could cause Cl in the soil to accumulate. The nitrate also accumulates in the upper UZ (4 m) for soil profiles (Fig. 6). The maximum value can reach 3583 mg/L. However,

groundwater nitrate content is rather low, ranging from 0.1 to 9.3 mg/L. If this is a reasonable assumption, solute contents and isotopic composition of soil moisture below certain depth not affected by recent climate change and human interference, should be similar with that in groundwater. The unpublished data from two deep UZs (45 m and 62 m) from the Zhengning loess plain, ~30 km from the Xifeng loess plain, shows that the solute contents and isotopic composition of soil moisture in the deep UZ is similar with that in spring and groundwater.

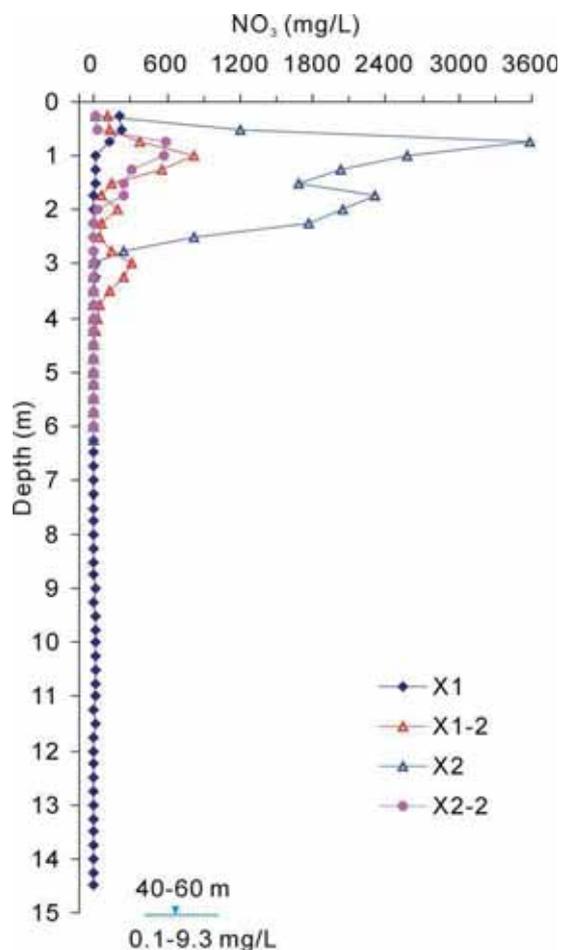


FIGURE 6
Nitrate distribution of soil profile and groundwater content

2) Focused recharge as main recharge sources for groundwater. If there is focused recharge through stream channels or topographic depressions with little evapo-transpiration and diffuse recharge contribution little to present groundwater, the solute contents and isotopic composition of groundwater could be low. In loess gully area, this may be reasonable [10]. However, in loess plain, as the study area, the gully and stream is the discharge area for groundwater system.

3) Groundwater is mainly derived from regional lateral recharge with low values of solute and isotopic composition. However, in the upstream of groundwater, the land use is similar and groundwater recharge is mainly derived from diffuse recharge.

In summary, the assumption 1) seems to be reasonable and the present groundwater was mainly

recharged before hundreds of year ago without much human interferences.

Impact of land-use change. As mentioned in the introduction, the local governments want to develop apple orchards to increase the income of farmers in most areas of the Loess Plateau. An important issue arises that the impacts of such land use change (from crops to orchard) on groundwater recharge. The soil profiles (Fig. 7) show planation of orchard results in depletion in soil moisture content. The changes in recharge can also be estimated from soil solute profile. From the equation (1) $R=J / C_s$, the changes in recharge correspond with C_s , as J is a constant parameter in the same area. Fig. 7 shows the conversion from winter wheat to apple orchard results in C_l increase, suggesting that diffuse recharge is decreasing.

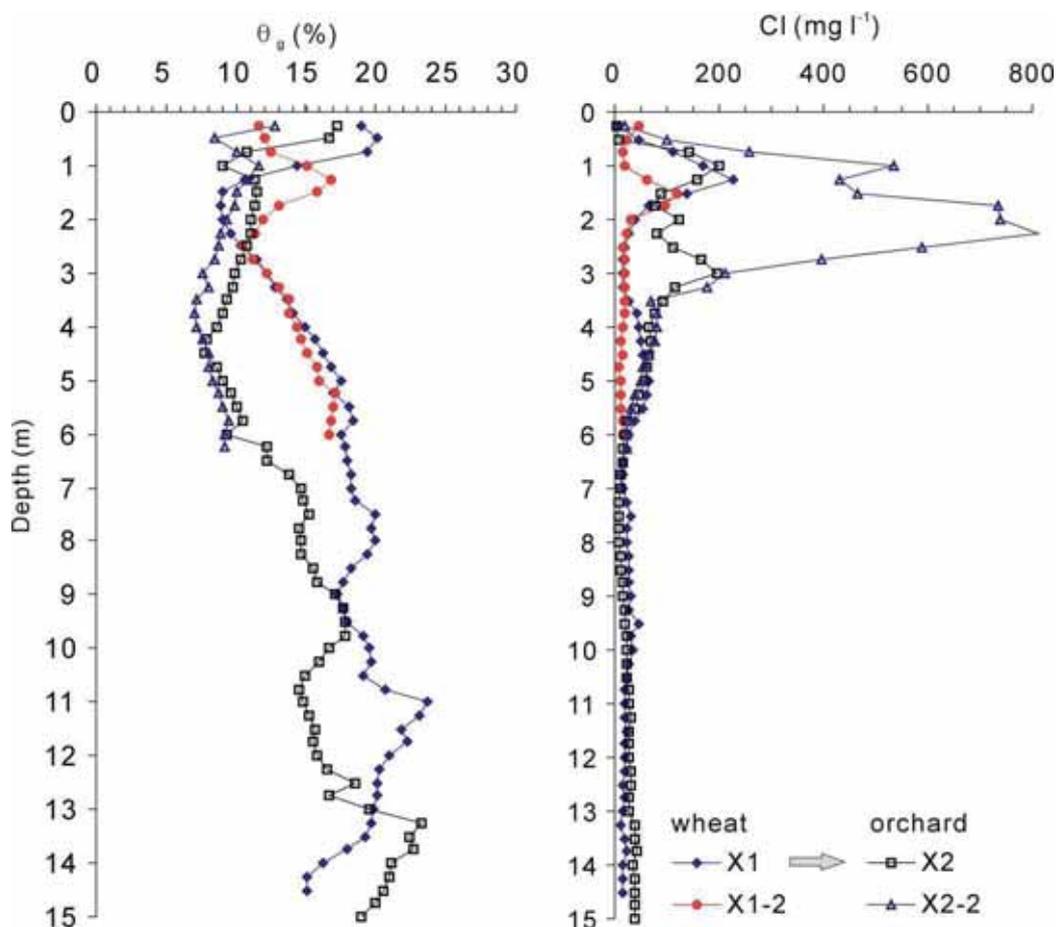


FIGURE 7

The changes in soil moisture and Cl content for wheat (X1 and X1-2) and orchard (X2 and X2-2)

This is easy to understand that the conversion from winter wheat to apple orchard results in decrease in recharge. Commonly, the forest (e.g., orchard) has deeper root and higher evapotranspiration, compared with crop or pasture [38]. When the precipitation remains constant (same region), water mass balance ($R=P-E$) results in decreases in deep drainage (diffuse recharge). Example from Changwu, the other loess plain and ~60 km south of Xifeng, shows that below 2.5 m, the average soil moisture content is 18.5% for crops (mainly winter wheat and maize) and 12.6% for more than 10-year old apple orchard (Fig. 8) [18]. About 6% of soil moisture depletion suggests that decrease in diffuse recharge. However, it is difficult to quantitatively assess the decrease using soil moisture data and the CMB also cannot do it unless new steady-state forms, which need hundreds of years for forest in arid area [9, 39].

CONCLUSIONS

Groundwater recharge and impact of land use change on recharge rate in a semiarid rain-fed agricultural loess plain in the Chinese Loess Plateau has been examined using multiple tracers and the unsaturated zone.

1) The present diffuse recharge beneath crops as the main land use in the Xifeng loess plain is 33-56 mm/yr based on the CMB method. The infiltration rate is 0.13 to 0.27 m/yr. Due to the limited recharge rate and thick unsaturated zone, it takes 110 to 460 years for annual precipitation to reach the water table, as confirmed by the absence of tritium (<0.3 tritium unit) of groundwater.

2) Groundwater Cl, NO_3 content and stable isotopic composition is lower than that in the upper unsaturated zone, which is possibly related to recent human activities. This suggests that the shallow groundwater has not been in hydraulic equilibrium with near-surface boundary conditions.

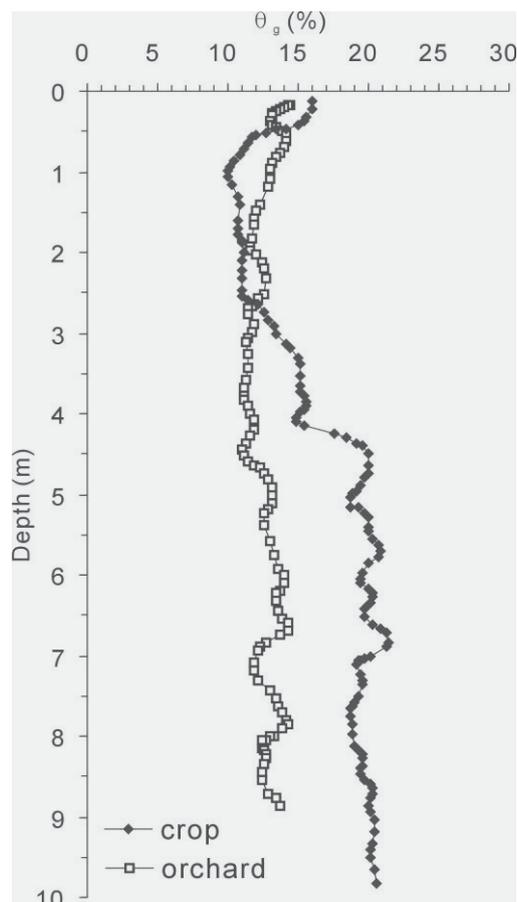


FIGURE 8
Soil moisture profile for crop and apple orchard in the Changwu, modified from reference [18]

3) The conversion from crops to orchard in the study area has resulted in decrease in soil moisture and increase in Cl content, suggesting decrease in deep drainage. This does not appear to be favourable to the groundwater resources, and could accelerate depletion of the soil reservoir in the Loess Plateau.

4) Soil Cl can be used to assess groundwater recharge and impact of land use change on diffuse recharge when new steady-state forms.

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EFFECT OF SALICYLIC ACID AND SELENIUM ON ANTIOXIDANT SYSTEM OF AVENA SATIVA L. UNDER FENOXAPROP-P-ETHYL STRESS

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ABSTRACT

The present study focuses on understanding the role of different antioxidants in plant defense against oxidative stress caused by fenoxaprop-p-ethyl herbicide. Besides ameliorative effect of exogenously applied salicylic acid (SA) and selenium (Se) on fenoxaprop-p-ethyl-induced toxicity was examined in *Avena sativa* L. It was determined that the MDA (malondialdehyde) content in the groups to which only herbicide was applied was higher than that of the groups to which SA and Se applied together with the herbicide. In plants exposed to fenoxaprop-p-ethyl, the activities of peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione reductase (GR) and content of glutathione (GSH) increased in the treatment groups compared to the control groups. The antioxidant activity increased in the control and treatment groups that were pre-treated with SA and Se compared to the groups that were not pre-treated with SA and Se. While catalase activity (CAT) decreased in SA pre-treated plants, it increased in Se pre-treated plants. These results indicate that the fenoxaprop-p-ethyl induces various plant defense responses in target plants and that treatment with exogenous SA and Se can increase stress resistance by altering these responses.

KEYWORDS:

Antioxidant system, fenoxaprop-p-ethyl, Salicylic acid, Selenium, Avena

INTRODUCTION

All organisms have evolved complex signalling pathways in reaction to various stimuli, such as biotic and abiotic stress factors, and have acquired acclimatization in metabolic and developmental functions to get over environmental stresses. Herbicide application is one of the most effective method to control weeds. Herbicides have

been determined to generate reactive oxygen species (ROS), either by direct involvement in radical production or by inhibition of biosynthetic pathways [1]. Fenoxaprop-p-ethyl is an aryloxyphenoxypropionate herbicide and is used to control annual grass weeds such as black-grass (*Alopecurus myosuroides*) and wild-oats (*Avena* spp.) [2, 3]. The application of fenoxaprop-p-ethyl causes chlorosis, necrosis and finally the death of plant tissue [4, 5].

Fenoxaprop-p-ethyl, 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy] propanoate, ethyl ester, is herbicide for postemergence control of annual and perennial grasses in paddy fields; its site of action is acetyl-coenzyme A carboxylase (ACCase), an enzyme in fatty acids biosynthesis [6]. Generally fenoxaprop-p-ethyl is rapidly de-esterified by hydrolysis in plants to yield fenoxaprop acid, which is the active herbicide. Although both compounds are phytotoxic, and is followed by the formation of water soluble metabolites. Metabolism is a major mechanism for species selectivity [7]. Fenoxaprop acid is metabolized in rice to major nontoxic metabolites via glutathione (GSH) or cysteine-mediated ether bond cleavage to yield nonphytotoxic GSH or cysteine conjugates of benzoxazolone (CDHB) [8].

Various subcellular organelles such as chloroplast, mitochondrion and peroxisome are the common sites of ROS production. The production of ROS is ubiquitous during metabolism and all plants can cope with them. However, if the ROS production becomes significantly high, it can cause a substantial cellular damage [9-11]. Overaccumulation of ROS results in cell death which can result from oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition, DNA and RNA damage [9]. The question arises as to how a plant controls and speeds up its rate of ROS production and ROS scavenging when it is exposed to an abiotic stress [11]. ROS damage is prevented by the antioxidative system. The antioxidative system comprises of numerous enzymes, such as POD, APX, SOD, CAT,

GST, GR etc. and compounds of low molecular weight, such as glutathione, carotenoid [12, 13]. Although ROS are highly toxic compounds, they are also known to fulfill important positive roles in physiological functions. For example, ROS are produced in response to a number of different physiological stimuli and they likely function as second messengers to directly or indirectly modulate many proteins [14]. Thus, sublethal doses of photosynthetic inhibitor herbicides may generate low levels of ROS, which in turn could cause a multitude of downstream effects not related to toxicity [15].

Some researchers reported that exogenous SA and growth regulators protected contrast to a number of stress factors (e.g. herbicide, heavy metals) in plants [16-18]. Under severe stress conditions, however, the antioxidant capacity may not be sufficient to minimize the harmful effect of oxidative injury. Therefore, the search for signal molecules that mediate the stress tolerance is an important step in our better understanding how plants acclimate to adverse environments [1]. Low concentrations of SA may enhance the antioxidant capacity in plants, but high concentrations of SA may cause cell death or sensitivity to abiotic stresses. To defense responses, SA plays an important role in the response to abiotic stresses, including drought, low temperature, and salinity stresses [19]. Moreover, SA has emerged as a critical signaling molecule that regulates plant responses to pathogen infection [20]. Plant roots can take up Se from soil as selenate, selenite, or organoselenium compounds. The biosynthesis of most selenium compounds in nature follows the pathways leading to isologous sulfur compounds in plants as well as yeast, bacteria or animals. Roots take up selenate faster than selenite at the same concentration but acquire organoselenium compounds, such as selenocysteine (SeCys) and selenomethionine (SeMet), most avidly [21]. Thereafter it is metabolized (via sulfur assimilation pathway) in that selenocysteine, SeMet and other Se analogues of various S metabolites [22, 23]. Se might help plants to cope with the environmental stresses by regulating the antioxidant level of them [24-26].

In this research the effect of SA and Se on antioxidant system of fenoxaprop-p-ethyl in *Avena* was evaluated. MDA content as a marker of lipid peroxidation, the content of non enzymatic antioxidants (total GSH levels) and antioxidant enzyme activities (GR, GST POD, APX, CAT, SOD) which was caused by herbicide were investigated.

MATERIALS AND METHODS

Plant growth and treatments. In this study, fenoxaprop-p-ethyl was provided from “Safa Agriculture”. Seeds of *A. sativa* were divided into three groups and the first group was kept at distilled water, the second group at 0.5 mM SA solution (pH 5.7) and the third group at 4 mM Se solution for six hours and then these seeds were planted. In the evaluation after preliminary trials it was observed that SA response is better in 0.5 mM and Se response is better in 4 mM concentration concerning stress response.

The plants were grown in 2-L pots containing perlite, using Hoagland solution [27] in a climate room with a temperature of 23 ± 2 °C and a humidity of 60%. On the 21st day of the growth, the plants were treated with fenoxaprop-p-ethyl with the spraying method (post-emergence application). In the preliminary trials performed with solutions in different concentrations prepared by taking the application dose of fenoxaprop-p-ethyl to the terrain into consideration, the toxic doses were determined for *A. sativa* and the upper and lower concentrations of this dose was applied to *A. sativa* by considering the possible residue in the soil depending on the half life of herbicide. Therefore, we used the doses (2.4, 3.6, 5.4, 8.1 and 12.15 mM) in fenoxaprop-p-ethyl herbicide treatment. The leaves of plants were gathered on the 10th day after the treatment and were stored in a freezer at a temperature of -80 °C.

The novel synthetic organoselenium compound 1,3-di (*p*-methoxybenzyl)-1,3-diazepan-2-selenone (SeI) was synthesized in organic chemistry laboratory at Inonu University by methods described in the literature and identified by ¹H NMR (300 MHz), ¹³C NMR (75.5 MHz) and microanalysis [28] (Fig. 1).

Determination of MDA content. MDA content was measured according to the method of Heath and Packer (1968) [29]. Absorbance of the supernatants was measured at 532 and 600 nm. MDA amount was calculated with $155 \text{ mM}^{-1} \text{ cm}^{-1}$ extinction coefficient.

Extraction of plant leaves. 0.5 g leaf tissue was homogenized in Tris HCl tampon (0.1 mM), EDTA (0.1 mM) and PVP (1%). The homogenates were centrifuged at 4 °C and 18.000 rpm and for 30 min. The supernatants were stored in a -80 °C deep freezer [30]. The activities of all of enzymes were expressed as specific activities.

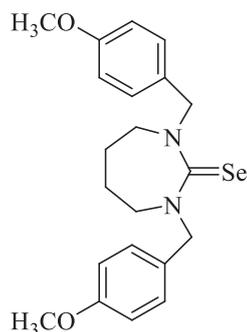


FIGURE 1
Structure of 1,3-di(*p*-methoxybenzyl)-1,3-diazepan-2-selenone (SeI) (28)

Determination of GST activity. The GST activity was measured according to the method of Habig et al. (1974) [31]. The reaction was monitored at 344 nm.

Determination of GR activity. GR activity was determined according to the method of Carlberg and Mannervik (1985) [32]. The enzyme activity was determined at 340 nm.

Determination of Total Glutathione content. The GSH content was determined according to the method of Akerboom and Sies (1981) [33]. The reaction was monitored at 412 nm.

Determination of POD activity. The POD activity was measured according to the method as previously described by Mac Adam et al. (1992) [34]. The enzyme activity was measured at 43 nm.

Determination of APX activity. The APX activity was determined by following the methods of Nakano and Asada (1981) [35] and Cakmak (1994) [36]. The enzyme activity was determined at 290 nm.

Determination of CAT activity. The CAT activity was determined according to the method of Luck (1963) [37] by measuring the decrease of absorbance at 240 nm attributed to the decomposition of H₂O₂.

Determination of SOD activity. The SOD activity was measured according to the method of McCord and Fridovich (1969) [38], in terms of the ability of the enzyme to prevent the reduction of cytochrome-c by superoxide generated by the

xanthine oxidase system. The reactions were monitored at 550 nm.

Determination of total soluble protein. The total soluble protein content was determined according to the method of Bradford (1976) [39] using BSA as a standard. The reaction was measured at 595 nm.

Statistical analysis. Statistical analysis was performed using SPSS 15.0 software. Duncan's (1955) [40] and t-tests were used to determine the differences between averages. In the analyses, $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Effect of SA and Se pre-treatments on lipid peroxidation. The MDA content increased depending on the increasing herbicide concentrations in all treatment groups. Exogenous SA and Se pre-treatments decreased the MDA content as compared to plants that were not pre-treated. The highest MDA content was determined as 19.37 $\mu\text{mol MDA/g FW}$ in the 12.15 mM treatment group in the not pre-treated plants ($p < 0.05$) (Table 1).

Effect of SA and Se pre-treatments on antioxidant enzymes. The GST activity increased depending on the increasing herbicide concentrations. Alongside with this, exogenous SA and Se pre-treatments were determined to have increased the GST activity. The highest GST activity was observed in the 12.15 mM treatment group as 0.151 $\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ in the plants pre-treated with SA ($p < 0.05$) (Table 2).

TABLE 1

Changes in MDA content in *A. sativa* exposed to fenoxaprop-p-ethyl. The different lower case letters indicate significant differences ($p < 0.05$) among the different concentration of fenoxaprop-p-ethyl according to Duncan's test. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of fenoxaprop-p-ethyl according to independent samples *t* tests.

| Treatments (mM) | MDA Content ($\mu\text{mol MDA/g FW}$) | | |
|-----------------|--|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | A4.92f | B2.24f | B2.93f |
| 2.4 | A9.37e | B6.13e | B6.01e |
| 3.6 | A12.19d | B8.21cd | B7.99cd |
| 5.4 | A14.66c | B9.13c | B8.71c |
| 8.1 | A16.99b | B11.67b | C10.01b |
| 12.15 | A19.37a | B12.99a | C11.48a |

TABLE 2

Changes in GST, GR and GSH activity in *A. sativa* exposed to fenoxaprop-p-ethyl. The different lower case letters indicate significant differences ($p < 0.05$) among the different concentration of fenoxaprop-p-ethyl according to Duncan's test. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of fenoxaprop-p-ethyl according to independent samples *t* tests.

| Treatments (mM) | GST activity ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$) | | |
|-----------------|---|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | C0.042f | A0.064e | B0.050f |
| 2.4 | B0.073e | A0.096d | A0.091de |
| 3.6 | C0.087d | A0.121c | B0.101d |
| 5.4 | C0.102c | A0.136bc | B0.112c |
| 8.1 | C0.120b | A0.145a | B0.130b |
| 12.15 | B0.135a | A0.151a | AB0.142a |

| Treatments (mM) | GR activity ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$) | | |
|-----------------|--|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | C0.036e | A0.075e | B0.061e |
| 2.4 | C0.083d | A0.119d | B0.101d |
| 3.6 | C0.099c | A0.133c | B0.121c |
| 5.4 | C0.122b | A0.151ab | B0.137b |
| 8.1 | C0.119b | A0.143b | B0.132b |
| 12.15 | B0.130a | A0.165a | AB0.156a |

| Treatments (mM) | GSH content ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$) | | |
|-----------------|--|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | C1.19e | A2.56d | B2.34e |
| 2.4 | C2.01d | A2.89c | B2.71d |
| 3.6 | B2.18c | A2.97c | A2.89c |
| 5.4 | C2.40b | A3.24b | B2.92c |
| 8.1 | B2.36b | A3.17b | A3.10b |
| 12.15 | C2.63a | A3.43a | B3.31a |

The GR activity increased depending on the increasing herbicide concentrations. The GR activity was found to be significantly increased in plants subject to pre-treatment with SA and Se. The

highest GR activity was determined in plants which were subject to pre-treatment with SA in the 12.15 mM treatment group as $0.165 \mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ ($p < 0.05$) (Table 2).

It was determined that the GSH content increased in the treatment groups compared to the control groups. GSH content was found to be higher in plants pre-treated with SA and Se. The highest GSH content was determined in the 12.15 mM treatment group as $3.43 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein in the plants pre-treated with SA ($p < 0.05$) (Table 2).

The POD activity increased depending on the increasing herbicide concentrations in the treatment groups. SA and Se pre-treatments of the plants were determined to have increased the POD activity. The highest POD activity was determined as $3.90 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein in the 12.15 mM treatment group in SA pre-treated groups ($p < 0.05$) (Table 3).

The APX activity was found to be significantly increased in plants subject to pre-treatment with SA and Se. The highest APX activity was determined in the 2.4 mM treatment group as $1.61 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein in the plants pre-treated with SA ($p < 0.05$) (Table 4).

It was statistically determined that the CAT activity decreased in the treatment groups, no matter if they are SA and Se pre-treated or not, compared to the control groups. The highest CAT activity was determined in plants which were subject to pre-treatment with Se in the control group as $14.56 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein. The CAT activity, in the plants pre-treated with SA, decreased in the control and treatment groups compared to the plants not pre-treated with SA ($p < 0.05$) (Table 5).

TABLE 3

Changes in POD activity in *A. sativa* exposed to fenoxaprop-p-ethyl. The different lower case letters indicate significant differences ($p < 0.05$) among the different concentration of fenoxaprop-p-ethyl according to Duncan's test. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of fenoxaprop-p-ethyl according to independent samples *t* tests.

| Treatments (mM) | POD activity ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) | | |
|-----------------|---|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | A0.95e | A1.08d | A1.00e |
| 2.4 | C2.40d | A2.90c | B2.68d |
| 3.6 | A3.20c | B2.90c | B2.98c |
| 5.4 | A3.88a | B3.66b | C3.55b |
| 8.1 | B3.70b | A3.86a | A3.87a |
| 12.15 | B3.65b | A3.90a | A3.84a |

TABLE 4

Changes in APX activity in *A. sativa* exposed to fenoxaprop-p-ethyl. The different lower case letters indicate significant differences ($p < 0.05$) among the different concentration of fenoxaprop-p-ethyl according to Duncan's test. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of fenoxaprop-p-ethyl according to independent samples *t* tests.

| Treatments (mM) | APX activity ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) | | |
|-----------------|---|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | A0.41d | A0.48e | A0.51d |
| 2.4 | C0.80a | A1.61a | B1.30a |
| 3.6 | C0.75a | A1.20c | B1.04c |
| 5.4 | C0.60b | A1.33b | B1.00c |
| 8.1 | C0.63b | B1.01d | A1.14b |
| 12.15 | C0.54c | A1.23c | B1.03c |

TABLE 5

Changes in CAT activity in *A. sativa* exposed to fenoxaprop-p-ethyl. The different lower case letters indicate significant differences ($p < 0.05$) among the different concentration of fenoxaprop-p-ethyl according to Duncan's test. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of fenoxaprop-p-ethyl according to independent samples *t* tests.

| Treatments (mM) | SOD activity ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$) | | |
|-----------------|---|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | C6.43e | A9.43e | B7.89f |
| 2.4 | C7.21de | A11.12d | B9.11e |
| 3.6 | C8.89d | A12.98cd | B10.49d |
| 5.4 | BC10.11c | A14.56c | B11.73c |
| 8.1 | BC12.19b | A16.41b | B13.01b |
| 12.15 | C13.47a | A17.89a | B15.12a |

The SOD activity increased in all treatment groups compared to the control groups. The SOD activity was found to be significantly increased in plants subject to pre-treatment with SA and Se. The highest SOD activity was determined in plants which were subject to pre-treatment with SA in the 12.15 mM treatment group as $17.89 \mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ (Table 6).

Plants are exposed to biotic (such as pathogens, wounding) and abiotic stress factors (such as salinity, UV radiation, drought, heavy metals, air pollution, herbicides) [41, 42]. Many abiotic stress factors can induce accumulation of ROS in plants [9]. Thus, plants have an antioxidant defense system to protect themselves against ROS [24]. SA, an endogenous plant growth regulator has been

found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development [43]. Exogenous application of SA can influence the antioxidant capacity of plant cells [1, 44]. In our study, similarly SA induced the antioxidant capacity of *Avena* (Table 2, 3, 4, 6). Se, an essential element for animals and humans, has also been found to be beneficial to plants [45, 46]. Se applications increase the activity of some antioxidant enzymes in plants subjected to various stresses [24, 25, 47]. We observed altered defense parameters in *A. sativa* due to treatment Se with the fenoxaprop-p-ethyl (Table 2-6). In this study, we observed altered defense parameters in *A. sativa* due to treatment with the fenoxaprop-p-ethyl.

TABLE 6

Changes in SOD activity in *A. sativa* exposed to fenoxaprop-p-ethyl. The different lower case letters indicate significant differences ($p < 0.05$) among the different concentration of fenoxaprop-p-ethyl according to Duncan's test. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of fenoxaprop-p-ethyl according to independent samples *t* tests.

| Treatments (mM) | SOD activity ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$) | | |
|-----------------|---|------------------------|------------------------|
| | Fenoxaprop-p-ethyl | Fenoxaprop-p-ethyl +SA | Fenoxaprop-p-ethyl +Se |
| Control | C6.43e | A9.43e | B7.89f |
| 2.4 | C7.21de | A11.12d | B9.11e |
| 3.6 | C8.89d | A12.98cd | B10.49d |
| 5.4 | BC10.11c | A14.56c | B11.73c |
| 8.1 | BC12.19b | A16.41b | B13.01b |
| 12.15 | C13.47a | A17.89a | B15.12a |

MDA is a product of lipid peroxidation and an indicator of plant membrane stress. There are studies showing that herbicide application affects MDA content in plants [18, 48]. It was determined in our study that, MDA content increased

considerably in all plants exposed to the herbicide. Fenoxaprop-p-ethyl induced ROS formation can increase membrane lipid peroxidation and, thus, MDA content. Similar to our findings, Lukatkin et al. (2013) [49] determined that when Topic

(clodinafop-propargyl) was treated to the wheat, corn and rye leaves in different concentrations, MDA content increased depending on the concentration of the herbicide. On the other hand, exogenous SA and Se application caused a decrease in MDA content (Table 1). In SA and Se treated plants, the reason of this decrease can be explained as development of resistance against stress by SA and Se.

Many plant species respond to herbicide stress by inducing detoxifying enzymes, and the systems and pathways are well characterized [50-52]. In general, detoxification occurs via multistep biotransformation processes known as cometabolism, in which foreign organic compounds are subjected to reactions such as oxidation, reduction, hydrolysis and conjugation. The overall metabolism of herbicides in plants can be divided into four phases. Phase I is a direct change in the herbicide structure brought about by oxidation, reduction or hydrolysis reactions. Phase II is conjugation to cell constituents such as glucose, glutathione or amino acids. During Phase III, conjugates are transported across cell membranes into the vacuole or cell wall where further processing (Phase IV) can yield insoluble and bound residues. All of these processes result in a loss of herbicidal activity. These enzymes bind molecular oxygen, catalyze its activation and incorporate one of its atoms onto the herbicide molecule [15].

GST catalyzes the conjugation of glutathione to xenobiotic substances and plays a crucial role in the herbicide detoxification [53]. GR efficiently maintains the reduced pool of glutathione [54]. There are reports showing that GST and GR activities and GSH content increased in various plants due to herbicide application [55, 56]. In our study, parallel to these findings, the GST and GR enzyme activities as well as GSH content increased in all the plants, regardless of SA and Se treatment, in the treatment groups compared to the control groups. Our analysis revealed that both GST and GR were involved in detoxification of fenoxaprop-ethyl. Besides, the exogenous application of SA and Se increased the GST and GR activities and total GSH content in the control and treatment groups compared to plants that were not pre-treated with SA and Se (Table 2).

The key enzymes of glutathione conjugation are GST, which catalyze nucleophilic attack of the thiol group onto electrophilic groups of oxidized herbicides. GST isozymes are found in large families that differ in number of members, herbicide specificity and inducibility across plant families. In many cases, these differences account for the range of susceptibility among plant species toward different herbicides. GST activity is

inducible by safeners and some herbicides. Metabolic pathways and specificities are highly diverse across the plant kingdom, and depend on the chemical structure of the xenobiotic, environmental conditions, metabolic factors and the ability of individual species to regulate these pathways. Interestingly, the so-called natural tolerance of crop species to selective herbicides is mostly due to their catabolic abilities, while the majority of cases of evolved resistance in weedy plants are due to mutations encoding altered target sites. Although this distinction may be due to the traditional methods of discovering herbicides [57], it may also reflect the relative frequencies of mutations for these respective phenotypes in native populations [15]. Both SA and Se content were observed to be effective on GSH, GST and GR activities. However it was also observed that SA is more effective than Se (Table 2).

Although oxygen (O_2) is essential for life, its reduction by any means results in the production of ROS that can easily perturb a variety of metabolic processes within a plant [58, 59]. When molecular O_2 undergoes reduction, it gives rise ROS such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\bullet OH$). Singlet oxygen (1O_2), which may arise due to reaction of O_2 with excited chlorophyll, is also considered as one of the potential ROS [9, 11, 60].

Antioxidant system plays a major role in plant's tolerance to various stresses. It was shown that the activities of antioxidant enzymes increased in various plant species under different stress conditions [16, 61, 62]. POD catalyze the dehydrogenation of structurally diverse phenolic and endiolic substrates by H_2O_2 and are thus often regarded as antioxidant enzymes [63]. CAT removes the H_2O_2 produced, preventing an increase in cytosolic H_2O_2 , which is potentially toxic to the plant cell, leading to oxidative stress and cell death [64]. In our study, POD activities increased in especially high concentrations compared to the control groups (Table 3). APX activities increased in all treatment groups compared to the control groups (Table 4). However, exogenous pre-treatment with SA and Se increased the activities of POD and APX in the control and treatment groups compared to the plants that were not pre-treated with SA and Se. Similar to our findings, previous studies reported that, exogenous SA and Se applications significantly increased POD and APX activities in plants [16, 25, 65]. The reason of this increase may be related with metabolism in the induction of antioxidant activity by SA and Se.

The most logical result of photosynthetic inhibition is that affected plants would starve due to the loss of fixed carbon. While this effect would

eventually happen, more rapid toxicity results from blocking electron transfer, preventing the conversion of light energy into electrochemical energy and resulting in the production of high-energy. Singlet oxygen and other ROS are highly toxic compounds that cause acute membrane damage [66]. Although these molecules have very short half-lives and can therefore travel only submicron distances before decaying, they can nonetheless accumulate and cause significant local damage. If unchecked, ROS can be converted by SOD into H₂O₂, which diffuses over much greater distances and causes further oxidative damage [15].

The CAT activity decreased considerably in SA treatment groups exposed to herbicide compared to the control ($p < 0.05$) (Table 5). This situation can be explained with the ROS accumulation, which results from the increase of the herbicide concentration, inhibiting the enzyme activity. Se pre-treatment increased the activity of CAT in the control and treatment groups compared to the plants that were not pre-treated with Se. Similar to our findings, Djanaguiraman et al., (2010) [24] expressed that the activity of CAT increased in the Se-treated plants compared to the plants that were pre-treated. On the other hand, application of exogenous SA decreased the CAT activity. In this study, decrease in CAT activity can result from the SA-mediated mechanism underlying the accumulation of H₂O₂.

SOD plays a role at the scavenging of superoxide radicals [67]. It was determined that in the plants exposed to herbicide, SOD activity changed generally with the effect of stress [48, 56]. In this study, SOD activity increased as a response to the stress compared to the control in all plants to which herbicide was applied. Exogenous SA and Se pre-treatment induced SOD activity in the control and treatment groups (Table 6). Hayat et al. (2010) [43] have explained that exogenous application of SA enhances the activities of antioxidant enzyme system. Similar to our findings, Krantev et al. (2008) [68] reported that SOD activity increased in the corn plant exposed to the Cd stress and this increase was more apparent in the plants treated with SA. Besides, Djanaguiraman et al. (2010) [24] reported that SOD activity decreased in the *Sorghum* plant which was exposed to heat stress while exogenous Se application increased SOD activity at the rate of 11.6 % compared to the plants not treated with Se. They explained SOD activity increase with Se application with the probability of Se affecting SOD enzyme at the level of transcription. In groups where application is made with SA, significant decrease was observed in increasing herbicide concentrations. On the other hand a significant increase is observed in groups to which Se was

applied. In groups to which SA was applied, the increase in SOD activity is considered to be explainable by the decrease in CAT activity (Table 5-6). Here it is observed that SA suppresses CAT activity while it increases SOD activity. According to our findings we can say that Se is more effective than SA on CAT activity. In our findings, it is observed that SA is effective on SOD compared to Se. Here we can say that SA is more effective on antioxidant system.

When we evaluate our findings in general it was determined that although SA and Se play effective role in systemic gained resistance against fenoxaprop-p-ethyl groups, SA was determined to be more effective.

Exogenous application of SA induces the systemic acquired resistance in plants, thereby provides a significant protection against various stresses. When the groups to which only fenoxaprop-p-ethyl were applied were compared to groups to which SA and Se was applied, it was determined that SA and Se increased the herbicide tolerance.

CONCLUSION

This study demonstrated that antioxidant defense responses were induced at *A. sativa* against toxicity induced by fenoxaprop-p-ethyl. However, the exogenous treatment with SA and Se increased enzymatic and non-enzymatic antioxidants. Here SA was generally determined to be more effective than Se (Table 1-6). Despite of the effect of Se on CAT activity, SA demonstrated more effective result compared to Se in all parameters except CAT. Depending on our findings we think that both SA and Se pre-treatment on seeds can be effective for developing resistance against herbicide and this can play an important role in protecting plants in agricultural areas.

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VARIATION OF DISSOLVED ORGANIC MATTER COMPOSITION IN EFFLUENT FROM SWINE WASTE-RETENTION BASINS: A CASE STUDY OF YUJIANG COUNTY, CHINA

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ABSTRACT

The main purpose of this study was to elucidate the variation of dissolved organic matter (DOM) composition in effluent from swine waste-retention basins of different concentrated swine feeding operations (CSFOs) located in Yujiang County of Jiangxi Province, China. Three fluorescence components were identified using three-dimensional excitation-emission matrix and parallel factor analysis, including tryptophan-like C1, tyrosine-like C2, and humic-like C3. For all samples examined, percentage of C1 varied from 0.49 to 0.61, and the range of C2 and C3 was 0.05–0.31 and 0.09–0.45, respectively. Moreover, C1 linked positively with C2 ($R^2 = 0.285$, $p < 0.05$), while negatively related to C3 ($R^2 = 0.577$, $p < 0.001$). Humification degree of DOM was adverse to biological activities ($R^2 = 0.946$, $p < 0.001$), and humic substances were predominantly derived from terrestrially DOM. In the present study, dissolved organic carbon concentration and C/N ratio significantly affected DOM composition in swine effluent.

KEYWORDS:

Concentrated swine feeding operations; dissolved organic matter; three-dimensional excitation-emission matrix; parallel factor analysis; redundancy analysis

INTRODUCTION

In 2008, concentrated swine feeding operations (CSFOs) accounted for 56 percent of pig farms in China, and the ratio has approached to 65% by the year 2015. With the increase of CSFOs, more and more public concerns have been focused on their potential environmental effects [1]. At

present, industrial-scales increased the risks of water contamination owing to large amounts of swine effluent containing high organic loading generated at a central place [2-7].

In swine effluent, dissolved organic matter (DOM) accounted for the most fractions of nutrients [6]. As one kind of organic polymer, DOM is generally composed of a complex mixture, ranging from low molecular weight (MW) substances, such as amino acids and carbohydrates, to high MW components, such as humic substances [8, 9]. Meanwhile, DOM properties differed with pig farms owing to the different farming scales, and feed formulas [4]. Furthermore, microorganism also played a more important role in the degradation of organic matter in wastewater [10, 11]. So, it is often difficult to characterize DOM component with traditional methods. Since the works of Stedmon and Bro (2008), fluorescent spectroscopy has been used effectively to characterize DOM components with parallel factor analysis (PARAFAC) [12-15]. Two major fractions of fluorescence DOM found in swine slurry were humic-like and protein-like components [16-18]. To date, although piles of studies focused on DOM have been carried out, knowledge about the variation of DOM composition between different CSFOs is still limited.

In the present study, a case study was carried out to elucidate the variation of DOM composition in swine effluent from waste-retention basins of different CSFOs, which encompassed a scale gradient ranged from 2000 to 24000 head, located in Yujiang County. The main purpose of this study was to characterize the DOM components in swine effluent using the combined technique of three-dimensional excitation-emission matrix (3DEEM) and PARAFAC, and made certain of the main environmental parameters that affected the variation of DOM composition.

MATERIAL AND METHODS

Study area and sample collection. Yujiang County (116°41'–117°09' E, 28°04'–28°37' N), located in the northeast of Jiangxi Province, China, is characterized by a subtropical humid monsoon climate with annual average temperature, rainfall and sunshine hours of 17.6°C, 1766 mm, and 1809 h, respectively [19]. In 2010, the percentage of pig farms with ≤ 2000, 2000–10000, and > 10000 heads accounted for 78.6%, 19.9%, and 1.2% of the total CSFOs, respectively, with the number of pigs being 41.4%, 43.4%, and 12.3%, respectively [20].

Eight CSFOs were selected randomly for study, and the abbreviation and annual pig marketing rate were embodied in the parenthesis, respectively: Guan Zhucheng (GZC, 2000 head), Wu Yanggao (WYG, 2000 head), Yong Sheng (YS, 4000 head), Cheng Lin (CL, 5000 head), Pan Luping (PLP, 5000 head), Zhong Tong (ZT, 12000 head), Wan Gu (WG, 20000 head), and Zheng Bang (ZB, 24000 head). More detail information about the geographical locations of these CSFOs is shown in the Supplemental file Fig.S1. In general, a swine feed formula, which consisted of 63–70% corn flour, 20–30% soybean pulp, and 5–20% wheat bran, was applied to these CSFOs. A mean of 2 kg solid manure, 3.3 kg urine and 8.0 kg wastewater (plus rinsed water) was generated by a fattening swine every day [20].

Swine effluent samples were collected in 500-mL acid-cleaned polyethylene bottles from the surface of waste-retention basins in triplicate at each CSFOs in December 2013. Sampling points were close to the center of basins at 5-meter intervals in a north-south line, and all samples were stored in an ice box and delivered back to laboratory within 24 hours. Prior to analyze DOM, all samples were filtered through pre-combusted 0.7 μm GF/F filters (Whatman, UK), and the filtrate was stored in pre-combusted (550 °C for 6 h) glass bottles and preserved in refrigerator (4 °C) for a further analysis. All measurements were completed within 7 days.

UV-Visible absorbance and fluorescence analysis. The absorption features of DOM between 200 and 600 nm were measured using an ultraviolet and visible spectrophotometer (Eppendorf), with a 1 cm quartz cuvette and MilliQ water as a reference. In the present study, the ultraviolet absorbance at 254 nm (UV₂₅₄) was applied to indicate the aromatic character of the DOM in swine effluent [21].

Prior to the fluorescence analysis, all samples were diluted and adjusted them comparable to each other with a dissolved organic carbon

(DOC) concentration in 7 mg/L [16]. Fluorescence analysis was completed using a Hitachi F-7000 fluorescence spectrometer, and the excitation/emission scanning ranges were 200–450 nm and 250–600 nm, respectively; the readings were collected at 5-nm intervals for excitation and at 1-nm intervals for emission with a scanning speed of 2400 nm/min, and the band-passes of excitation and emission were both set at 5 nm [22]. A MilliQ water (18.0 Ω) blank was subtracted from fluorescence excitation-emission matrices (EEMs) to eliminate the Raman scatter peaks.

In order to remove the inner-filter effect thoroughly, the EEMs were revised for absorbance by multiplying every reading in the EEMs with a revision modulus, based on the premise that the average path length of absorption of the emission and excitation light is 1/2 of the cuvette length [23]. The revision was expressed as follows:

$$F_{Re} = F_{Obs} \times 10^{(A_{Ex} + A_{Em})/2} \quad (1)$$

where, F_{Re} and F_{Obs} are revised and unrevised fluorescence intensities, and A_{Em} and A_{Ex} are the absorbance values at the current emission and excitation wavelengths, respectively.

Fluorescence intensity was normalized as quinine sulfate units (QSU), where 1 QSU is defined as the maximum fluorescence intensity of 0.01 mg/L of quinine in 1 N H₂SO₄ at the excitation/emission wavelength of 350 nm/450 nm [24]. Moreover, the Rayleigh scatter effects were eliminated from the data matrices by excluding any emission measurements in the two triangle regions (emission wavelength ≤ excitation wavelength + 5 nm, and ≥ excitation wavelength + 300 nm), and the missing values of the two triangle regions were replaced with zeroes [22].

PARAFAC modeling. Without any assumptions on the spectral shape or the number of fluorescent components, the complex mixture of DOM has been decomposed into individual fluorescent components using PARAFAC [22]. Compared with bilinear solutions (e.g. PCA), PARAFAC decodes the data matrix into a set of trilinear terms and a residual array, and the modeling has been completed by the processes of minimizing the sum of square residuals [25]:

$$x_{ijk} = \sum_{f=1}^F a_{if} b_{jf} c_{kf} + \epsilon_{ijk} \quad (2)$$

where, x_{ijk} represents the fluorescence intensity of the i th sample at emission wavelength j and excitation wavelength k , and F is the number of components. The a_{if} is directly proportional to the f th component concentration in the i th sample; both b_{jf} and c_{kf} are linearly linked to the emission and excitation spectra at wavelengths j and k

respectively for the f_{th} component, and ε_{ijk} is the variability not accounted for by the model [26].

The PARAFAC analysis was performed using MATLAB (R2009a, USA) with the DOMFluor toolbox according to the works carried out by Stedmon and Bro (2008) [12]. A total of 24 EEMs of effluent samples from these CSFOs were applied for PARAFAC analysis, and the fluorescent spectra of components were drawn using MATLAB

Fluorescence indices. Humification index (HIX) was introduced by Zsolnay et al. (1999) and applied to determine the maturation degree of soil DOM initially [27]. It was defined as the ratio of two spectral region areas (300 nm and 345 nm, 435 nm and 480 nm) from the emission spectrum scanned for excitation both at 254 nm [28]. In the present study, to decrease the inner filtration effects, HIX was defined as the ratio of the fluorescence intensity from 435 to 480 nm divided by the sum of fluorescence intensity from 300 to 345 nm and from 435 to 480 nm, both excited at 255 nm and the reading of excitation determined at 5-nm intervals mentioned earlier [22, 29]. Moreover, HIX values are in the range of 0–1, increasing with the degree of aromaticity of DOM.

Biological index (BIX) is often used to determine the presence of the β fluorophore, characteristic of autochthonous biological activity in water samples, and defined as the ratio of the fluorescence intensity at an emission wavelength of 380 nm divided by the maximum fluorescence intensity within 420–435 nm in this study, both excited at 310 nm [28]. High BIX value (> 1) indicated more autochthonous origin of DOM, whereas a low value (0.6–0.7) indicated lower autochthonous DOM production in natural waters [22].

To distinguish sources of isolated aquatic fulvic acids, McKnight et al. (2001) has introduced

a fluorescence index (FI_{370}): the ratio of fluorescence intensity at emission wavelength of 450 nm divided by fluorescence intensity at emission wavelength of 500 nm, both excited at 370 nm [23]. The index value of microbial-derived fulvic acids is ≥ 1.9 , and the value of terrestrial-derived fulvic acids is ≤ 1.4 [22].

Chemical measurement and statistic analysis. Electrical conductivity (EC) and pH were measured using a FE30 conductivity meter (Mettler Toledo, Switzerland) and a pH meter (HANAN, Italy), respectively. Chemical oxygen demand (COD) was analyzed according to the standard method [30]. DOC was measured from the difference between TC and IC using a Multi N/C 3100 analyzer (analytikjena, Germany), and total dissolved nitrogen (TDN) was determined by UV digestion method using flow analysis and spectrometric detection (Skalar San++, Dutch). In the study, the C/N ratio is defined as the ratio of DOC divided by TDN [31].

Statistical analyses, including the mean value, coefficient of variation, and analysis of variance, were completed with SPSS 20.0 software (Statistical Program for Social Sciences). All linear regression analyses were completed using OriginPro 9.1 (USA), and the redundancy analysis (RDA) was performed using R 3.0.3 statistic software (New Zealand) with a vegan package. All statistically significant differences were at the level of $p < 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

Environmental parameters in swine effluent. The variation of environmental parameters in swine effluent is summarized in Table 1.

TABLE 1
Variations of environmental parameters in swine effluent

| CSFOs | pH | EC (mS/cm) | COD (O ₂ , mg/L) | UV ₂₅₄ (cm ⁻¹) | DOC (mg/L) | TDN (mg/L) | TP (mg/L) | C/N ratio |
|--------|-------------|-------------|-----------------------------|---------------------------------------|---------------|--------------|-------------|--------------|
| GZC | 7.62±0.01 e | 2.27±0.02 f | 898.0±129.5 b | 2.07±0.13 cd | 136.6±13.5 de | 185.7±3.9 d | 47.8±1.1 a | 0.74±0.08 b |
| WYG | 8.44±0.05 a | 6.01±0.07 a | 1628.3±20.7 a | 6.37±0.16 a | 574.8±33.6 a | 518.9±2.8 a | 14.9±2.9 de | 1.11±0.06 a |
| YS | 7.93±0.01 d | 3.30±0.01 e | 168.6±44.9 de | 1.99±0.12 cd | 213.1±39.0 bc | 315.7±3.0 c | 24.0±1.1 c | 0.67±0.12 b |
| CL | 8.34±0.01 b | 5.28±0.07 c | 1003.0±194.1 b | 4.21±0.65 b | 238.5±34.6 b | 476.8±2.4 b | 16.6±0.5 d | 0.50±0.07 c |
| PLP | 8.09±0.01 c | 5.42±0.03 b | 272.3±0.0 de | 2.35±0.08 c | 164.7±70.3 cd | 478.6±12.4 b | 31.4±0.7 b | 0.34±0.14 d |
| ZT | 8.06±0.10 c | 0.88±0.01 h | 138.9±25.2 e | 0.64±0.12 e | 12.1±1.1 f | 54.9±7.9 e | 13.1±1.1 e | 0.22±0.03 de |
| WG | 7.98±0.02 d | 2.17±0.01 g | 730.3±115.5 c | 1.75±0.24 d | 88.2±3.4 e | 178.7±3.7 d | 24.8±0.3 c | 0.49±0.01 c |
| ZB | 7.92±0.02 d | 3.41±0.00 d | 340.0±36.2 d | 1.69±0.12 d | 26.8±13.2 f | 312.9±2.0 c | 25.1±0.2 c | 0.09±0.04 e |
| CV (%) | 3.1 | 48.5 | 77.0 | 66.0 | 97.3 | 50.9 | 43.4 | 60.5 |

CSFOs, concentrated swine feeding operations; EC, electrical conductivity; COD, chemical oxygen demand; UV₂₅₄, ultraviolet absorbance at 254 nm; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; TP, total phosphorus; C/N, the ratio of DOC divided by TDN; CV, coefficient of variation. The error was measured using three replicates; different lowercases within the same column indicate the significant at the level of $p < 0.05$.

For all samples examined, pH and EC ranged from 7.62 to 8.44 with a coefficient of variation (CV) of 3.1% and 0.88 mS/cm to 6.01 mS/cm (CV = 48.5%), respectively, indicating a low alkalinity and high salinity. The concentration of COD varied from 138.9 mg/L to 1628.3 mg/L (CV = 77.0%), suggesting a high organic loading in swine effluent. The range of UV₂₅₄ and DOC was 0.64–6.37 cm⁻¹ (CV = 66.0%) and 12.1–574.8 mg/L (CV = 97.3%), respectively. TDN and TP varied from 54.9 mg/L to 518.9 mg/L (CV = 50.9%) and from 13.1 mg/L to 47.8 mg/L (CV = 43.4%), respectively. The range of C/N ratio was 0.12–1.06 (CV = 60.5%). In all environmental parameters, DOC had the largest coefficient of variation followed by COD, indicating a huge variation of organic loading between these CSFOs.

The high free ammonia concentration accounted for the slightly basic pH for all effluent samples examined [32]. EC was linked with TDN significantly ($R^2 = 0.991$, $p < 0.001$, data not shown), and it had been applied to predicate the nutrients value in swine slurry as positive relationships were noticed between EC and NH₃-N [33]. A positive relationship was observed between

UV₂₅₄ and DOC ($R^2 = 0.895$, $p < 0.001$, data not shown), and both can be used as surrogate indices for the DOM in wastewater [21]. Moreover, the TDN varied as same as DOC ($R^2 = 0.418$, $p < 0.01$, data not shown), suggesting a common source or the same variation [34]. The range of TP concentration was consistent with the previous study. In Yujiang, the range of phosphorus was 4.1–66.6 mg kg⁻¹ in the wastewater discharged from biogas system of the CSFOs [20]. In swine effluent, a high concentration of TDN was apt to bias a low C/N ratio. According to the previous studies, a low C/N ratio would restrict the removal efficiency of organic matter from swine effluent [35, 36].

PARAFAC modeling of DOM components. In the present study, although two protein-like components and one humic-like component were identified by PARAFAC analysis based on a total of 24 EEMs of effluent samples, it did not mean that only three fluorophores exist in all the samples or all the three fluorophores were present in each sample examined. The EEMs spectra of these fluorescence components are shown in Fig. 1.

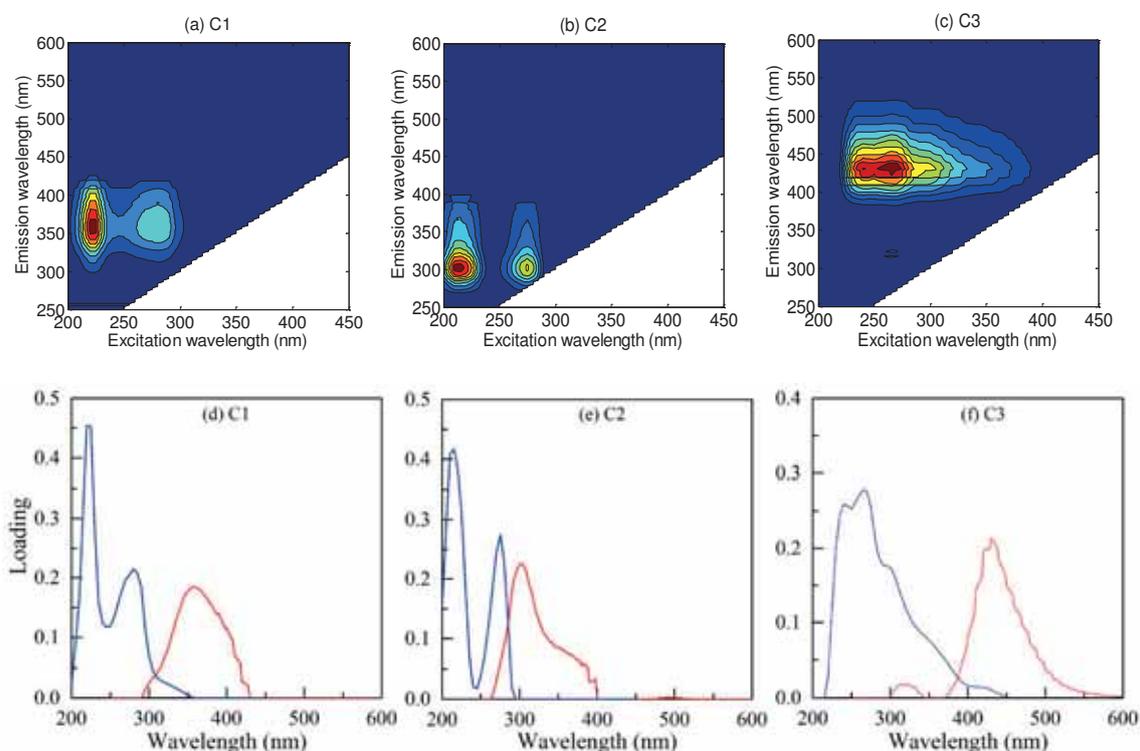


FIGURE 1

PARAFAC model output showing fluorescence signatures of three fluorescence components (a-c), split-half validation results of the three components, excitation (left, blue) and emission (right, red) spectra were estimated from two independent halves of the dataset; C1-tryptophan-like component, C2-tyrosine-like component, C3-humic-like component.

C1 exhibited two fluorescence peaks, with the major and minor peaks at 225/350 and 275/350 nm, respectively (Fig.1a, d), similar to the tryptophan-like fluorophores observed in seawater or lakes and was named as peak T generally [22, 37, 38]. The documented sources of tryptophan-like fluorescence were both autochthonous products and bio-available substrates [39, 40]. C2 also presented two excitation maxima (at 200 nm and 350 nm) at the corresponding maximum emission wavelength of 300 nm (Fig.1b, e). These fluorescence characteristics were identical to the previously reported tyrosine-like fluorescence and named as peak B generally [37], which were derived from autochthonous processes [22, 40]. In swine effluent, the protein-like components would come from the swine forage containing a higher percentage of crude protein. C3 displayed a primary fluorescence peak at an excitation/emission wavelength of 265 nm/425 nm (Fig.1c, f), similar to the UVA humic-like components whose main constituent was fulvic-like acid and noted in many aquatic systems [16, 25, 37, 40]. Moreover, the blue shift of C3 with respect to peak A, is observed, which may be attributed to the high concentration of artificial materials (e.g. N-containing compounds) in swine effluent [40]. Compared with humic-like component, tryptophan-like component was the predominant fluorophore in all examined samples, mostly in GZC and WYG.

Fluorescence components percentage. The relative concentration of each component was defined as the ratio of the maximum fluorescent intensity (F_{\max}) of each component divided by the sum of F_{\max} of all identified components. Compared with tyrosine-like C2 and humic-like C3, tryptophan-like C1 had a higher F_{\max} generally in all samples examined. The percentage of C1 was in the range of 0.49–0.61 (CV = 7.7%). C2 and C3 varied from 0.05 to 0.31 (CV = 55.4%) and from 0.09 to 0.45 (CV = 53.6%), respectively. Moreover, with the increase in the farming scale, the percentage of protein-like components decreased roughly, while humic-like component increased

accordingly. More detail information is shown in the Supplemental file Table S1.

In swine effluent, high concentrations of protein-like components were attributed to autochthonous biological degradation of plentiful terrestrial DOM [25, 40], and its presence indicated a low humification degree and a high level of bio-available organic matter in swine effluent [41]. In general, the tryptophan-like peak was more pronounced than the tyrosine-like peak at the same molar concentration, due to the higher quantum yield of former [42, 43]. Meanwhile, compared with humic-like components, the protein-like components were apt to be used by the heterotrophic bacteria as its ephemeral nature in aquatic system [39-41]. Through linear regression, a positive relationship was observed between C1 and C2 ($p < 0.05$, Fig.2a), and a negative relationship existed between C1 and C3 ($p < 0.001$, Fig.2b). It had been reported that both of the fluorescence intensities of protein-like components were significantly correlated with the concentration of amino acids [39], suggesting a common source. Moreover, as one possible mechanism, amino acids and proteins can be incorporated into humic substances for avoiding the microbial degradation [39]. Thus, the concentration of humic-like component would be strengthened as the microbial degradation of protein-like components [41].

Variation of fluorescence indices. For all samples examined, the HIX ranged from 0.92 to 0.99 (CV = 3.1%), and the BIX and FI_{370} were varied within 0.74–2.42 (CV = 46.2%) and 1.14–1.69 (CV = 12.3%), respectively. In general, with the increase in the farming scale, HIX increased gradually, while BIX decreased accordingly. Moreover, the values of FI_{370} indicated the primary source of humic substances was terrestrially-derived DOM in swine effluent, except for WG, which was a mixture of terrestrially- and microbially-derived DOM. More detail information is shown in Supplemental file Table S1.

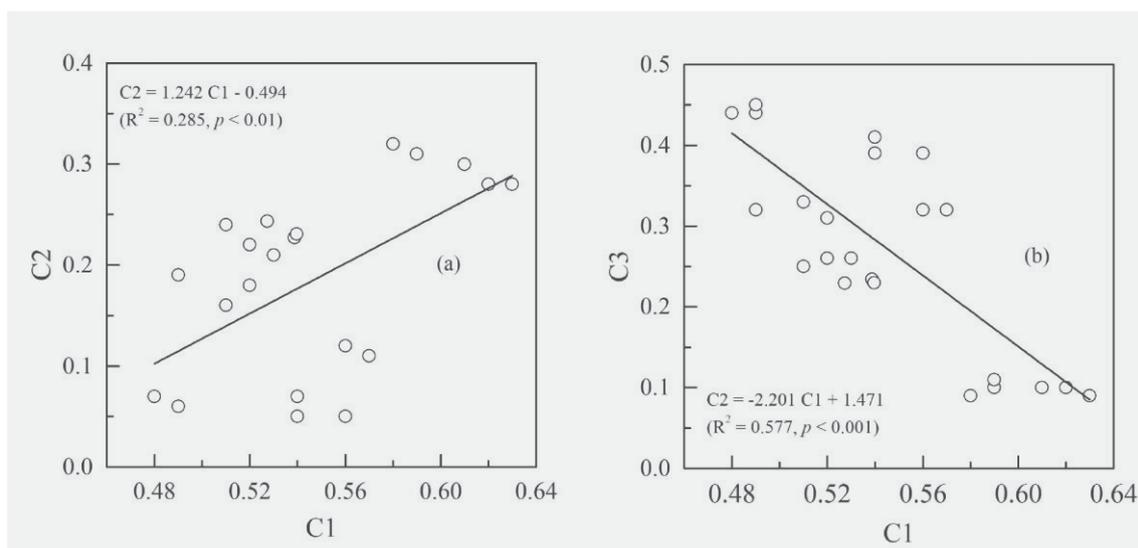


FIGURE 2

Linear relationships between C1 and C2 (a), C1 and C3 (b); C1-tryptophan-like component, C2-tyrosine-like component, C3-humic-like component.

Through linear regression, the BIX correlated significantly with both of HIX and FI_{370} ($p < 0.001$, Fig.3a-b), respectively, and a significant positive relationship was observed between HIX and FI_{370} ($p < 0.001$, Fig.3c). As the aforementioned, a high concentration of protein-like components indicated a low humification degree and a high level of bio-available organic matter in swine effluent [41], which could favor a high degree of biological activities [44-46]. The humification degree of DOM would be strengthened as the microbial degradation of organic matter during the process of anaerobic or aerobic digestion [18, 32, 47]. In general, DOM containing a higher aromaticity was difficult to be utilized by microorganisms and induced a lower microbial activity subsequently [48, 49]. Thus, a high humification degree was adverse to biological activities in aquatic systems. Meanwhile, compared with previous studies, the conclusions about relationship between FI_{370} and BIX were controversial. Guo et al. (2012) studied that the FI_{370} increased with the production of microbially-derived organic matter and linked negatively with the BIX [16]. However, a significantly positive relationship had also been observed between BIX and FI_{370} in lakes of Yungui Plateau [22]. In general, FI_{370} increased with the formation of microbially DOM consisted of a low aromaticity, and decreased with the production of terrestrially DOM commonly contained a higher aromaticity [23]. Thus, the

possible reasons for the inconsistent were that the rapid metabolism and self-degradation of a high level of microorganisms contributed to the increase of microbially-derived DOM in eutrophic systems (e.g. swine slurry), whereas more terrestrially-derived DOM were utilized for the maintenance of microbial metabolism in oligotrophic streams [49]. Moreover, the FI_{370} increased with the HIX owing to the decreased biological activities as the aforementioned.

Relationships between environmental parameters and DOM components. Fig.4 had visualized the potential relationships between environmental parameters and DOM components across all effluent samples examined through the ordination of redundancy analysis (RDA), and pH, DOC, TP, and C/N were remained as the main constrained factors. All the four constrained factors had explained a total of 51.6% of the variance of DOM composition between different CSFOs, and only RDA1 was the significant and explained 48.6% of the total variance ($p < 0.01$). The relative significance of each factor was elucidated in the ordination diagram by the length of their corresponding centrifugal lines, and two significant factors were screened out, namely DOC ($R^2 = 0.41$, $p < 0.001$) and C/N ($R^2 = 0.47$, $p < 0.001$), respectively. The variance explained by the two ordination axes was a bit low, which was attributed to the absent of biological factors.

treatments (e.g. anaerobic or aerobic digestion) have often been compulsively applied to process the swine effluent at large-scales (WG and ZB). Anaerobic or aerobic digestion has been proved to be effective methods to remove chemical oxygen demand and organic pollutants from wastewater^[52]. Thus, high DOC concentration favored a high level of protein-like components and biological activities. In general, the rate of carbon consumption was 25–30 times faster than that of nitrogen during the course of anaerobic digestion^[53]. Compared with large-scale CSFOs, the C/N ratio of small-scale CSFOs was higher owing to a higher content of undigested or fresh swine slurry. Moreover, it was well established that an optimal anaerobic or aerobic microbial metabolism could achieve with a higher C/N ratio^[54]. Thus, the high concentration of DOC and C/N ratio accounted for the variation of DOM composition in swine effluents.

CONCLUSIONS

In swine effluent, there existed discrepancies in DOM properties between different CSFOs. In the present study, three fluorescence components (tryptophan-like, tyrosine-like, and humic-like) were identified using the combined technique of 3DEEMs and PARAFAC. Compared with humic-like component, protein-like components were the predominant fluorophores generally in swine effluent, mostly in small-scale CSFOs. Meanwhile, the biological activities linked negatively with humification degree of DOM, and the primary source of humic substances was a mixture of terrestrially-derived DOM in all samples examined. Through redundancy analysis, both the DOC concentration and C/N ratio significantly affected the DOM composition. In sum, the combined technique of 3DEEM and PARAFAC could provide a feasible and convenient method for analyzing the variances of DOM composition in swine effluent.

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SPATIAL RELATIONSHIPS BETWEEN SUBMERGED AQUATIC VEGETATION AND WATER QUALITY IN HONGHU LAKE, CHINA

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ABSTRACT

Water environment characteristics play a significant role in wetland submerged aquatic vegetation (SAV). With increased human activities, water quality conditions change significantly; such change affects the distribution and diversity of SAV. Revealing the relationship between SAV and water environmental characteristics at a spatial scale to assess the impact of human activity is necessary. Canonical correspondence analysis (CCA) and redundancy analysis (RDA) were employed to investigate the spatial relationship between SAV distribution and water environmental variables in Honghu Lake, China. Results reveal that obvious spatial variations exhibit in the diversity and abundance of SAV and water quality characteristics. The diversity indices and wet biomass of SAV are significantly higher in the middle-southern region than in the northern regions. Accordingly, the middle-southern region has a higher value of transparency and lower concentrations of nitrogen and phosphorus than the northern regions. The results of CCA and RDA indicate that water transparency is the most important variable that affects SAV distribution in Honghu Lake. The relationship between diversity and water transparency differs in the regions. The sensitive nutrient that influences the SAV community in the northern regions is total phosphorus. The results of this research can serve as an important basis for decision making on wetland ecosystem management.

KEYWORDS:

submerged aquatic vegetation; water quality; ordination analysis; Honghu Lake

INTRODUCTION

Submerged aquatic vegetation (SAV) plays an essential role in aquatic ecosystems. Aquatic plants absorb large amounts of nitrogen and

phosphorus from sediments and water in the process of growth and limit phytoplankton development. They reduce water velocity, limit sediment resuspension and increase water transparency. SAV has become a commonly used ecological engineering method for nutrient retention to manage shallow eutrophic lakes [1,2]. The major factors that affect the growth of SAV are light availability, nutrients, sediments, suspended solids, water flow and water temperature [3-8]. The physical and chemical properties of a water body are important factors that affect the biomass and community distribution of SAV, which in turn affect the growth, reproduction and distribution of submerged aquatic plants [9-11].

Honghu Lake is the largest lake in Hubei Province and the most typical shallow lake in the middle reaches of Yangtze River in China. As one of the "Species Gene Pool", Honghu Lake is important wetland with rich biodiversity and genetic diversity, and is listed in the Ramsar Wetlands of International Importance. The lake is also the breeding and wintering habitat for some rare or endangered species, such as *Ciconia boyciana* and *Mergus squamatus*. Recently, large-scale fish farming and increased discharge of agricultural and industrial wastewater from the upstream region have resulted in the drastic deterioration of water quality [12]. Several studies have shown that human activity greatly influenced the macrophyte growth and water quality in Honghu Lake. Before 2004, the trophic state of Honghu Lake began to change from mesotrophic to eutrophic. The distribution area of SAV significantly decreased. Total phosphorus (TP), permanganate index (COD_{Mn}) and total nitrogen (TN) were believed to be the major pollutants in Honghu Lake [13]. Li et al. [14] respectively evaluated the effects of different fishery development intensities on two categories of aquatic plants (favoured or not favoured by fish). This study summarized the importance of both of fish-open-culture scale and the utilizing way of aquatic plants favoured by fish for the sustainable development. Cheng and Li [15] analysed the

eutrophication process and characteristics of Honghu Lake, both of which caused the deterioration of the water quality of the lake. In 2004, the Demonstration Project of Aquatic Vegetation Protection and Restoration was implemented to reduce the impact of human activity, and the scale of fish farming was regulated. SAV coverage increased, and the concentration of nitrogen and phosphorus nutrients gradually reduced [13]. Mo et al. [16] evaluated the ecosystem health of Honghu Lake before and after the project was implemented in 2004 and demonstrated that the project was effective for the wetland protection. Ban et al. [17] indicated that the water quality of Honghu Lake deteriorated from 2001 to 2005 and gradually improved after 2006 because the measures implemented. Since 2008, with the rebound of human activity interference, the wetlands' ecological environment has been under threat once again [18].

Many studies have examined the relationship between water quality and SAV through different methods. Most studies adopted laboratory-scale approaches [19]. Through a numerical simulation, Rasmussen et al. [20] developed a combined hydrodynamic–ecology numeric model to analyse the changes in water quality and simulated the response of submerged rooted vegetation by reducing the nutrient loads. Traditional numeral statistics based on field surveys have also been employed in previous research. Scheffer et al. [21] studied the response of *Potamogeton pectinatus* and *Potamogeton perfoliatus* to water quality by using multiple logistic regressions. The results concluded that both species were predominantly related to rooting depth and water transparency. Grzybowski [22] analysed the similarities and differences in eutrophic water quality index and aquatic macrophytes between dimictic and polymictic lakes and found that aquatic vegetation in lakes may have a delayed response to phosphorus nutrient supply.

Previous studies based on field surveys often focused on the relationship between environmental parameters and SAV in different lake types at a large spatial scale [23–24]. Few studies emphasised the relationship between SAV communities and water environmental characteristics that intensively considered the influence of human activities. This study aims to identify the interaction mechanism between SAV distribution and water environmental variables in different regions in Honghu Lake, where the water ecological environment is complex and typical. The study also attempts to determine the promotion and restriction impacts of water environmental variables to SAV diversity through correlation analysis. The spatial variations of SAV

abundance, diversity and water environmental variables are analysed, the key water environmental factors that influence SAV distribution in different regions are elucidated and the correlation between SAV diversity and key water environmental variables is identified. The results are expected to provide scientific basis for restoring aquatic vegetation and enacting regional lake management measures, which is important for reducing the damaging human activities influences and sustaining ecological balance in Honghu Lake.

MATERIAL AND METHODS

Study site. Honghu Lake (29° 49' N and 113° 17' E), which is located in Honghu County and Jianli County in the southeast Hubei Province, is the largest natural freshwater lake in Jiangnan Plain and Four-lake Basin in central China (Figure 1). The lake has an area of 308 km², a mean depth of 1.34 m and a maximum depth of 2.3 m [11]. Honghu Lake was formed as a natural dammed lake within the depression between the Yangtze River and Dongjing River (a tributary of the Han River). The lake is also a catchment that collects the flow from the Four-lake Basin, which originally connected with Yangtze River and had been separated from the river by sluices since the 1950s. Honghu Lake has become a controlled lake with main function of flood regulation, water supply and fisheries [25]. The lake is a shallow aquatic system with abundant aquatic organism resources, which includes SAV species such as *Potamogeton maackianus*, *Hydrilla verticillata*, *Myriophyllum verticillatum*, *Potamogeton crispus*, *Potamogeton pectinatus* and *Ceratophyllum demersum*. Most SAV species in the lake are perennial, except for *Potamogeton crispus*, which is a hibernal annual plant.

Data collection and laboratory procedure. Water environmental characteristics and plant community structure were measured in spring and autumn from 2011 to 2014 with the support of Xiaogang Wetland Ecological Station, Chinese Academy of Sciences. Such seasonal sampling was based on the comprehensive consideration of hydrological environment stability and vegetation growth. The two seasons (spring and autumn) with equal water level periods were selected. Twenty-three sampling sites were set up based on a rough outline of Honghu Lake (Figure 1). Ten optional quadrats of SAV were collected in each SAV sampling site by using a 20 cm × 20 cm tailor-made iron clamp. The samples from the area were representative of the plant community. SAV data

was gathered at species level. The wet weight biomass (B , g/m^3) of each species was weighed on site.

Thirteen elementary physico-chemical parameters were collected. These parameters included water depth (WD), water temperature, pH, conductivity, dissolved oxygen (DO), chlorophyll a (Chl- a), water transparency (SD), TN, TP, COD_{Mn} , nitrite nitrogen (NO_2^- -N), ammonia (NH_4^+ -N) and nitrate nitrogen (NO_3^- -N). SD (in meters) was measured with a Secchi Disc. The sampling depth at each point was approximately 0.5 m below the water surface. Temperature, pH, conductivity, DO and Chl- a were determined with a portable calibrated Hydrolab DS5 Multiprobe Water Quality Sonde made by Hach Co., USA. Preservation, transportation and analysis of the water samples were performed based on the Environment Quality Standard for Surface Water [26]. NH_4^+ -N was determined through Nessler's reagent colorimetric method. NO_3^- -N was analysed through the ultraviolet spectrophotometric method, and NO_2^- -N was measured through the colorimetric method [27].

Analytical Methods. The importance value index (IVI) for each species in each sample site was calculated to indicate the relative dominance of species through the following formula [28].

$$\text{IVI} = (\text{Relative frequency} + \text{Relative Biomass}) / 2$$

Relative frequency refers to the percentage of the number of the quadrats containing a species over the total quadrat number in a sample site. Relative biomass refers to the percentage of a species' biomass over the total SAV biomass in a sample site.

The richness, heterogeneity and evenness index of each sample site were measured with the following diversity indices.

- (1) Margalef richness index (Ma)
 $Ma = (S-1)/\ln N$

- (2) Simpson heterogeneity index (DS)
 $DS = 1/\sum P_i^2$

- (3) Shannon–Wiener heterogeneity index (H')
 $H' = -\sum (P_i * \ln P_i)$

- (4) Pielou evenness index (JP)
 $JP = -\sum (P_i * \ln P_i) / \ln S$

Here, in all the above indices, $P_i = N_i/N$. N is the sum of the IVI of all species in a sample site, N_i is the IVI of the i th species and S is the species number for a sample. The spatial variations in SAV diversity and water environmental variables were analysed through an independent sample t -test. The relationship between SAV community distribution and water environmental factors was explored by using the ordination methods, which concentrate the effects of water environmental variables on species on a synthetic ordination axis [29]. Statistical significance test of the ordination analysis between species composition and environmental variables was conducted by applying the Monte Carlo permutation test. Detrended correspondence analysis (DCA) of the species' IVI conducted as preliminary processing revealed that the maximum length of the gradient was 2.64 for species data in northern regions of Honghu Lake. With regard to the rare species in the samples, a unimodal model (canonical correspondence analysis, CCA) was more suitable for the ordination analysis of the northern regions' datasets [29,30]. The maximum length of the gradient in DCA results was 1.32 for species data in middle-southern region, indicating that a linear model (redundancy analysis, RDA) was more suitable for the middle-southern datasets [29]. Prior to the analysis, the datasets were transformed to enable them to have a normal distribution. Ordination analysis was performed with CANOCO Version 5.0.

Two-tailed Pearson's correlation analysis was conducted to reveal the variations in the aforesaid four diversity indices along with the environment variable gradients. Statistical analysis was performed with the SPSS (PASW) Statistics 18.0 software package.

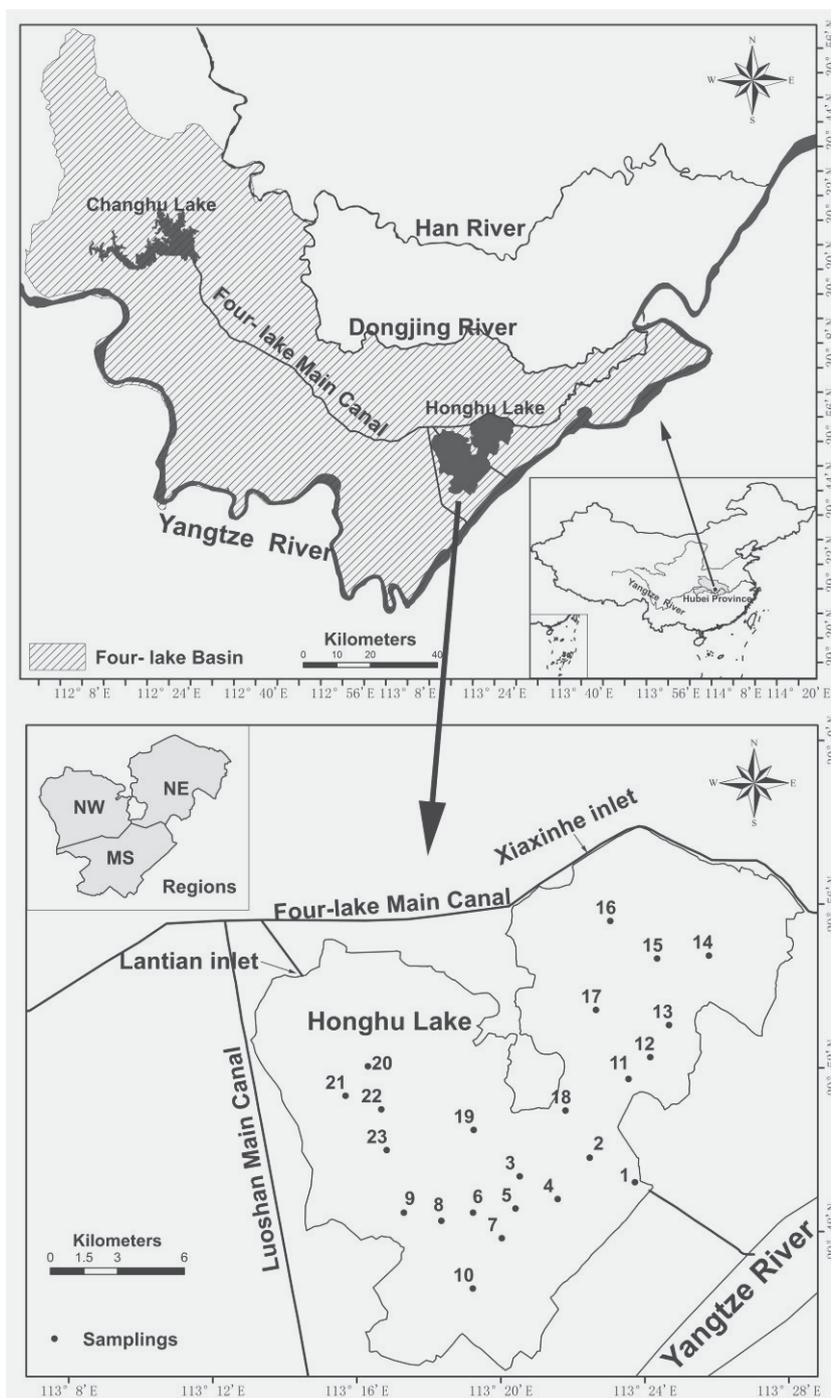


FIGURE 1

Study area and location of sampling sites, including 23 sites (in dots) where both water environmental variables and SAV were investigated. MS represented the middle-southern region; NE represented the north-eastern region; NW represented the north-western region.

RESULTS

Species Composition of SAV. The statistics of IVI reveal that *P. maackianus* has the highest IVI in Honghu Lake (Table 1); the mean IVI is 62.3 in spring and 67.7 in autumn. *M. verticillatum* and *P.*

crispus are the second-dominant species. The mean IVI of *P. crispus* is 55.3 in spring and 39.4 in autumn. The IVI of *P. crispus* declines in autumn; this hibernant annual plant gemminates in winter and gradually diminishes in autumn of the next year.

TABLE 1

IVI of SAV in two seasons. Sd is standard deviation. Analysed communities: *P. maackianus*—*Potamogeton maackianus*; *P. crispus*—*Potamogeton crispus*; *M. verticillatum*—*Myriophyllum verticillatum*; *P. pectinatus*—*Potamogeton pectinatus*; *H. verticillata*—*Hydrilla verticillata*; *C. demersum*—*Ceratophyllum demersum*; *P. malaianus*—*Potamogeton malaianus*; *N. marina*—*Najas marina*; *V. spiralis*—*Vallisneria spiralis*; and *P. lucens*—*Potamogeton lucens*. These abbreviations are also used in the text and figures.

| Species | Spring | | | Autumn | | |
|-------------------------|--------|------|------|--------|------|------|
| | Max | Mean | Sd | Max | Mean | Sd |
| <i>P. maackianus</i> | 95.6 | 62.3 | 31.2 | 99.6 | 67.7 | 29.9 |
| <i>P. crispus</i> | 100.0 | 55.3 | 34.5 | 93.4 | 39.4 | 24.2 |
| <i>M. verticillatum</i> | 71.9 | 42.4 | 19.6 | 76.5 | 41.8 | 19.7 |
| <i>P. pectinatus</i> | 87.1 | 35.6 | 22.9 | 98.0 | 36.3 | 29.6 |
| <i>H. verticillata</i> | 58.5 | 33.3 | 16.4 | 68.7 | 33.3 | 16.2 |
| <i>C. demersum</i> | 61.5 | 30.2 | 17.7 | 79.2 | 32.2 | 20.8 |
| <i>P. malaianus</i> | 56.4 | 19.0 | 17.3 | 72.8 | 26.5 | 21.1 |
| <i>Charetea</i> | 20.3 | 10.2 | 7.8 | 32.0 | 10.3 | 8.0 |
| <i>N. marina</i> | 5.6 | 5.4 | 0.4 | 38.2 | 14.2 | 14.5 |
| <i>V. spiralis</i> | 11.2 | 7.4 | 3.3 | 22.6 | 11.3 | 6.7 |
| <i>P. lucens</i> | 12.4 | 12.4 | - | 10.5 | 8.3 | 3.1 |

Spatio-temporal Analysis of SAV. To study the spatial variation in SAV community state, we subjectively divided Honghu Lake into three regions based on the geographical position and the utilization patterns. They are respectively the middle-southern region (MS, samples 1[#]–10[#], Figure 1), north-eastern region (NE, samples 11[#]–18[#]) and north-western region (NW, samples 19[#]–23[#]). The average values of the four diversity indices and wet biomass at different sampling times and regions are shown in Figure 2.

The spatial variations in species diversity indices and wet biomass are obvious. The Margalef richness index is significantly the highest in the middle-southern region and the lowest in the north-western region (Figure 2a, $p < 0.05$). The Simpson and Shannon–Wiener heterogeneity indices, as well

as the wet biomass in the middle-southern region are significantly higher than that in the two northern regions (Figure 2b–2c, Figure 2e, $p < 0.01$). The regional difference in Pielou evenness index is not significant (Figure 2d, $p > 0.05$).

The temporal variation range of the four SAV diversity indices in the middle-southern region is distinctly lower than that in the other regions. However, no significant trend of diversity indices in seasonal difference is indicated between spring and autumn ($p > 0.05$) (Figure 2a–2d). The wet biomass in the middle-southern region is significantly higher in autumn than in spring (Figure 2e, $p < 0.01$). Conversely, in the north-eastern region, the wet biomass is significantly lower in autumn than in spring (Figure 2e, $p < 0.05$).

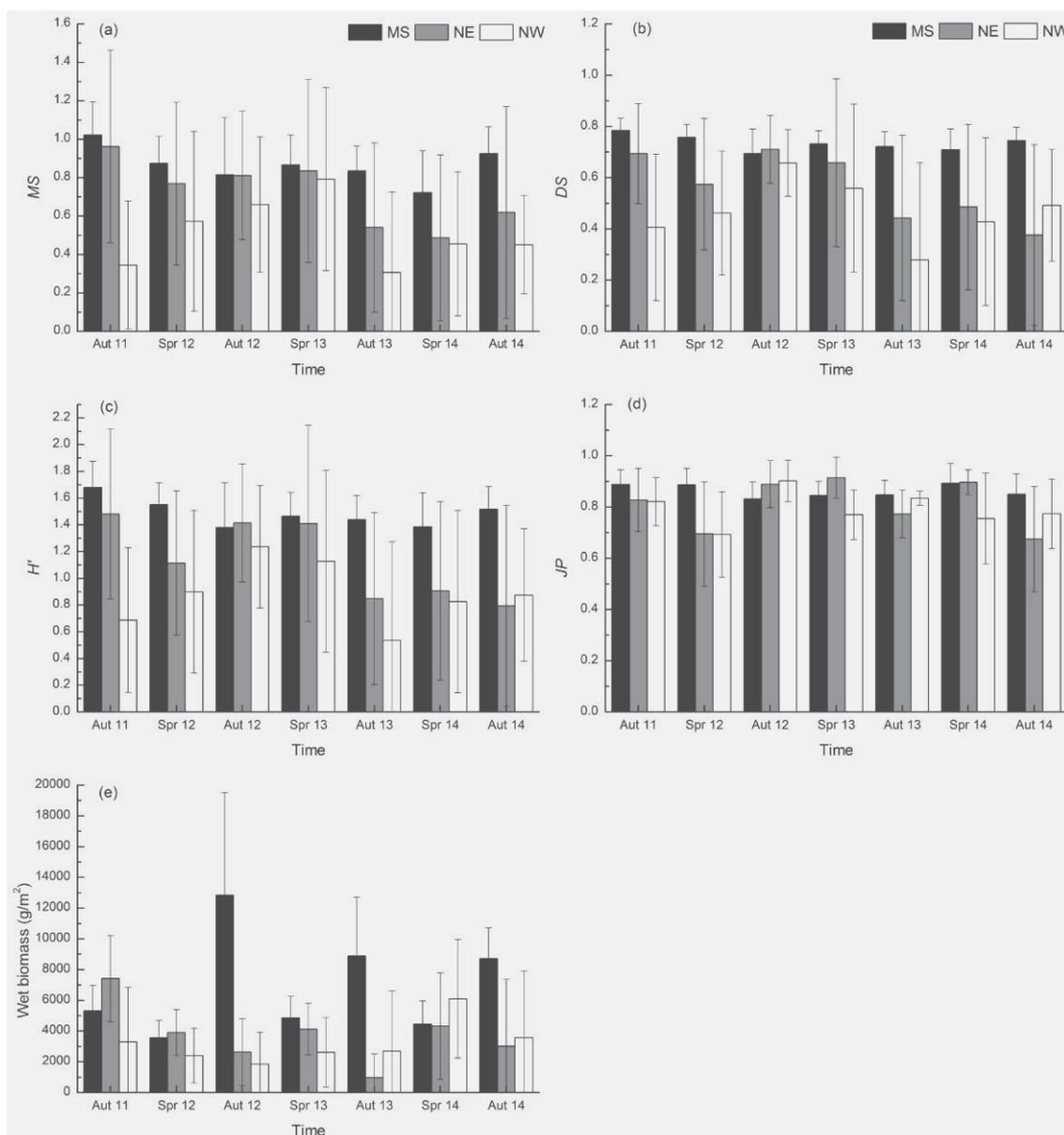


FIGURE 2
Margalef richness index (Ma , a), Simpson heterogeneity index (DS , b), Shannon–Wiener heterogeneity index (H' , c), Pielou evenness index (JP , d) and wet biomass (e) with respect to sampling times and regions. MS: middle-southern region; NE: north-eastern region; NW: north-western region.

Spatial Variations of Water Environmental Variables. Based on the spatial variations in water environmental characteristics, the middle-southern region is distinguished from the northern regions (Table 2). The middle-southern region has a significantly higher value of transparency, a lower value of NO_3^- -N and a lower ratio of WD to transparency ($Z_{(WD/SD)}$) than the two

northern regions. The spatial differences in the three regions become more obvious when nutrients and algae are considered. Chl-*a*, conductivity, TN, TP and NO_2^- -N are the lowest in the middle-southern region and the highest in the north-western region. Furthermore, the north-western region has the highest concentration of COD_{Mn} and NH_4^+ -N.

TABLE 2
Mean values of water environmental variables (mean \pm standard deviation) in the three regions compare through an independent sample *t*-test. Differences are signified by letters ($p < 0.05$).

| Variables | Middle-southern region | North-eastern region | North-western region |
|--|------------------------|------------------------|------------------------|
| WD (m) | 1.55 \pm 0.29 (a) | 1.79 \pm 0.35 (b) | 1.64 \pm 0.28 (a) |
| SD (m) | 1.34 \pm 0.36 (a) | 0.87 \pm 0.39 (b) | 0.77 \pm 0.36 (b) |
| $Z_{(WD/SD)}$ | 1.24 \pm 0.44 (a) | 2.67 \pm 1.63 (b) | 2.67 \pm 1.46 (b) |
| Temperature ($^{\circ}$ C) | 20.6 \pm 4.3 (a) | 20.14 \pm 3.89 (a) | 20.00 \pm 4.37 (a) |
| pH | 9.27 \pm 0.53 (a) | 9.20 \pm 0.7 (a) | 9.02 \pm 0.55 (a) |
| Conductivity (μ S/ cm) | 296.34 \pm 35.99 (a) | 341.33 \pm 50.19 (b) | 389.95 \pm 78.85 (c) |
| DO (mg/L) | 8.11 \pm 1.60 (a) | 7.89 \pm 2.09 (a) | 8.39 \pm 1.70 (a) |
| Chl- <i>a</i> (μ g/L) | 4.99 \pm 3.8 (a) | 13.82 \pm 10.59 (b) | 21.77 \pm 19.09 (c) |
| COD _{Mn} (mg/L) | 5.74 \pm 0.88 (a) | 5.97 \pm 0.98 (a) | 6.69 \pm 1.42 (b) |
| TN (mg/L) | 0.68 \pm 0.24 (a) | 1.05 \pm 0.69 (b) | 1.60 \pm 1.31 (c) |
| TP (mg/L) | 0.05 \pm 0.05 (a) | 0.07 \pm 0.04 (b) | 0.12 \pm 0.07 (c) |
| NH ₄ ⁺ -N (mg/L) | 0.27 \pm 0.09 (a) | 0.35 \pm 0.40 (a) | 0.56 \pm 0.49 (b) |
| NO ₃ ⁻ -N (mg/L) | 0.16 \pm 0.16 (a) | 0.29 \pm 0.28 (b) | 0.35 \pm 0.39 (b) |
| NO ₂ ⁻ -N (mg/L) | 0.00 \pm 0.01 (a) | 0.01 \pm 0.02 (b) | 0.04 \pm 0.06 (c) |

Ordination Analysis of Species Composition and Environmental Variables.

Automatic forward selections of CCA/RDA are conducted to identify the principal water environmental variables that affect the distribution of SAV (Table 3). Owing to the lack of samples in the north-western region and the presence of a similar trend with the north-eastern region (Figure 2), we combine the north-western dataset with the north-eastern dataset and then compare the

combined datasets with the middle-southern region's dataset. The conditional effects show that in the northern regions, SD ($p < 0.01$), pH ($p < 0.01$), TP ($p < 0.05$) and WD ($p < 0.05$) are the key water environmental factors that affect SAV distribution. In the middle-southern region, the principal water environmental factors are conductivity ($p < 0.01$), SD ($p < 0.01$), WD ($p < 0.01$), Chl-*a* ($p < 0.05$) and water temperature ($p < 0.05$).

TABLE 3
Conditional effects obtain from the forward selection procedure in two regions. The conditional effects show that the environmental variables are in order of their inclusion in the model. Together with the additional variance, each variable explains the time it is included. The *P* value is the significance level of a variable using a Monte Carlo permutation test.

| Northern regions | | | Middle-southern regions | | |
|---------------------------------|--------------------------|----------------|---------------------------------|--------------------------|----------------|
| Variable | Explanatory variance (%) | <i>P</i> value | Variable | Explanatory variance (%) | <i>P</i> value |
| SD | 11.0 | 0.002 | Conductivity | 25.6 | 0.002 |
| pH | 6.6 | 0.004 | SD | 9.3 | 0.002 |
| TP | 3.3 | 0.038 | WD | 9.1 | 0.002 |
| WD | 2.5 | 0.049 | Chl- <i>a</i> | 4.6 | 0.026 |
| TN | 2.7 | 0.076 | Temperature | 3.6 | 0.038 |
| NO ₂ ⁻ -N | 3.0 | 0.076 | COD _{Mn} | 2.5 | 0.170 |
| Conductivity | 3.2 | 0.044 | pH | 2.4 | 0.184 |
| Chl- <i>a</i> | 1.3 | 0.454 | DO | 2.3 | 0.224 |
| NO ₃ ⁻ -N | 2.1 | 0.182 | NO ₃ ⁻ -N | 1.2 | 0.580 |
| DO | 1.0 | 0.620 | TN | 1.1 | 0.634 |
| Temperature | 0.8 | 0.736 | TP | 0.9 | 0.756 |
| COD _{Mn} | 0.6 | 0.814 | NH ₄ ⁺ -N | 1.1 | 0.634 |
| NH ₄ ⁺ -N | 0.5 | 0.928 | NO ₂ ⁻ -N | 0.8 | 0.832 |

In the northern regions, the eigenvalues (λ) of the first and second ordination axes are 0.186 and 0.042, respectively (Figure 3), which explain approximately 85.2% of the cumulative variance in the relationship between SAV composition and water environmental factors. The eigenvalues of the first and second ordination axes in the middle-southern region are 0.353 and 0.102, respectively (Figure 4), which explain 86.1% of the cumulative variance in the relationship between SAV composition and water environmental variables. The Monte Carlo permutation tests of the first and second ordination axes in both regions reach the significance level ($p < 0.05$). The results of CCA reveal that the first and second ordination axes can

sufficiently illustrate the effects of water environmental factors on the distribution of SAV communities.

The first canonical axis can be explained as the gradients of SD and TP in the northern regions (Figure 3), which is significantly correlated with SD and TP ($p < 0.01$, Table 4). The value of TP concentration decreases from left to right; the *P. crispus* triangle situates near the arrow is positively correlated to TP concentration. The SD value decreases from left to right. The triangles of *P. maackianus*, *H. verticillata* and *M. verticillatum* situate near the arrow of SD show that the important values of these species increase with the increase in the SD value.

TABLE 4
Pearson's correlation coefficients between environmental variables and the first CCA/RDA axes in two regions. ** means the correlation is significant at the 0.01 level (two tailed).

| Correlation coefficients | | SD | TP | Cond | Chl-a |
|--------------------------|------------------------|----------|----------|---------|---------|
| The first CCA/RDA axes | Northern regions | 0.792** | -0.517** | - | - |
| | Middle-southern region | -0.555** | - | 0.879** | 0.737** |

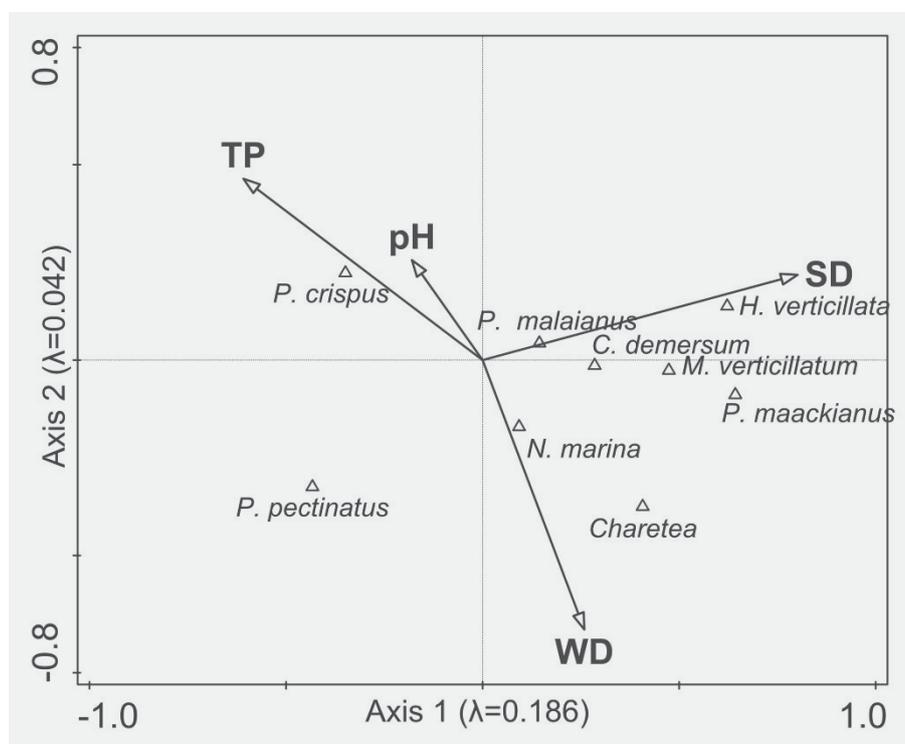


FIGURE 3
Ordination diagram between water environmental factors (in arrows) and SAV species (in up triangles) in the northern regions.

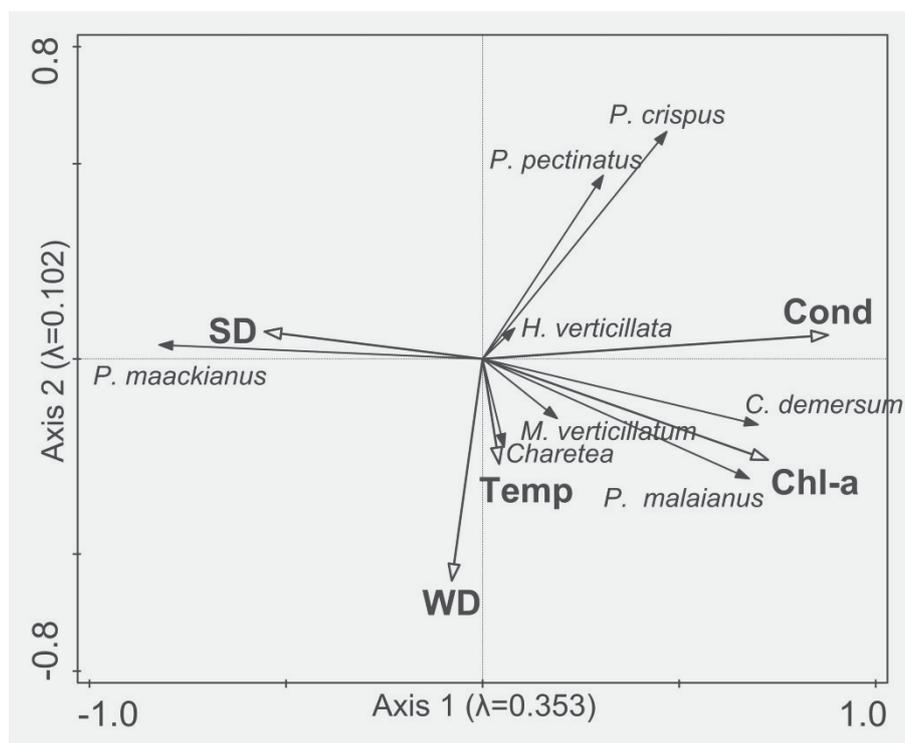


FIGURE 4

Ordination diagram between water environmental factors (in hollow arrows) and SAV species (in solid arrows) in the middle-southern region.

The first canonical axis is gradients of conductivity, SD and Chl-*a* in the middle-southern region (Figure 4), which is significantly correlated with these environmental variables ($p < 0.01$, Table 4). The concentration of conductivity and Chl-*a* increase from left to right. *C. demersum* and *P. malaianus* situating near the arrow of Chl-*a* potentially have a weak ability to limit phytoplankton development. In the middle-southern region, the first canonical axis is also a gradient of SD; the important value index of *P. maackianus* increases as the value of SD increases.

Change in Species Diversity Indices. The first CCA/RDA axes are utilised to represent the environmental gradients. Based on the CCA/RDA results, the correlative relationship between species

diversity indices and the first CCA/RDA axes were determined through Pearson’s correlation analysis method (Table 5).

The richness and heterogeneity indices (*Ma*, *DS* and *H'*) have significant positive correlations with the first CCA axis that represents the gradients of TP and SD ($p < 0.01$). The diversity indices increase with the increase in the SD value and the decrease in TP in the northern regions. The changes in species diversity in the middle-southern region reveal a different pattern. The four diversity indices have significant positive correlations with the first RDA axis that represents the gradients of conductivity, SD and Chl-*a* ($p < 0.01$). The higher the values of conductivity and Chl-*a* are and the lower the transparency value is, the greater species diversity is in the middle-southern region.

TABLE 5

Pearson’s correlation coefficients between species diversity indices and the first CCA/RDA axes in two regions. ** means the correlation is significant at the 0.01 level (two tailed).

| Correlation coefficients | | <i>Ma</i> | <i>DS</i> | <i>H'</i> | <i>JP</i> |
|--------------------------|------------------------|-----------|-----------|-----------|-----------|
| The first CCA/RDA axes | Northern regions | 0.400** | 0.377** | 0.444** | 0.279 |
| | Middle-southern region | 0.605** | 0.737** | 0.763** | 0.448** |

DISCUSSIONS

Spatial Variations of SAV and Water Quality. The diversity and abundance of SAV exhibit distinct spatial variations in Honghu Lake, as well as water quality. The wet biomass, richness and heterogeneity indices of SAV in the middle-southern region are significantly higher than those in the northern regions (Figure 2). The middle-southern region has a significantly higher water transparency value and a lower nitrogen and phosphorus nutrients concentration than the northern regions (Table 2). A fishing ban has been strictly implemented in the middle-southern region, which is located in the core area of Honghu Lake National Wetland Nature Reserve, since 2004. Simultaneously, large areas of submerged vegetation, such as *P. maackianus* and *V. spiralis*, were transplanted into the region [31]. These measures gradually increased SAV diversity and improved the stability of the aquatic ecosystem. The northern regions are close to the inlets from the Four-lake Main Canal (Figure 1), which adopt agricultural and industrial wastewater from the Four-lake Basin. A previous study and field data both reveal that the TN concentrations of Lantian inlet and Xiaxinhe inlet from the Four-lake Main Canal exceed to 2.0 mg/L [32]. The north-western region is close to the Lantian aquaculture area and the Lantian ecotourism scenic spot. Li et al. [33] reported that the water quality in Honghu Lake deteriorated after enclosure aquaculture was established. The remanent fish bait and waste have accelerated the eutrophication of the region, as well as the emissions of fisherman's domestic waste and spent fuel. Moreover, the extreme factors such as grass mowing have uncertain impacts on the abundance and diversity of SAV, which may result larger temporal fluctuation of diversity indices in the northern regions. Intense human activities aggravate the deterioration of water quality and ecology in the northern regions.

Dominant SAV Species. *P. lucens* and *V. spiralis* are not included in the ordination plots because the probabilities of their occurrence are less than 10% (Figure 3 and 4). SAV is tolerant with a wide range of nutrients concentration [34]. A previous study demonstrated that *P. crispus* could efficiently avoid NH_4^+ accumulation in plant tissues and was notoriously abundant in eutrophic water [35]. *P. crispus* also plays an important role in the retention of phosphorus nutrients [36]. In the current study, as a stain-resistant SAV species, *P. crispus* is found to be more tolerant of high concentration of nutrients than other species in the northern regions (Figure 3). On the other hand, as

an annual plant, the yearly decay of *P. crispus* may increase the release of nutrients. Ni [37] found that *P. maackianus* was strongly inhibited by the consequences of nutrient enrichment in water in Donghu Lake in the 1970s; the species disappeared because of eutrophication. In the current study, *P. maackianus* is found to be less tolerant of high concentration of nutrients in the entire region of the lake (Figure 3 and 4) and is the dominant species in the core region of Honghu Lake, which is scarcely exploited by human activities.

The wet biomass of SAV exhibit significant seasonal differences. In the middle-southern region, it is significantly higher in autumn than in spring. Conversely, in the north-eastern region, the wet biomass is significantly lower in autumn (Figure 2e). It can be explained by the growth rhythm of the dominant species in different regions. The dominant SAVs in the middle-southern region, such as *P. maackianus*, belong to perennial vegetation that germinates in spring. *P. crispus*, which is the dominant SAV in the north-eastern region, is a hibernant annual plant that germinates in winter and grows well in spring of the following year.

Key Environmental Factors Affect SAV.

Controversy has always surrounded the issue of whether nitrogen or phosphorus is the key factor that affects the composition and distribution of SAV [19, 38, 39]. Many researchers studied the N:P ratio in freshwater lakes and revealed that eutrophication was possibly limited by P nutrients [40, 41]. The influence of water body eutrophication on SAV is mainly caused by the decline of water transparency, which also results in light attenuation [42]. Eutrophication of freshwater lakes may be caused by frequent human activities, such as watershed non-point pollution and development of culture fishery. In the present study, the results of relationship between species and environmental variables conducted in the northern regions indicate that the distribution of SAV communities in Honghu Lake can be significantly explained by TP (Table 3), the SAV diversity indices are negatively correlated with TP (Table 5), where large amounts of P nutrients are carried by surface runoff and chemical fertilizers are retained. This support the facts illustrated by May [43] that the changes in maximum growing depth of aquatic plants have a close relationship with the external P load.

The abundance and distribution of SAV in shallow lakes are closely related to water depth and transparency [21, 44]. Liang [45] found that SAV would not survive if $Z_{(WD/SD)}$ was permanently over 2.50–5.59. Conversely, the growth of SAV has a positive effect on wave action restriction and

sediment resuspension, which both increase water transparency. Most SAV are shade plants, in which photosynthetic light saturation and compensation points are lower than those in terrestrial plants [46]. For instance, the photosynthetic light saturation and compensation points of *P. maackianus* in Lake Donghu were measured at depths of ca. 0.9 m and ca. 1.5 m; all the growth indices of the plant peaked at 1.2–1.3 m simulated depths [47]. The experiments on *P. crispus* growth at transparency of 15, 20, 35 and 70 cm indicated that *P. crispus* grew well when the SD values were 20 and 35 cm [48]. Water transparency in the middle-southern region of Honghu Lake is generally high, with most of the $Z_{(WD/SD)}$ values reaching 1.0 (Table 2). Unlike in the northern regions, the light intensity obtained at the bottom in this region may exceed the light saturation points. A distinct correlation between SD value and species diversity in the two regions would probably be the results. This study thus reveals the promotion and restriction of nutrients and transparency to SAV growth from a statistical perspective.

Management Suggestions and Study Improvements. The deterioration of water quality and decline of SAV diversity in the northern regions by anthropogenic nutrients inputs can be relatively important ecological problems in Honghu Lake. The sources of loads mainly include enclosure aquaculture inputs and runoff discharge from upstream. As is mentioned above, the Honghu Lake National Wetland Nature Reserve has implemented a list of measures to reduce the impact of human activities, which focus on reducing the enclosure aquaculture inputs. Furthermore, it is suggested that transplanting other perennial plants (e.g. *P. maackianus*) into northern regions as an ecological restoration approach to reduce the dominance of *P. crispus*. In the long term, measures could facilitate improvement in the Honghu Lake ecosystem through reductions in nutrients load from the upstream basin. For one thing, treatment of point and nonpoint sources should be reinforced, such as domestic and industrial wastewater and agriculture runoff; for another, it will be effective that constructing engineering in the inlets of Honghu Lake to retain the nutrients input. These management measures will undoubtedly bring about major improvements for water quality and biodiversity in Honghu Lake wetland.

This study has provided a comprehensive understanding of the relationships between water environmental variables and SAV in a typical shallow macrophytic lake. Such understanding is important for the clarification of the interactional patterns between wetland environment and ecology.

It also provides theoretical basis for implementation of effective ecological restoration measures and protection of the aquatic ecosystem. However, several problems remain. Owing to the lack of data and the complexity of human activities, this study did not consider other relevant environmental factors, such as sediments, suspended solids and water flow. Other pervasive human activities, such as mowing grass and snail fishing, were also not included. The results can be improved if additional influences are considered.

CONCLUSIONS

Descriptions of the relationship between water environmental variables and SAV in Honghu Lake are presented considering the impacts of human activities. The diversity and abundance of SAV, as well as water quality characteristics, reveal obvious spatial variations because of the influence of human activities. The wet biomass, richness (*Ma*) and heterogeneity (*H'*, *DS*) indices of SAV in the middle-southern region are significantly higher than those in the northern regions. The temporal fluctuation in SAV diversity in the middle-southern region is lower. Accordingly, the middle-southern region has a significantly higher water transparency value and a lower nitrogen and phosphorus nutrients concentration than the northern regions. The results of RDA/CCA reveal that water transparency is the main factor that influences SAV distribution in the two regions. The diversity indices increase as SD increases in the northern regions; opposite trends are observed in the middle-southern region where the human activities are less intensive. The sensitive nutrient that influences the SAV community in the northern regions is found to be total phosphorus. *Potamogeton crispus* is found to be more tolerant of high concentration nutrients than other species in the northern regions. The IVI of *Potamogeton maackianus* increases with the increase in SD. Pollutants from the upstream tributary and enclosure aquaculture are found to be the main causes of the degradation of the wetland ecosystem in the northern regions of Honghu Lake. The results of this study provide an important reference for aquatic ecological environment studies that compare other shallow macrophytic lakes and can serve as an empirical basis for wetland ecosystem management.

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CORPORATE ENVIRONMENTAL BEHAVIOUR: FROM LITERATURE REVIEW TO THEORETICAL FRAMEWORK

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ABSTRACT

The enterprise–environment debate is growing and is of great importance in the context of climate change. Enterprises face huge environmental challenges, and adoption of positive environmental behaviour can help in building a corporate green competitive advantage. This paper examines enterprise–environment interdependence and the significance of corporate environmental behaviour (CEB). An analytical review of the literature provides insight into the CEB evolution path. The factors that drive a firm’s environmental behaviour are explored. A theoretical framework for CEB provides a useful representation of relationships among factors affecting CEB, CEB itself, and CEB performance for enterprises. The suggested logical relationships lay a foundation for the next step in empirical research and can help enterprises in adopting positive CEB to build a green competitive advantage.

KEYWORDS:

corporate environmental behaviour; green competitive advantage; literature review; theoretical framework

INTRODUCTION

The ecological environment (EE) is a global concern. Because of their major role in resource consumption and pollution discharge, enterprises have negative EE impacts while providing products and services. Therefore, corporate environmental behaviour (CEB) is of great significance for realisation of EE objectives and is the basis for sustainable corporate development. Here we

construct a three-dimensional CEB conceptual model and conduct an analytical review of the literature on CEB evolution, influential factors, and performance. We also suggest a theoretical framework for CEB that describes the relationships among its three dimensions, lays a foundation for empirical research in this field, and help enterprises form green competitive advantage.

Understanding the interdependence between enterprises and the environment is the main premise of this paper. The environment provides enterprises with physical resources for production, and the amount of such resources that can be utilised depends on the production capacity of the environment. Wastes produced by enterprises are discharged into the environment. The EE faces a huge threat if the physical resources and waste emissions required by enterprises are greater than the production capacity and carrying capacity of the environment. As enterprises are the largest source of pollutant discharge, CEB is of great importance for global EE protection.

ENTERPRISES AND THE ENVIRONMENT

Enterprises, regardless of their size, technology level, or activity, all produce wastes that can pollute the EE (D’Souza & Peretiatko, 2002). Environmental pollution resulting from enterprise production activities is increasingly apparent (Table 1). According to Perrow (1997), few significant man-made EE problems do not have organisations behind them. Enterprises can cause potential EE problems, including water, air, solid waste, and noise pollution.

TABLE 1
Environmental pollution arising from enterprise production.

| <i>Type of pollution</i> | <i>Example</i> |
|--------------------------|---|
| Water pollution | Chemical oxygen demand and ammonia nitrogen discharge |
| Air pollution | Sulfur dioxide and nitrogen oxide emissions |
| Solid waste pollution | Various waste residues, dust and other wastes; can be divided into general industrial waste and hazardous solid waste |
| Noise pollution | Mechanical equipment can generate noise in industrial and mining enterprises, including fans, blowers, air compressors, internal combustion engines, electric motors, weaving machines, pressing machines, rolling mills, saws, punches, ball mills, vibration sieves, and exhaust pipes for boilers and other pressure vessels |

Industrial economic development has had a serious negative impact on the global environment. According to Global Environment Outlook 5 (2012) published by the United Nations Environmental Programme, environmental protection measures adopted by all countries so far have failed to produce the desired result, and it is hard to achieve an agreed environmental goal. The report notes that climate change and unsustainable human production and business activities are putting ever-increasing pressure on land, water, and forest resources. The global economy loss caused by deforestation and forest degradation is greater than that caused by the 2008 financial crisis. Chemicals

and waste also pose a serious global EE threat: approximately 650 million tons of municipal waste were generated in OECD member countries in 2007 at an annual growth rate of 0.5–0.7%, with electronic waste accounting for 5–15%.

Because of the role of enterprises in resource consumption and pollution discharge in industrial economic development, their adoption of active CEB is key in mitigating global EE problems. CEB involves all aspects of business, including manufacturing, raw material procurement, energy utilisation, marketing, finance, human resources, and product development (Table 2).

TABLE 2
Concrete manifestations of corporate environmental behaviour

| <i>Area</i> | <i>Example</i> |
|--------------------------|---|
| Manufacturing | Green production, waste reduction, and adoption of green technology |
| Raw material procurement | Green procurement |
| Energy utilization | Improve energy efficiency and use alternative energy |
| Marketing | Green marketing, green supply chain management |
| Product development | Development of green products |
| Finance | Total environmental cost accounting |
| Human resources | Green human resources management |

Although enterprises are aware of the seriousness of global EE problems and recognise the strategic importance of their environmental

responsibility, there is no consensus on definitions of relevant environmental issues and how enterprises practise environmental responsibility



through CEB. Practice and theoretical research in related fields have shown that enforcement of corporate environmental responsibility can be divided into four stages: (i) the resisting stage, when enterprises refuse to take on environmental responsibility; (ii) the compliance stage, when enterprises meet the minimum standard for environmental regulations; (iii) the proactive stage, when enterprises recognise that accepting environmental responsibility helps to foster competitive advantage and adopt active CEB; and (iv) the sustainable development stage, when companies integrate environmental protection into their long-term development strategy to achieve a win-win situation between environmental protection and economic development (Starik & Marcus, 2000).

CEB research started in the 1970s and has rapidly expanded since 2000, with widespread interest from academia and industry in this interdisciplinary research field encompassing environmental economics, management science, and behaviour science, among other areas. Here we present a systematic review of the relevant literature and propose a comprehensive theoretical framework as a basis for future empirical research.

CEB DEVELOPMENT AND EVOLUTION: FROM LINEAR PATH TO NONLINEAR PATH

LINEAR PATH

Most of the early CEB classification studies drew on the reactive, defensive, accommodative, and proactive response modes noted above (Carroll, 1979; Wartik & Cochrane, 1985) because they reflect the gradually increasing emphasis enterprises place on social responsibility. In this context, CEB can be regarded as a continuum ranging from reactive to proactive along a linear path. At one end of the continuum, enterprises choose a passive EE response and simply follow existing regulatory requirements, while at the other end, enterprises choose a positive EE response and adopt a strategy to create a competitive advantage. Such strategies include green production and marketing and environmental technology and management innovations. Some studies proposed that a dichotomy can exist in CEB evolution. According to the degree of compliance with environmental regulations, Sharma (2000) divided CEB into compliant and voluntary CEB. Compliant CEB comprises standard environmental practices that simply follow the minimum environmental standard, while voluntary CEB minimises the

negative EE impact of business operations. Moon (2008) and Aragon-Correa and Sharma (2003) divided CEB into reactive and active types. Winn and Roome (2007) classified CEB as follower, compliance, and leadership behaviours to reflect constant enhancement of the positive attitude of enterprises towards EE issues. Liu (2009) classified CEB into defensive, preventive, and enthusiastic behaviours. Hart (1995) took a natural resource view of enterprises and proposed four CEB strategy stages: end-of-pipe control, pollution prevention, product stewardship, and sustainable development. The study showed that under the action of path dependence and embeddedness, the different CEB strategy stages are interrelated and evolve from the lower to the higher stage under accumulation and evolution of firm resources and capabilities. Winsemius and Guntram (2002) divided CEB into four types: reactive, functional, integrated, and proactive. These environmental responses fit the assumption of a CEB continuum and indicate that corporate environmental responses are a gradual process from reactive to proactive. On the basis of enterprise resources and capabilities, Christmann et al. (2002) classified CEB into five types: reactive, capability-building, defensive, accommodative, and proactive. Their study included an analysis of reasons for the emergence of international voluntary environmental initiatives and of the importance of the trend for enterprises, and a theoretical framework for CEB with evolution from a low level to an advanced level to build competitive advantage.

CEB classification in most of the above studies was confined to theoretical analysis; few studies have carried out empirical tests (Liu, 2009; Liu and Ye, 2012). Using a structured questionnaire for 321 enterprises in the Yangtze River Delta, Liu (2009) tested the relationship between external environmental pressures and CEB via path analysis and found significant correlation. The results indicated that pressure due to government regulation is the most important factor affecting defensive CEB, market pressure is the most important factor affecting preventive CEB, and pressure from community groups and non-governmental organisations (NGOs) is the most important factor affecting enthusiastic CEB. Liu and Ye (2012) used an adaptive agent-based model to analyse CEB evolution and influential factors. Using a structured questionnaire for 167 Chinese enterprises, simulations showed that CEB follows a linear evolutionary path from defensive to preventive and then enthusiastic behaviour.

Colby (1991) proposed five CEB paradigms related to the correlation between economic development and environmental management

orientation: a frontier economy; environmental protection; resource management; eco development; and deep ecology. Among these, a frontier economy and deep ecology are at the two extremes of the environmental behaviour continuum, with environmental protection, resource management, and eco development in the continuous evolution process between these extremes. Johnson and Macy (2001) established a

model to replace the division proposed by Colby (1991) to evaluate an organisation's environmental perception and analyse how this perception affects the response to stakeholder pressure. They proposed four dimensions (point of view, principle, process, and result) to distinguish organisational social and ecological responsibility in relation to various stakeholders. Table 3 summarises research on CEB evolution stages.

TABLE 3
Division of evolutionary stages of corporate environmental behaviour

| <i>Study</i> | <i>Standard</i> | <i>Evolutionary stages</i> |
|----------------------------|---|--|
| Hunt and Auster, (1990) | Extent and manner of corporate environmental pressure response | (1) beginner; (2) firefighter; (3) citizen caring for society; (4) pragmatist; (5) proactivist |
| Colby(1991) | Correlation with economic development and environmental management orientation | (1) frontier economy; (2) environmental protection; (3) resource management; (4) eco development; (5) deep ecology |
| Roome (1992) | Response mode of enterprise to environmental regulations and social pressure | (1) noncompliance; (2) compliance; (3) compliance plus; (4) environmental leader |
| Hart (1995) | Interaction between enterprise and environment | (1) end-of-pipe control; (2) pollution prevention; (3) product stewardship; (4) sustainable development |
| Venselaar (1995) | The way enterprises respond to environmental issues | (1) reactive; (2) active; (3) proactive |
| Richards (1997) | The way enterprises respond to environmental issues | (1) unprepared; (2) reactive; (3) anticipative; (4) highly integrated |
| Donaire (1999) | Extent and manner of corporate environmental response | (1) environmental control of output; (2) environmental control of industrial practice; (3) environmental control of management process |
| Sharma (2000) | Degree of enterprise compliance with environmental regulations | (1) compliance; (2) voluntary |
| Christmann et al. (2002) | Importance of corporate environmental management behavior and resources and capabilities available | (1) reactive; (2) capability-building; (3) defensive; (4) accommodative; (5) proactive |
| Winsemius & Guntram (2002) | Way in which environmental affairs are integrated into the general strategy and operation of the enterprise | (1) reactive; (2) functional; (3) integrated; (4) proactive |
| Jabbour & Santos (2006) | Way in which environmental affairs are integrated into corporate operation strategy | (1) functional specialization; (2) internal integration; (3) external integration |

In summary, despite differences in the terms used to describe CEB evolutionary stages, the essential features of the early stages are reaction and basic compliance, while active participation is typically characteristic of the last stage. Many CEB studies have only been at a theoretical analysis level and agree that CEB evolution is linear (Roome, 1992; Grant & Campbell, 1994). Although such studies do not explicitly define the concept of a continuum, some have linked models to the quality

management concept of continuous improvement (Roome, 1992). Although some models involve a taxonomy, they still imply that a continuum exists, and deepen understanding of how CEB evolves along such a continuum. Hass (1996) noted that these models conclude that CEB is a sequential continuous process. These studies provide enterprises with a basis for developing environmental strategies on the basis that CEB is a gradual process.

NONLINEAR PATH

Some studies raised doubts about the traditional model of linear CEB evolution through surveys and proposed critical assumptions for a nonlinear evolution model (Ghobadian et al., 1995, 1998). In an empirical study of the environmental policy of 78 top UK enterprises, Ghobadian et al. (1995) raised doubts about the assumption of linear evolution. They noted that existing models did not fully consider how CEB evolution is affected by key behavioural moderating factors, including changes in the operational environment and in managerial perception. Viney et al. (1997) used the same survey data for UK firms to construct a spatial model of the effects of key factors (external, mediating, and moderating factors) on CEB evolution. The authors argued that the response of an enterprise to these key factors is infinite, and they proposed seven prototype CEB states. The study emphasises distinctive company characteristics, especially with distinctions among active, reactive, and pragmatic company types. The model indicates how key factors encourage enterprises to make noncontinuous movements among the prototype states. However, the authors did not explore the inherent reasons why CEB follows nonlinear and noncontinuous evolution driven by these key factors. Ghobadian et al. (1998) proposed three possible types of CEB strategy positions beyond the linear evolution model (limited, speculative, and conditional commitment), and indicated that these CEB strategy positions are influenced by external, mediating, and moderating factors. The authors proposed a nonlinear CEB evolution path that takes different internal and external factors into consideration, and argued that the comprehensive effect of these factors leads to a series of different strategic environmental policies and behaviours. The advantage of a nonlinear method over the traditional linear method is that it recognises typical gaps between proposed CEB policy objectives and operational reality, and confirms that actual operational considerations may be more important than environmental issues, which naturally leads to three types of CEB strategy beyond the linear evolution model. In suggesting further research, Ghobadian et al. (1998) proposed four key hypotheses for nonlinear CEB evolution. First, the real decisive factor in CEB is the interaction between mediating factors and external factors, and this interaction does not necessarily lead to a linear response. Second, as determined by the interaction between external and internal factors, enterprises can make noncontinuous movements between different positions. Third, companies operating in

different environments can differ in personality and strategic environmental policies. Fourth, there are usually gaps between the environmental strategy position advocated by enterprises and actual strategic environment policies and behaviours.

Since 2000, empirical studies on CEB evolution have found evidence of both linear (Liu and Ye, 2012) and nonlinear evolution (Kolk & Mauser, 2002; Lee & Rhee, 2005; Jabbour, 2010). Liu and Ye (2012) showed that CEB follows a linear evolution path from defensive to preventive to proactive behaviour. Lee and Rhee (2005) investigated CEB evolution in Korea under institutional and resource changes from institutional theory and resource-based view of enterprises, respectively, using a survey of 85 paper-making enterprises during 2001–2004. The study revealed that CEB in South Korean enterprises exhibited nonlinear evolution from ignorance to compliance and then strategic compliance. In a survey of 94 enterprises with ISO 14001 certification, Jabbour (2010) found that the traditional division of CEB into reactive, preventive, and active stages along a continuous linear evolution path does not apply to Brazilian companies. Factor analysis revealed that the final two stages are synergy for eco-efficiency and environmental legislation view. These two stages can coexist in an organisation because they have different objectives, operate along different pathways, and are linked to different corporate environmental management (CEM) activities. In addition, the stages combine variables that belong to different stages in theory (CEM activities), confirming the nonlinearity of CEB evolution. This confirms the proposal by Kolk and Mauser (2002) that nonlinearity can best describe CEB evolution and that variables (CEM activities) can be grouped according to enterprise characteristics.

Wilson (1975) divided corporate response to CSR into four types: reactive strategic behaviour to escape CSR through various means; defensive strategic behaviour to simply follow the minimum regulatory standard; accommodative strategic behaviour to respond to the expectations of the various stakeholders; and proactive strategic behaviour to predict the expectations of various stakeholders and actively take measures to meet them. McAdam (1973) described four types of CSR response that match the strategies proposed by Wilson (1975): (1) fight all the way; (2) do only what is required; (3) be progressive; and (4) lead the industry.

Following Wilson (1975) and McAdam (1973) and considering the large body of literature on CEB evolution, we assume that CEB evolution is linear and divide Chinese CEB into three stages: defensive, accommodative, and proactive (Table 4).

These hypotheses are the basis for a confirmatory study using survey data from Chinese enterprises.

TABLE 4
Classification and subdivision of evolutionary stages of corporate environmental behaviour

| <i>Corporate environmental behaviour facets</i> | <i>High scores indicate that firms demonstrate...</i> |
|--|---|
| Form the required structure for raw materials | Defensive behaviour by doing only what is required |
| Target reductions in resource consumption | Defensive behaviour by doing only what is required |
| Realise obvious reductions in pollutant emission | Defensive behaviour by doing only what is required |
| Implement clean production audits | Accommodative behaviour by being progressive |
| Special environmental department | Accommodative behaviour by being progressive |
| Achieve ISO 14000 certification | Accommodative behaviour by being progressive |
| Integrate environmental issues | Accommodative behaviour by being progressive |
| Establish an environmental monitoring system | Accommodative behaviour by being progressive |
| Write environmental rules and regulations | Accommodative behaviour by being progressive |
| Conduct ecological technical innovation | Proactive behaviour by leading the industry |
| Provide human resources support for environmental issues | Proactive behaviour by leading the industry |
| Provide financial resources support for environmental issues | Proactive behaviour by leading the industry |
| Involvement of management | Proactive behaviour by leading the industry |
| Provide R&D support for cleaner technology | Proactive behaviour by leading the industry |

FACTORS INFLUENCING CEB

Enterprises make a series of different strategic responses to EE problems. What are the main factors that drive different enterprises to make different responses to the same environmental issues? This section focuses on the factors that affect CEB.

The literature on management shows that the corporate strategic response to EE problems is largely determined by the environment in which an organisation operates (Pettigrew, 1987), which can be divided into external and internal environments. The external environment may include factors such as law, policy, community, and industry and capital markets. The internal environment may include internal policies, structure, enterprise values, attitudes and beliefs of employees, and the resources and capabilities available.

Lu and Lake (1997) noted that enterprises experience exogenous (external) pull and endogenous (internal) motivation for CEB implementation. Organisational policies, structure, rules, values, and culture constitute internal motivation, while environmental regulations, higher public EE awareness, and the growing demand from environmental pressure groups constitute the external pull. The green strategy trend that often diffuses among competitors in a sector is also exogenous, whereby an organisation follows the higher environmental standards implemented by the leading enterprise to ensure continued operation.

EXTERNAL ENVIRONMENTAL FACTORS

Most of the research on external environmental factors affecting CEB is based on two related theories, new institutional theory (DiMaggio and Powell, 1983) and stakeholder theory (Freeman, 1984). New institutional theory

considers an organisation as an open system whose structure and operation are subject to the institutional environment (Zhou, 1999; Scott, 2002). The institutional environment refers to generally accepted elements such as concepts, external norms, and values that induce an organisation to adopt generally accepted structures and operational methods to obtain organisational legitimacy (DiMaggio and Powell, 1983). Therefore, stakeholders, social factors, and political factors in the institutional environment have important effects on CEB (Raedeke et al, 2001; Delmas, 2002; Bray et al, 2002). From this point of view, CEB can be regarded as a social construction process in which enterprises obtain organisational legitimacy. According to stakeholder theory, CEB is influenced by various stakeholders (Liu and Anbumozhi, 2009; Frooman, 1999; Donaldson and Preston, 1995). Many studies have taken this perspective, and external stakeholders for CEB may include the government, the surrounding community, industry associations, NGOs, investors, creditors, consumers, competitors, and suppliers (Dong et al., 2014; Lin et al., 2014; Qi et al., 2011; Zeng et al., 2010; Liu et al., 2010; Lu and Abeysekera, 2014).

GOVERNMENTAL REGULATION

Henriques and Sadorsky (1996) suggested that governmental regulation is an important factor affecting CEB. Researchers taking a traditional point of view found that government inspection activities have an important effect on CEB (Dasgupta et al., 2001; Gray & Deily, 1996; Helland, 1998; Laplante & Rilstone, 1996; Magat & Viscusi, 1990; Nadeau, 1997; Saether, 2000; Ytterhus & Synnestvedt, 1995; Dobers, 1997; Larsson et al., 1996; Lindell & Karagozoglu, 2001). Reijnders (2003) found that a permit system based on full rules and a consultation agreement may be a more specific policy tool for encouraging enterprises to implement cleaner production. Stafford (2002) examined the impact of new enforcement of US Environmental Protection Agency (EPA) regulations on enterprises complying with a toxic waste management system. These studies initially explored the effect of government intervention on CEB in two sectors, the pulp industry and the steel industry (Gray & Deily, 1996; Helland, 1998). These studies confirmed the positive relationship between enterprise compliance and implementation of government decisions. Later research expanded to other industrial sectors. Evangelinos and Oku (2006) evaluated regulatory and environmental issues for mining operations in the Cyclades islands. Mendivil et al. (2005) used a

systematic approach to assess the effects of technological evolution, markets, and regulation on the past and future environmental performance of Swiss chemical manufacturing enterprises. Triebswetter and Hitchens (2005) examined whether stringent environmental legislation will have a negative impact on German industrial plants using three case studies. They found that pollution reduction behaviour can be implemented without obvious economic damage.

COMMUNITY GROUPS

Community groups are playing a more positive role in environmental protection in developed countries and have become a key factor affecting CEB (Chen & Soyeze, 2003; Blackman & Bannister, 1998; Pargal & Wheeler, 1996). Henriques and Sadorsky (1996) and Dasgupta et al. (2000) suggested that self-reported community pressure has a significant effect on CEB. Studies have also explored the impact of community characteristics on industrial wastewater emissions at firm level, decisions on enterprise location, pollution abatement costs for manufacturing plants, and the extent of polluter compliance with regulations (Hamilton, 1993; Pargal & Wheeler, 1996; Wolverton, 2002; Becker, 2004; Earnhart, 2004).

Gunningham et al. (2003) discussed the effects of community pressure on CEM. Maxwell et al. (2000) extended the economic theory of regulation to allow for strategic self-regulation that preempts political action. Results showed that an increased threat of regulation (as represented by increased membership in conservation groups) induces firms to reduce toxic chemical releases. Becker (2004) examined whether community characteristics can explain pollution abatement costs for US manufacturing enterprises. Estimations showed that community characteristics had a significant impact on regulatory intervention and corporate environmental performance. In China, communities are playing an increasingly important role in environmental protection. In fact, some communities are playing an active role in urging companies to improve environmental performance (Zhang et al., 2008). However, some studies did not find that community is a key factor affecting the environmental behaviour of Chinese enterprises (Wang et al., 2007; Cai, 2002).

CAPITAL MARKET STAKEHOLDERS

In developed and emerging market economies, the capital market can react to environmental information and motivate enterprises to adopt

pollution control behaviour (Lanoie et al., 1998; Konar & Cohen, 2001; Dasgupta et al., 2006). Using evidence from the USA and Canada, Lanoie et al. (1998) found that the capital market can react to release of pollution information, with large enterprises affected to a greater degree than smaller enterprises. Dasgupta et al. (2006) examined the reaction of investors in South Korea to publication of the names of enterprises failing to comply with national environmental laws and regulations, and found that such enterprises experienced a significant decline in market valuation. These results have important policy implications. In stimulating enterprises to adopt pollution controls, the role of government regulation can be leveraged through the release of corporate environmental performance information, which is very important for developing countries with weak environmental supervision and implementation (Dasgupta et al., 2001). This is supported by empirical research by Gupta and Goldar (2005) in analysing the effects of environmental ratings on the stock price for large pulp and paper, automobile, and chlor alkali enterprises in India. The authors found that disclosure of weak environmental performance leads to negative abnormal returns (up to 30%), so the capital market usually penalises environmentally unfriendly behaviour and there is a positive correlation between abnormal stock returns and environmental performance.

In contrast to studies showing that capital markets react to disclosure of enterprise pollution information, some research yielded different conclusions. Carpentier and Suret (2015) analysed the stock market reaction to 161 major environmental and non-environmental accidents reported on the front page of the New York Times over 50 years. They found that the estimated average cumulative abnormal return (CAR) following environmental accidents does not differ from zero after 1 year, so the significant negative CARs estimated immediately after an environmental accident in previous studies do not persist. The authors concluded that the deterrence effect of the stock market is likely to be weak in markets driven by institutional investors.

INDUSTRIAL MARKET STAKEHOLDERS

Many studies have found that industrial market pressure affects CEB (Chase, 1991; Saether, 2000; Ytterhus & Synnestvedt, 1995; Henriques & Sadorsky, 1995, 1996; Chen & Soye, 2003). Economic research into the impact of non-regulatory external factors on CEM has revealed that interactions with suppliers and customers may provide manufacturers with more effective

solutions to environmental challenges (Prakash & Potoski, 2006; Vachon & Klassen, 2006). Zhu et al. (2007) found that market pressures have become a strong factor in stimulating Chinese automobile enterprises to adopt green supply chain management (SCM). Kuei et al. (2015) used a partial least squares method to identify factors prompting Chinese enterprises to adopt green practices. Aggregate data showed that external environmental factors (including consumer and regulatory pressure, government support, and environmental uncertainty) are the most important in enterprise adoption of green SCM.

Studies have shown that many consumers tend to choose environmentally friendly products (Weber, 1990; Bermmmer, 1989). Wen and Chang (1998) regarded market demand as a powerful force inducing Taiwan enterprises to achieve better environmental management. In an empirical analysis of 4000 companies in seven OECD countries, Johnstone and Labonne (2009) found that larger enterprises have stronger motivation to adopt environmental management systems to gain market advantage. However, a Japanese empirical study found that consumers of final products did not have a significant effect on enterprise adoption of ISO 14001 (Kimitaka, 2009).

INTERNAL ENVIRONMENTAL FACTORS

According to new institutional theory, enterprises adopt the same or similar CEB behaviour when facing the same or similar institutional pressures. However, in reality some enterprises take positive CEB, while other enterprises in the same industry have not yet reached the minimum standards of environmental regulation; even within the same business group, great differences in CEB exist between different subsidiaries (Jennifer A, 2005; Prakash, 2000). To explain the actual situation, some studies examined the effect of internal stakeholders and enterprise characteristics on CEB. Most of this research has been from the perspective of stakeholder theory (Freeman, 1984) and organisation and management theory. Enterprises that have recognised the benefits of environmental management have already adopted positive CEB to realise green-strategy revenue (Paulraj, 2009).

Internal stakeholder pressure may be more important than pressure from external stakeholders in promoting CEB (Tatoglu et al., 2015). Internal stakeholders include corporate managers, environmental leaders, and employees (Andersson & Bateman, 2000; Dong et al., 2014; Lin et al., 2014; Qi et al., 2011; Zeng et al., 2010; Lu and Abeysekera, 2014). Environmental management

leading to real performance improvements depends on the commitment of executives and the participation and commitment of employees at all levels in CEB implementation (O'hEocha, 2000).

In addition to internal stakeholders, enterprise characteristics are important internal environmental factors. In contrast to new institution theory, organisation and management theory focuses on the internal effects of enterprise characteristics on environmental behaviour. These characteristics include enterprise scale (Hayami, 1984; Welch et al., 2002; Liu & Anbumozhi, 2009), financial status (Earnhart & Lizal, 2002; Lu & Abeysekera, 2014), corporate resources and capability (Hart, 1995; Sharma, 2000; Christmann, 2000; Buysee & Verbeke, 2003; Marcus & Nichols, 1999; Zhu et al., 2008), and enterprise culture (Ágnes, 2007; Wang & Juslin, 2009).

Following Ghobadian et al. (1998), we divide internal environmental factors into mediating and moderating factors to review relevant research results. According to Ghobadian et al. (1998), mediating factors actively influence the relationship between two participants (or sources of influence) in CEM decision-making. In this context, mediating factors can actively influence the relationship between external pressures and the capabilities necessary for enterprises to implement a successful strategic environment policy. The most important mediating factor is the leader because leaders may choose to emphasise a company's traditional methods or challenge them (Ghobadian, et al., 1998). The leader factor can be subdivided into environmental awareness, commitment, support, and values. Leaders in CEB implementation can be enterprise managers or environmental leaders. Other mediating factors include the attitudes, beliefs and expectations of employees, the enterprise culture, and the organisational structure.

Moderating factors such as process complexity and resource availability affect the environmental behaviour of enterprises in decision-making (Ghobadian et al., 1998). In setting successful strategic environmental policy goals, moderating factors limit the potential response of enterprises when facing external pressures or restrict the promoting effect of mediating factors.

Compared with mediating factors, moderating factors involve more practical considerations such as the availability of resources and capability for CEB adoption. Moderating factors mainly determine whether enterprises have the ability necessary to pursue a goal. Capability analysis requires assessment of the availability of resources and capability, scale, financial status, and human resource management when pursuing environmental policy targets.

MEDIATING FACTORS

Enterprise managers. Many studies have found that strong executive support and commitment to continuous improvement in environmental management, pollution prevention, and compliance with government regulatory standards are key factors affecting CEB (Brío et al., 2001; Gattiker & Carter, 2010). Chin and Pun (1999) showed that the commitment of corporate executives is a decisive factor affecting implementation of ISO 14001 in the printed circuit board (PCB) industry in Hong Kong. Henriques and Sadorsky (1999) showed that regardless of CEM system preparation and the criteria used, it is unlikely that an enterprise will achieve its expected environmental goals unless CEM implementation has full support and strong commitment from all members of the organisation, especially the top management. Some studies found that the environmental awareness of enterprise managers plays a role in prompting enterprises to make CEB decisions (Azzone et al., 1997). According to Stone et al. (2004), truly effective CEB implementation is difficult if enterprise managers have a traditional defensive approach. Conversely, strong commitment and support by managers can avoid negative public concerns and help to build stakeholder support through active responses to EE issues (Bansal & Roth, 2000). Some studies emphasised the effects of the environmental values, attitude, and perception of top management on CEB. Papagiannakis and Lioukas (2012) proposed a corporate environmental response model based on planned behaviour and values-beliefs-norms theories. Empirical tests using this model with survey data for 142 companies in Greece showed that the environmental values of the top management have a significant indirect impact on corporate environmental responses through the formation of environmental attitudes, but have no significant direct impact. Tung et al. (2014) found that effective CEM requires higher levels of top management support.

Environmental leaders. Studies of the influence of environmental leaders on corporate EE responses found that CEB changes are usually triggered and driven by environmental leaders in management and functional departments, including formal and emergent environmental leaders who exert top-down and bottom-up effects on the organisation (Benn et al., 2006a,b). Bansal (2003) found that attention to environmental issues and organisational values by individuals is a necessary condition for positive enterprise responses to EE issues. Therefore, enterprises should cultivate

champions among individuals with higher environmental awareness. Support of such champions through further education and training will affect the scope, degree, and speed of corporate EE responses via positive CEB (Andersson & Bateman, 2000). Research in the municipal wastewater treatment industry found that a key factor in positive CEB adoption is the emergence of environmental leaders (Cashman, 2008). Such environmental leaders have been called champions (Andersson & Bateman, 2000; Taylor, 2009), policy entrepreneurs (Brouwer et al., 2009; Meijerink & Huitema, 2010), and change agents (Benn et al., 2006a; Dunphy et al., 2007).

Employees. Positive CEB can stimulate employee enthusiasm and pride. Under the premise of enhancing public awareness of environmental protection and the green development strategy of the global enterprises, many employees increasingly want their employer to have good environmental performance. Dechant and Altman (1994) showed that the viewpoints of employees on corporate environmental performance affect their willingness to work for the enterprise. In a survey of global senior managers, 68% agreed that organisations with adverse environmental performance will find it increasingly difficult to recruit and retain high-quality staff (Fischer and Schot, 1993). People want to work for enterprises with good environmental performance, and an enterprise is more likely to retain and attract high-quality staff by urging employees to participate in practices to improve environmental performance (Coopers & Lybrand Consultants, 1993). Therefore, adoption of positive CEB is fundamental to attracting and retaining not only customers but also talented employees.

Enterprise culture. Many studies have suggested that an enterprise culture oriented towards sustainable development has a significant impact on adoption of active CEB to achieve sustainable development (Post & Altman, 1994; Welford, 1995; Crane, 1995). Kitazawa and Sarkis (2000) suggested that transformation of enterprise culture is an essential factor for successful implementation of environmental pollution reduction measures. Top management should cultivate a deep-rooted strong culture under which employees are free to implement practices to improve environmental performance without much management intervention (Daily & Huang, 2001; Daily, et al., 2003, 2007; Govindarajulu & Daily, 2004). Linnenluecke and Griffiths (2010) investigated the relationship between enterprise culture orientation and CEB and found that

enterprises are unlikely to show a unified organisational culture oriented towards sustainable development. According to the differentiation perspective, different subcultures can exist in an organisation, and members of these subcultures can differ in their attitudes to sustainable CEB. The results also showed that although there are some important obstacles and restrictions, transformation of their culture will prompt enterprises to adopt active CEB, such as publication of sustainable development reports and inclusion of sustainability criteria in employee performance appraisal and staff training. Jennifer A (2006) discussed the role of organisational culture and subculture in CEB and the interpretation of EE issues in particular, and suggested that organisational culture affects the way in which individuals identify EE problems and strategies to solve them. A 9-month survey of a high-tech manufacturer showed that multiple subcultures led to different explanations and behaviour strategies, and the relative subculture force influenced interpretation of EE problems and the CEB strategy adopted. Thus, differences among subcultures and the characteristics of their relationships in enterprises can explain behavioural differences an organisation exhibits when dealing with EE issues.

Some studies focused on the effects of elements of enterprise culture on CEB. Ágnes (2007) stressed the effects of environmental values on CEB. Empirical results indicated that clear and stable integration of environmental values into the enterprise culture is required to realise consistent CEB.

The effects of specific cultural types on corporate environmental responses have also been addressed. Using a questionnaire for 276 members of the Iran Industrial Research Organisation, Akhavan et al. (2014) studied the effects of enterprise culture on environmental responsive capability (ERC), including direct and indirect effects with knowledge management (KM) as the intermediary. They found that enterprise culture has significant positive effects on ERC, both directly and indirectly via KM. In addition, compared to other types of enterprise culture, innovativeness culture has the highest correlation with ERC, both directly and indirectly via KM.

Organisational structure. Hoffman (2001) noted that organisational structure plays an important role in explaining different enterprise responses to institutional pressure, and the form of an environmental response is similar to external institutional pressure reflected in the organisational structure. From this perspective, an organisation makes a variety of responses to institutional

pressure. In this context, using more than 500 responses from an original survey, Delmas and Toffel (2005) focused on how a company's functional departments interact with different institutional constituents and affect environmental decisions by the organisation. The results show that a company's functional organisation and internal power structure influence sensitivity to and interpretation of institutional pressures and subsequent response via CEB adoption. Rosana (2003) suggested that a change in organisational structure leads to better corporate environmental performance, and that active participation by stakeholders accelerates the process.

MODERATING FACTORS

Corporate resources and capability. Using resource and capability theory, this section considers the influence of enterprise resources and capabilities on CEB. Hart (1995) took a natural-resource-based view of enterprises and theoretically explored the notion that competitive advantage can only be obtained when CEB is supported by specific disposable resources and capabilities. The author proposed three types of corporate strategy for obtaining competitive advantage: pollution prevention, product stewardship, and sustainable development. Specific disposable resources and capabilities may include technology, management skills, a comprehensive environmental capacity to prevent pollution, sustained innovation, and stakeholder relations (Sharma, 2000; Buysee & Verbeke, 2003). From the perspective of contingency and dynamics, Aragon-Correa and Sharma (2003) took a natural-resource-based view of active CEB and analysed how changes in the business environment and existing resources and capabilities affect proactive CEB. According to contingency theory, changes in specific factors in the business environment (e.g., environmental strategy of competitors, pollution prevention technology, social EE values, and diversification of stakeholder EE values) will affect active CEB. Enterprises should adopt proactive CEB to maximise the likelihood of gaining a competitive advantage according to specific changes in their business environment and the resources and capabilities available.

Some studies that used a natural-resource-based view found that complementary resources and capabilities affect CEB via mediation of the business ethics attitudes of managers, such as promoting managerial perception of natural resources as a competitive opportunity (Christmann, 2000). There have been some studies on the influence of specific capabilities on CEB.

Marcus and Nichols (1999) and Zhu et al. (2008) suggested that learning ability is particularly important in a resource-based framework. In particular, for organisations with an emphasis on continuous improvement, learning ability helps in constructing more basic "complementary capabilities" (such as those associated with ISO 9000 and total quality management). Bhupendra and Sangle (2015) discussed the driving effect of innovation ability on CEB in relation to pollution prevention and cleaner technology strategies. Empirical results showed that innovation in technological processes and behaviour is necessary for implementation of a pollution prevention strategy. In addition, enterprises implementing a cleaner technology strategy need top management and personnel with high risk tolerance, as well as market, product, and strategy innovations.

Enterprise size. Enterprise size has an important effect on CEB (Hayami, 1984; Welch et al., 2002; Liu & Anbumozhi, 2009; Saleem & Gopinath, 2011). Compared to small and medium enterprises, large enterprises are more likely to adopt positive CEB (Zhu & Geng, 2001; Zhu et al., 2008; Zeng et al., 2010; Luethge & Han, 2012; Lu & Abeysekera, 2014), possibly for the following reasons. First, larger enterprises are more likely to be subject to pressure from governmental regulation and community groups and higher expectations for disclosure of environmental and social information. Second, because of their size, larger enterprises face more environmental problems and pressures (Christmann, 2004). Third, larger enterprises find it easier to obtain resources, capabilities, and external support. Welch et al. (2002) showed that enterprises with earlier adoption of ISO 14001 certification in Japan tended to be larger, greener, and less driven by regulatory, market, or media pressures; conversely, subsequent adopters of ISO 14001 tended to be smaller, less green, and more pressured by regulatory, competitive, and media forces.

Financial status. Using new institutional theory, Moon and Bae (2011) examined how state-level institutional pressure and organisational attributes influence decisions to participate in the US EPA Energy Star for Buildings (ESB) program. Binary probit analysis showed that the influence of state-level institutional pressure on ESB participation is weaker than that of organisational attributes. The empirical findings also suggest that a decision to participate in ESB is more likely to be influenced by economic factors (such as potential return on investment) than by institutional pressures. Earnhart and Lizal (2002) studied the link between corporate financial performance and

corporate environmental performance. An empirical analysis of unbalanced panel data for Czech companies during 1993–1998 showed that successful financial status motivates enterprises to adopt positive CEB to improve their future environmental performance. Within legitimacy and stakeholder frameworks, Lu and Abeysekera (2014) empirically investigated the effects of stakeholder power and corporate internal characteristics on social and environmental information disclosure practices of Chinese listed firms. Corporate internal characteristics such as enterprise size, financial status, and industry classification had a significant effect on CEB (represented by social and environmental information disclosure practices). According to legitimacy theory, firms that are more likely to be subject to public scrutiny, such as larger firms and firms with strong financial status, tend to disclose more social and environmental information to meet public expectations.

Human resource management.

Implementation of positive CEB requires employees with a high level of technology and management skills (Callenbach et al., 1993). Daily and Huang (2001) concluded that CEM systems can be effectively implemented only when the enterprise has the right staff with the right skills and capabilities. Thus, strategic human resource practices can contribute to CEB implementation because they may help companies to find and utilise knowledge and expertise within the organisation (Scarborough, 2003). Implementation of strict systems for employee recruitment, selection, and performance appraisal, introduction of training to improve staff awareness of environmental protection, and development of new technology and management skills will contribute to cultivation of environmental innovation and thus drive enterprises to adopt positive CEB (Renwick et al., 2008).

CEB PERFORMANCE

OVERALL PERFORMANCE

Early studies found that CEB improved overall enterprise performance (Montabon et al., 2007; Claver et al., 2007), but the results are not consistent (Primc and Cater, 2015). In empirical tests, Montabon et al. (2007) found a significant positive correlation between CEM practice and overall performance. They used innovative data analysis to test the hypothesis of Porter and van der Linde (1995a) that innovation can offset the costs of environmental regulations to achieve a win-win situation (Montabon et al., 2007). Claver et al. (2007) proposed that overall enterprise

performance is a combination of environmental performance, competitive advantage, and economic performance, and constructed a wider framework including environmental strategy and overall performance to demonstrate their relationship. A case study of the COATO farming cooperative showed that environmental management focusing on pollution prevention had a positive net effect on environmental performance. In addition, CEM can help an enterprise to develop new organisation ability, which will lead to a competitive advantage. In a survey of 27 Australian companies, Primc and Cater (2015) found that positive CEB is not always correlated with high corporate performance, and positive CEB is not as important as other causal conditions for high-performing firms in highly polluting industries. The study analysed the relationship between CEB and overall performance more holistically by including a series of external and internal factors regarded as important in past research.

ECONOMIC PERFORMANCE

The relationship between CEM and corporate economic performance has been investigated, but there are conflicting results (Dixon-Fowler et al., 2013; Horvathova, 2010; Nishiani et al., 2011). Studies identifying a positive correlation found that CEB yields a number of economic benefits, such as innovation and operational efficiency (Porter & van der Linde, 1995b; Aguilera-Caracuel & Ortiz-de-Mandojana, 2013), a better competitive advantage (Hart, 1995; Russo & Fouts, 1997), and enhanced environmental reputation and employee commitment (Welford, 1995; Dogl & Holtbrugge, 2014), reflecting strong organisational and managerial ability (Aragon-Correa, 1998; Aschehoug et al., 2012). Studies that observed a negative correlation found that non-voluntary improvement of corporate environmental performance may raise costs, which is not conducive to a corporate competitive advantage (Walley & Whitehead, 1994). A new measure of corporate economic performance was used to re-examine the relationship with CEM. Cordeiro and Sarkis (1997) found a significant negative relationship between CEB (using toxic release inventory data) and 1- and 5-year earnings-per-share performance forecasts for a sample of 523 US firms. Using a corporate earnings index for a survey of more than 120 companies in multiple industries, Stanwick and Stanwick (1998) found a significant positive correlation between corporate earnings and total data on pollution discharge, suggesting that corporate economic performance is negatively correlated with environmental performance. Some

studies argue that the relationship between CEM and economic performance is not significant (Levy, 1995; Fogler and Nutt, 1975). Therefore, the debate on whether positive CEM improves corporate economic performance is far from resolved and further in-depth research is required.

Why is the relationship between CEM and corporate economic performance opposite in different situations? What is the underlying reason for such differences in relationship for specific enterprises during specific periods? Neoclassical scholars hypothesised that environmental regulations impose additional enterprise costs (Palmer et al., 1995; Walley & Whitehead, 1994; Cordeiro & Sarkis, 1997). However, others suggested that positive CEM can yield actual benefits. Porter (1991) stated that environmental regulation could lead to a win-win situation in which both social welfare and enterprise benefits increase. Porter and van der Linde (1995a,b) suggested that reasonably designed environmental regulations lead to innovations that partly or completely offset the compliance costs. The study also suggested that environmental protection is likely because pollution is a marker for economic inefficiency. Some scholars attributed the benefits of CEM implementation to integration of environmental management factors in the corporate strategy (Shrivastava, 1995; Beaumont et al., 1993; Guimaraes & Liska, 1995).

TECHNOLOGICAL PERFORMANCE

PROGRESS

Many studies have explored technological progress in terms of the effects of an environmental management system (EMS) on adoption of environmental technology. To gradually decrease negative environmental impacts, an EMS should motivate enterprises to adopt the best available environmental technology in a timely manner taking economic feasibility and cost efficiency into consideration (ISO, 1996). This means that ISO 14001 and technological change are interdependent (Hillary, 1997). Hilson and Nayee (2002) studied the practicality of EMS integration in mining and related operations found that EMS can promote cleaner production and help mining enterprises to comply with environmental regulations and realise economic and technological benefits. Radonjic and Tominc (2007) empirically investigated the effects of EMS on adoption of cleaner energy technologies. Analysis of survey data from 36 Slovenian metal and chemical manufacturing enterprises revealed that ISO14001 certification can enhance the motivation to adopt cleaner energy technologies

and thus improve corporate environmental performance.

INNOVATION PERFORMANCE

Many studies have explored CEM innovation performance in terms of the role of EMS adoption in stimulating technological, organisational, management, and product innovations. Since environmental innovations consist of new or modified technology, organisation, management practices, and products to avoid or reduce environmental harms, EMS adoption is frequently regarded as a typical example of organisational innovation (Ziegler & Rennings, 2004; Halila, 2007). Carruthers and Vanclay (2012) concluded that inherent EMS features promote the adoption of new practices in natural resource management. Zhu and Sarkis (2006) suggested that green management represents a competitive advantage and green production processes can promote green product innovation and effective distribution of enterprise resources. Some studies have analysed the causal effect of EMS adoption on technological innovations and found positive impacts (Ziegler & Rennings, 2004; Wagner, 2007). However, other studies found negative impacts. Using Canadian facility-level manufacturing data, Henriques and Sadowsky (2007) found that EMS adoption reduces the likelihood of undertaking cleaner technological innovations. Other studies found that positive effects of EMS adoption on environmental technological innovation are not clear. Empirical estimations by Frondel et al. (2008) showed no association between technological innovation and EMS adoption, confirming the hypothesis of King and Lenox (2000) that EMS adoption does not necessarily trigger environmental technological innovation. Econometric analyses by Ziegler and Nogareda (2009) revealed a significantly positive effect of environmental technological innovations on EMS. Thus, the role of EMS in promoting environmental technological innovations is still unclear.

IMAGE PROMOTION PERFORMANCE

Berry & Rondinelli (1998) found that proactive CEM may insulate firms from stakeholder pressure and promote a positive corporate image. Proactive CEM to fulfil CSR yields economic benefits and reflects concern about social public interests, and thus can help in building a good green image, consumer brand awareness and loyalty, and a competitive advantage (Brammer & Millington, 2005; Sen & Bhattacharya, 2001). This viewpoint was confirmed by Ann et al. (2006), who found that

EMS certification has positive effects on the environmental and economic performance of enterprises, and enhances their corporate image.

THEORETICAL FRAMEWORK FOR CEB

On the basis of the CEB evolution path, influential factors, and performance, we propose a theoretical framework for CEB (Figure 2).

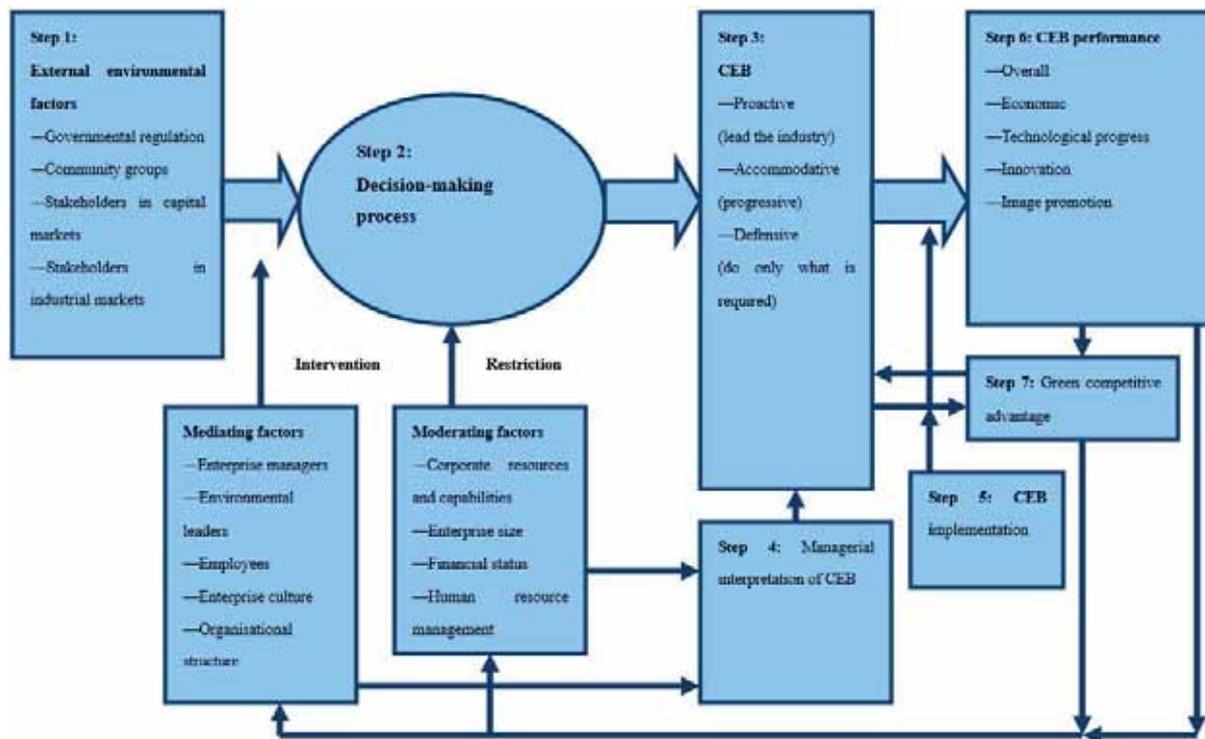


FIGURE 2
Theoretical framework for corporate environmental behaviour (CEB)

The theoretical framework suggests that the external environmental and mediating, and moderating factors play a significant role in CEB decision-making (Figure 2), but these factors differ in their mode of action. On the one hand, according to new institutional and stakeholder theories, CEB is influenced by external stakeholders in pursuing organisational legitimacy. This exogenous pull arises from external environmental factors that include governmental regulation, community groups, and stakeholders in capital and industrial markets. On the other hand, from the perspective of stakeholder theory and organisation and management theory, decision-making is influenced by mediating factors and restricted by moderating factors (endogenous motivation), which are actually a refinement and reclassification of internal stakeholder factors and enterprise characteristics. Mediating factors actively influence the relationship between external environmental factors and CEB decision-making, while moderating

factors restrict CEB decision-making because of the complexity of the process or the availability of resources and capabilities (Figure 2). Mediating factors include internal stakeholders (enterprise managers, environmental leaders, and employees) and enterprise characteristics (enterprise culture and organisational structure), among which enterprise managers and environmental leaders are the most important. Moderating factors include enterprise characteristics (corporate resources and capability, enterprise size, financial status, and human resource management), among which corporate resources and capability are the most important.

The CEB decision-making process determines the evolution path from defensive to accommodative to proactive CEB. Managerial interpretation of environmental issues may also regulate CEB choice. Sharma (2000) suggested that managerial interpretation of environmental issues as opportunities rather than threats is likely to influence CEB choices, and such interpretation is

influenced by internal environmental factors. Finally, CEB performance is affected by the CEB implementation mode. Successful CEB implementation will realise the expected performance and contribute to a green competitive advantage, with feedback influencing CEB choices. CEB performance in terms of financial benefits, technological progress, and innovations in environmental technology and management and the final objective will also have feedback effects on internal environmental factors (Figure 2).

CONCLUSIONS

CEB has involved three stages (Starik & Marcus, 2000):

- 1 **Resistance stage** During the emergence of the environmental protection movement in the 1960s and 1970s, the public were aware of the negative effects of enterprises on the environment. Enterprises operated within a strict cost-benefit framework and only adopted CEBs that yielded significant financial benefits (Walley & Whitehead, 1994), so most enterprises resisted environmental regulations in this stage.
- 2 **Compliance stage** Since the 1980s, increasing numbers of enterprises have changed from a resistance mode and have begun to comply with environmental regulations. This has included setting up environmental departments, making public commitments to comply with environmental regulations, developing environmental policies, and considering environmental protection in investment decision-making. However, enterprises regard CEB as a cost-increasing exercise in this stage, and activities mainly involve end-of-pipe control.
- 3 **Proactive stage** Increasing numbers of enterprises are recognising that proactive CEB contributes to a sustainable competitive advantage, and therefore adopt positive CEB beyond that required by environmental regulations. Such environmental management is reflected in the whole product life cycle, and mainly involves reduction and recycling activities.

From a macro perspective, CEB evolution is already in the proactive stage. However, from a micro perspective, the environmental behaviour of

an enterprise is affected by external stakeholder pressure and internal environmental factors. Therefore, individual enterprises differ in their CEB. Although increasing numbers of enterprises chose to adopt proactive CEB, some enterprises still chose accommodative or defensive CEB. Therefore, the relative influence of various factors on CEB must be identified for each specific enterprise and industry. What is the specific mode of action of various influential factors in CEB decision-making? How can managerial interpretation of environmental issues affect CEB choices under the influence of the three factor types? How are CEB choices affected by the mode of implementation and what are the effects on performance and a green competitive advantage? The theoretical framework in Figure 2 only lists the logical relationships among these elements, and empirical testing of these relationships is an avenue for future work.

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ASSESSING THE VISUAL QUALITY OF RURAL HIGHWAY LANDSCAPE RECLAMATION IN THE WESTERN BLACK SEA REGION OF TURKEY

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ABSTRACT

The purpose of this study was to assess the visual quality of proposed rural highway landscape reclamation of the new Bartın-Amasra highway via public perceptions and design approaches. The four objectives of this study were to: (1) analyze the study site's landscape to determine its characteristics, (2) evaluate three landscape reclamation concepts (open, semi-open, and closed) for eight research areas and generate 24 3-D simulations/visualizations, and (3) survey academic staff, public staff, students, and laypeople regarding their preferences of the new Bartın-Amasra highway. The study was conducted at eight sites where landscape reclamation is required on the highway. The survey sampled 300 highway users from four groups to determine their socio-demographic characteristics and use preferences. The data were analyzed by interpreting statistical correlations and frequency analysis. The results found that there were 75 plant species from 39 families in the eight sampled areas. Educational attainment was positively related to preferring to use the highway to watch the sea and scenery, although many highway locations were perceived as negative. The most striking remedies for mitigation were landscaping designs that counter noise and visual pollution and renewal of damaged vegetation. The most positive aspect of the highway was "having an open and closed scenery attribute."

KEYWORDS:

visual quality, rural landscape, highway landscape reclamation, visualization, user preferences, Bartın-Amasra.

INTRODUCTION

Highways are increasingly important landscape features [1] because transportation infrastructure interacts with surrounding landscapes and, thus, has a cultural dimension. In particular, highways are physical manifestations of social

connections as well as the political and economic decisions that led to the land-use changes that created them [2]. Highways dominate our daily surroundings and are important to people's access to the visual landscape [3]. According to Box and Forbes [4], roadside vegetation has become a significant element of highway environments with which people regularly interact because of their increasing use of roads for transportation in modern life [5].

During the twentieth century, the construction and maintenance of highways have been the most widespread types of modification to the natural landscape [1, 6-8]. However, the effects of these activities on biological diversity and ecological processes have been studied only recently (e.g., [2, 9, 10]). These studies have resulted in the emergence of a new scientific discipline, highway ecology [11, 12]. Other scholars have proposed that the experience of a landscape is cognitive and personal; that is, an individual reacts to and experiences a landscape based on that individual's personal experiences [13]. From this perspective, an individual gives meaning to and interprets the landscape, and is stimulated by it. However, it is likely that the evaluation of our aesthetic experiences is determined by cultural as well as individual factors [14], in which the cultural aspect builds on individual capacities, experiences, and learning [15].

Scale, naturalness, human influences, topographical variations, the presence or absence of water, and openness or density [13, 16] are landscape characteristics that are important to people's evaluations of a landscape. Its character as open, forested, or varied influences people's driving behaviors (such as average speed, variation in lateral vehicle position, and steering wheel grasp frequency) because drivers' perceptions of driving situations are influenced by landscape characteristics [15].

Zube et al.'s [17] review of landscape perception studies and Lothian's [18] study on landscape quality assessments provided thorough overviews and structure of the literature in the field

of landscape aesthetics. In this context, Tveit et al. [19] provided a comprehensive theory-based framework for analyzing the visual character of landscapes. However, the visual factors that were identified are not directly transferrable to highway projects. The ideas must be adapted to fit the nature of highways, which includes landscapes, landscape elements, and construction, with technical elements [20].

The National Cooperative Highway Research Program [21] evaluated methodologies used for visual impact assessments (VIA). The report is of broad interest to state, regional, and local planners, project development staff, and environmental staff. The report offered 10 criteria for evaluating VIA procedures that emerged from research findings. These criteria prescribe desirable overarching characteristics of VIA methods and procedures, as follows: objective, valid, reliable, precise, versatile, pragmatic, easily understood, useful, consistently implemented, and legitimate.

Landscape quality is important to quality of life [22, 23], although the landscape experience can be interpreted in many ways. Individual preferences for environments have been examined by environmental psychologists, who suggest that people tend to appreciate the visual experience of nature more than they appreciate city views [13, 15, 24].

Funderburg et al. [25] emphasized that the relationship between highway construction and urban development is an important research topic for modern regional planning [23]. Many ecological studies have assessed the ecological effects of highway construction [1, 23, 26-28].

The relationship between landscape and people's behavior is studied in environmental psychological and human physiological research. Some studies first expose research subjects to videos or photographs of a variety of landscapes as seen from the road and then measure their reactions to assess the types of preferred landscape [13, 15, 29-32]. Ecological information is shared with the public and other stakeholders using visualizations. Some studies have examined the effects of visualization techniques. Landscape visualizations have been successfully used to communicate landscape issues and discuss planning decisions with experts, stakeholders, and the public [33].

Highways that pass through some areas degrade the natural environments and cause a variety of ecological problems. According to Trombulak and Frissell [1], all types of roads influence the terrestrial and aquatic ecosystems through which they pass in seven basic ways: (1) increased wildlife mortality from road construction, (2) increased wildlife mortality from vehicular

impacts, (3) modification of animal behavior, (4) alteration of the natural environment, (5) alteration of the chemical environment, (6) spread of exotic species, and (7) increased human alteration and use of wildlife habitats. Highways often result in land-use conversions, loss of land cover, and fragmentation of remaining land cover into smaller and more isolated elements. Landscapes that are shaped by these elements likely have more and smaller habitat patches, decreased connectivity between or among patches, decreased complexity of patch shape, and higher proportions of edge habitat [27].

To minimize these effects, it is important to approach highway development by taking the natural assets of the proposed highway route and land-use developments into account at the planning stage [34]. According to Geneletti [35], a feature of road corridor planning is that plans assess a variety of alternatives based on well-defined territorial variables in the decision-making criteria [36]. Ecological management of highway corridors is important to the improvement of natural values and aesthetic appreciation as long as embankment management is linked to the management of the surrounding landscape [37].

One of the many efforts to enhance environmental quality is increased attention to taking advantage of the existing natural vegetation in urban and rural landscapes. However, regular maintenance of roadside plants can be ignored because it is costly, which is the case in Turkey. This problem can be partly addressed by taking advantage of the indigenous plants, which are relatively more suitable to the landscape design of sites that receive limited maintenance [38].

In 2000, the European Landscape Convention was adopted by the Council of Europe. The convention aims to promote "the protection, management and planning of European landscapes" [39] and it emphasizes the importance of exceptional as well as ordinary and degraded landscapes [20, 40].

This study had four main purposes. First, it analyzed landscapes to determine the characteristics of the area of interest, the new Bartın-Amasra highway. Second, it generated three landscape reclamation concepts (open, semi-open, and closed) and 24 three-dimensional (3-D) simulations/visualizations for eight study sites. Third, a survey was conducted to identify the socio-demographic characteristics of users of the new Bartın-Amasra highway. Fourth, the Semantic Differential Scale was employed to ascertain the visual quality values of the 3-D simulations/visualizations. The results yielded suggestions for future landscape reclamation

projects along rural highways and contributed to sustainable regional development.

MATERIALS AND METHODS

STUDY AREA

The Bartın-Amasra highway is a transit route of the Black Sea tours organized by national tourism companies. The museum city of Safranbolu has been a UNESCO Cultural Heritage city since 1994. The historical, archeological, ancient port city of Amasra was added to the UNESCO World Heritage Center Tentative List in 2012. According to Sertkaya [41], Cengiz [42], and Çorbacı [43], Amasra is a center of attraction along the western Black Sea shore, with a current tourism potential that is national as well as regional in scale. The city of Amasra is located on the western Black Sea coast of Turkey in Bartın Province. The city is on a peninsula extending northward with bays on either side [42]. Bartın, located along the Safranbolu-Amasra highway route. Bartın is a city that shows an inner harbor characteristic with the Bartın River that passes through it and its traditional settlement texture [44, 45]. Because of these features, Safranbolu, Bartın, and Amasra attract attention to their cultural landscapes and they are on the tourism itineraries of the western Black Sea Region.

The Bartın city center access to the Amasra district is accomplished either from the old road or the new road. The old and new roads are one road from the Bartın city center to the Kaman Village detour (about 3.5 km), after which it divides into the old and new roads. The new road connection to the Amasra district is the Bartın-Amasra-Çakraz route, which is a 25-km section of the Zonguldak-Bartın-Amasra-Cide-İnebolu-Çatalzeytin-Ayancık-Sinop state highway. Amasra town center is

reached about 9.5 km after the Kaman Village detour. The new Bartın-Amasra highway was completed in 2001.

The study sites are eight areas along the new Bartın-Amasra highway in the western Black Sea Region (between 5.6 - 16.6 km). The study area is in the Euxine (Eux) sub-region of the Euro-Siberian region [46].

The city of Bartın attracts attention to its forest and waters. About 56% of the city is forested and it has a shoreline of about 59 km, which attracts attention to the Bartın River. On the other hand, Amasra, a district of Bartın, is a typical coastal historical city with 3000 years of history. Historically, Amasra was ecologically (forest and water) oriented, but it is becoming a tourist destination and an industrialized region. Highway landscaping at the study sites is characteristically rural.

The region is dominated by the typical Black Sea climate. The Bartın summers are cool and its winters are rainy and mild. The highest measured temperature in Bartın was 42.8° C (in July) and the lowest recorded temperature was -18.6° C (in February). Annual average temperature is about 12.5° C. Rainfall in Bartın is highest in October, November, and December, and lowest in May, with an average of 1030 mm. Average annual relative humidity is about 78% [47]. The average annual temperature of 13.8° C is slightly higher in Amasra, average annual total precipitation is about 1035.22 mm, and average annual relative humidity is about 69.8%. The wind blows northeast between October 15 and March 15. Amasra also is exposed to winds from the north, southwest, and northwest [42, 48]. The geographic locations of the study sites are shown in Fig. 1.

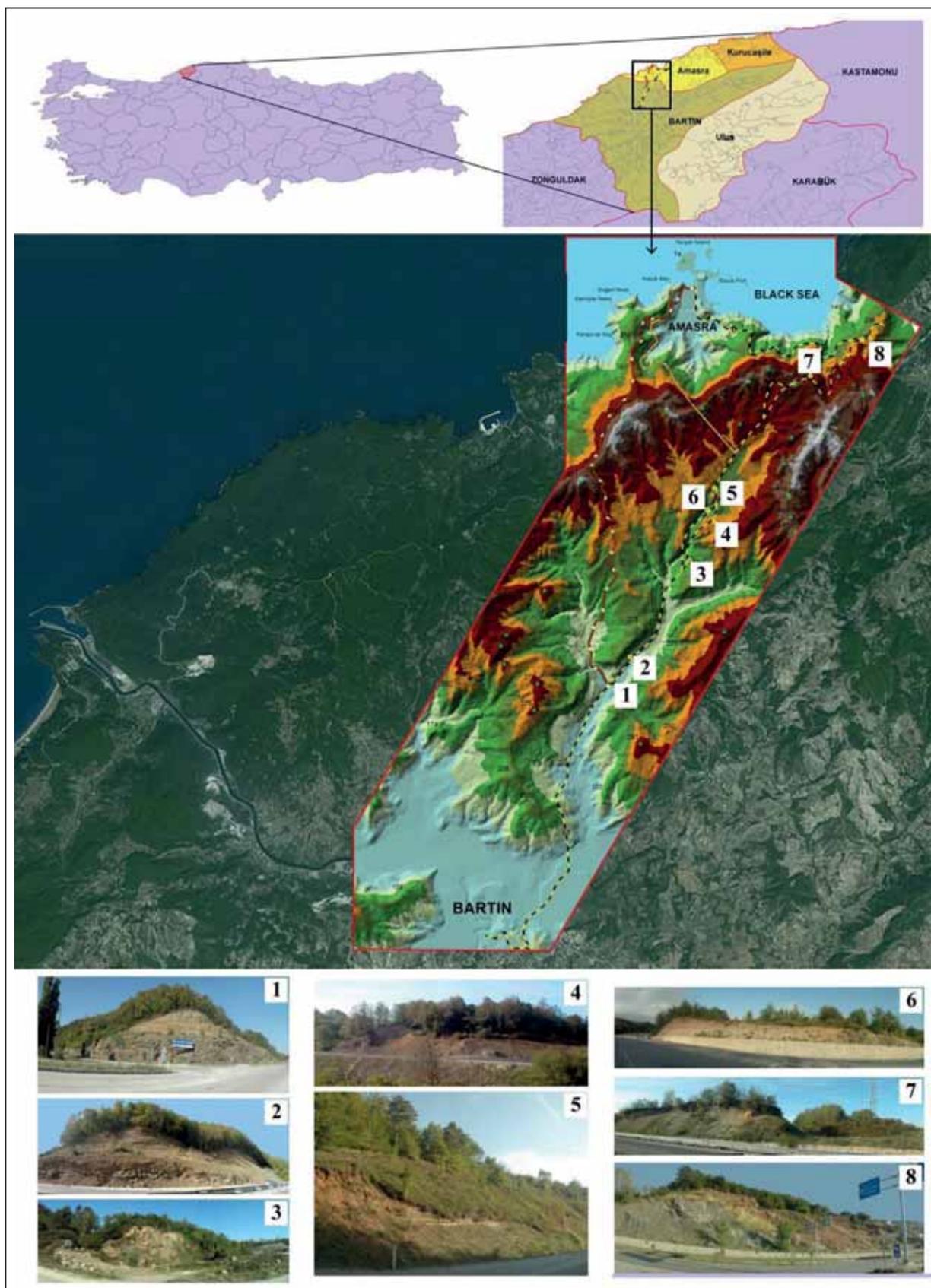


FIGURE 1
Geographical locations and close-up images of the study sites.

DATA COLLECTION

The research proceeded through three stages: (1) field study, (2) landscape visualization, and (3) survey study.

Stage 1: Field Study

Study Site Selection. The field study was conducted in eight areas along the new Bartın-Amasra highway route. The process of selecting the sites proceeded in four sequential steps [49]. First, the areas of the highway that required landscape reclamation were identified. Second, the identified areas were organized based on similarities in landscape components. Third, distance zones were observed. Fourth, the sites were organized in terms of landscape composition, landform pattern quality, and suitability for simulations. The steps were accomplished using data on numerical topography, forest properties, soil, geologic maps, satellite images from Google Earth, and photographic images obtained during field studies. Images obtained during visits to the field were created using a Canon IXUS 55 digital camera between August of 2012 and November of 2012.

Identifying the Landscape Characteristics of the Study Sites. ArcGIS 10.1 was employed to identify the characteristics of the study sites (e.g., [50-54]). These were:

- The 1:25,000-scaled Topographic Maps produced by the General Command of Mapping [55] determined promontory groups, slopes, aspects, peaks, contour lines, water body types (dry or wet streams), and current settlements;
- The 1:25,000-scaled Geology Maps published by the General Directorate of Mineral Research and Exploration [56] determined geological structures and fault statuses;
- The 1:25,000-scaled Soil Maps published by the General Directorate of Rural Affairs [57] determined the land groups, the land-use capacity classes, and erosion statuses;
- The 1:25,000-scaled Forest Management Planning Map from the General Directorate of Forestry [58] determined the forest features (stand type, forest type or operating class, and forest crown closure); and
- Current settlements were examined using Google Earth satellite images.

The specific characteristics were identified in the field studies of the eight study sites to determine the accuracy of the acquired data. A Global Positioning System was used to determine the coordinates of each site, an altimeter was used to determine their elevations, a compass was used to

determine the aspects, and an inclinometer was used to measure the inclines (slopes). Because forest plans do not provide information about forest sub-covers, plant samples were collected from each site during the field visits and identified.

Collection and Identification of the Plants.

The observation period was during the 2012 vegetation and flowering periods, during which time plant types, other nearby plants, cultivation characteristics, and cultivation media were separately observed and noted. The method of collecting the samples, their drying, and the labeling procedures were conducted following plant inventory forms created by Yaltrık and Efe [59], Cengiz et al. [60], Bekci et al. [61], and Cengiz et al. [62]. In addition, archives were created of plant samples with detailed photographic images of their locations. Accurate identification of the collected plants was accomplished by comparing them to samples at the Kahramanmaraş Sütçüimam University (KSU) Department of Forestry, Forest Botany herbarium, using the identification keys prepared by Davis [63]. In addition, the collected plants were compared to photographic images taken in the natural cultivation areas and in the herbarium. Through this process, the plant existence inventory was developed. Table 2 presents the inventory of plants at the eight study sites.

Stage 2: Landscape Visualization

First, the landscape opportunities provided by the new Bartın-Amasra highway were considered and three landscape reclamation options were proposed: (1) open, (2) semi-open, or (3) closed. These options were proposed for each of the eight study sites; then, 24 3-D simulations/visualizations were generated for each area [15, 64]. The important aspect of the 3-D simulations is the intensive use of plants (high density) in the “closed” option, in which the plants are tall and roughly textured. The “semi-open” option has relatively low plant density and there are small trees and plants with few branches. The lowest plant density is in the “open” option, consisting of thin-textured ground cover. The design criteria proposed by Cengiz et al. [60], Robinson [65] and Leszczynski [66] regarding natural-style planting designs were used for each 3-D simulation/visualization and tentative illustrations were created as 3-D drawings using CS6 Photoshop, Google sketchup, 3-D max, and Lumion.

Stage 3: User Survey

The preferences of people who use the new highway are important to the landscaping decisions that influence the driving experience. The socio-demographic characteristics and highway use preferences of users of the new Bartın-Amasra highway were investigated by means of survey data. A questionnaire was constructed to obtain information about users' preferences [67, 68]. The sample consisted of 300 users of the new highway, of which 50 were academic staff, 50 were public staff, 100 were landscape architecture students, and 100 were laypeople. The survey was conducted face-to-face and each questionnaire was completed in 48 minutes (average of two minutes for each project).

The data were analyzed using correlation analysis and statistics were assessed using Spearman coefficients (r) and statistical significance with SPSS (Statistical Package for the Social Sciences) 16.1 software. In addition, frequency analysis was employed. The Semantic Differential Scale method was used to assess the visual quality of the 24 simulations generated during the 3-D visualization stage [69, 70]. The questionnaire responses were analyzed in accordance with the Semantic Differential Scale to ascertain the ways that the respondents viewed the interactions among semantic properties, landscape elements, and space [64, 71]. Twelve pairs of opposing descriptive terms were selected to evaluate the visual quality of the 3-D simulations. These 12 pairs of term were:

- Beautiful/ugly: The respondent liked the space very much,
- Attractive/unattractive: The respondent was attracted to the space,
- Regular/irregular: The use of landscape planning and design criteria in the space,
- Relaxing/disturbing: The space provided the individual with an opportunity to comfortably move around,
- Safe/unsafe: The respondent felt safe in the space,
- Accessible/inaccessible: The respondent believed that the space was accessible,
- Practical/impractical: The respondent believed the space was practical for the users,
- Visible/invisible: Playing an effective role in the used space,
- Natural/artificial: The space was perceived as natural,
- Varied/monotonous: The space used a variety of plants,
- Rich in terms of species/poor in terms of species: Richness of plant species, and
- Sufficiently green/insufficiently green: Placement of plant material was in accord with the fullness/emptiness criteria.

The respondents were asked to rate the 3-D simulations/visualizations with respect to each descriptive pair of terms by scoring them on a scale ranging from -3 to +3 [71]. The differences among the respondents in their assessments of the visual quality of the new Bartın-Amasra highway by group (academic staff, public staff, students, and laypeople) were analyzed using analysis of variance (AOV) and Duncan tests with SPSS 16.1.

RESULTS

CHARACTERISTICS OF THE STUDY AREAS

Table 1 shows the specific characteristics that were identified in the field studies of the eight study sites to determine the accuracy of the acquired data (see Fig. 2).

Table 2 presents the inventory of plants at the eight study sites. The 75 plant species recorded in the eight sampled areas belonged to 39 families. In order of frequency, the first three family groups were as follows: *Astertaceae* (10 taxa), *Fabaceae* (9 taxa), and *Rosaceae* (5 taxa) (Table 2).

TABLE 1
Locations, topographical characteristics, geological and soil features, and aspects of forests of the eight study sites.

| Study site | Location | | Topography | | | Geology | Soil | | | Forest | | |
|--------------|------------------------------------|-------------------------------------|--------------------|---------------------|-----------|----------------------|--------------------------|----------------|-------------------------|-------------------------|----------------------|--------------------------------|
| | Coordinates | Round trip (km) | Elevation (meters) | Aspect ^a | Slope (%) | Geology ^b | Land groups ^c | Erosion status | Land-use capacity class | Stand type ^d | Closure ^e | Operational class ^f |
| I. Region | N: 41° 40' 25'' E: 32° 21' 54'' | 16.1 return trip on the right | 30 | S | 70.3 | Ky | M | Modetate | IV | Z-5 MGnDy3 | 3 | AA |
| II. Region | N: 41° 40' 31'' E: 32° 22' 04'' | 15.8 return trip on the right | 43 | SE | 58.4 | Ky | M | Modetate | IV | GnKsKn3 | 3 | AA |
| III. Region | N: 41° 41' 59'' E: 32° 23' 07'' | 10.3 going on the right | 136 | SW | 45.4 | Ky | M | Modetate | IV | KnGn1 | 1 | AA |
| IV. Region | N: 41° 42' 21'' E: 32° 22' 52'' | 11.3 going on the right | 190 | NW | 57 | Ky | P | Modetate | VI | Z-1 | 0 | - |
| V. Region | N: 41° 42' 37'' E: 32° 23' 31'' | 11.2 return trip on the left | 188 | W | 70 | Ky | M | Modetate | IV | GnMKn3 | 3 | AA |
| VI. Region | N: 41° 42' 27'' E: 32° 23' 25'' | 11.5 return trip on the right | 179 | SE | 56 | Ky | M | Modetate | IV | Kn3 | 3 | AB |
| VII. Region | N: 41° 43' 51'' E: 32° 24' 46'' | 7.7 return trip on the right | 320 | S | 44.4 | Ky | P | Modetate | VI | GnKs3 | 3 | AA |
| VIII. Region | N: 41° 44' 15'' E: 32° 25' 38'' | 5.6 return trip on the left | 215 | NW | 74.3 | Ky | P | Modetate | VI | Z | 0 | - |

^aN = North, NE = Northeast, E = East, SE = Southeast, S = South, SW = Southwest, W = West, NW = Northwest

^bKy = Yemişli Creek Formation

^cM = Alluvial soils, M = Non Calcic Brown forest soils

^dIV = Denoting land, where agriculture may be practiced under some restrictions, VI = Denoting land that should be allocated for forestry, settlement or industry type usage.

^eMGnDy3 = Oak, Hornbeam; GnKsKn3 = Hornbeam, Chestnut, Beech; KnGn1 = Beech, Hornbeam; Z-1 = Agricultural field; GnMKn3 = Hornbeam, Oak, Beech; Kn3 = Beech; GnKs3 = Hornbeam, Chestnut; Z = Agricultural field

^f1 = % 11-40 closure stands, 3 = Stands with more than 71% closure

^gAA = Grove, AB = Grove-Coppice

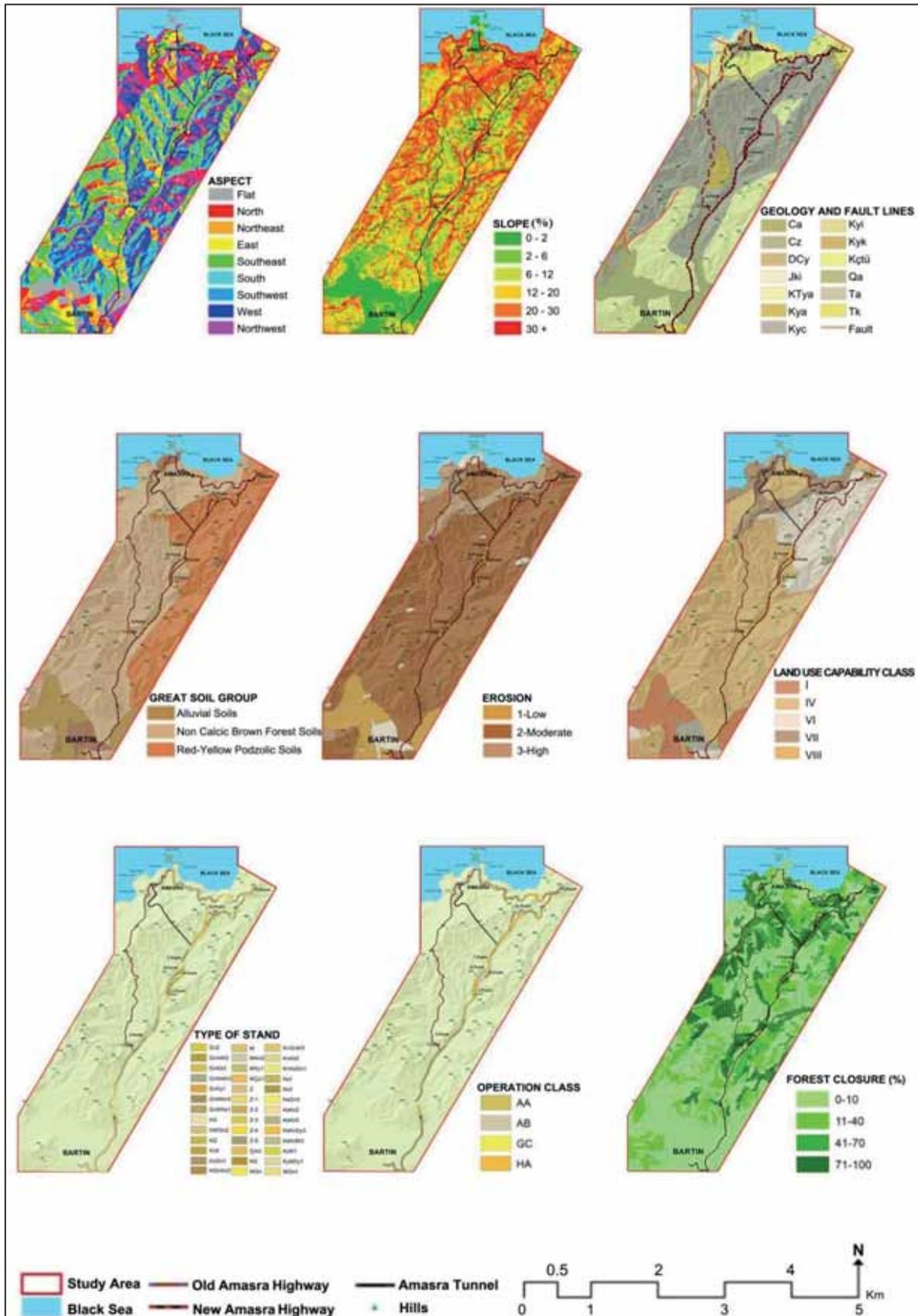


FIGURE 2
The important natural characteristics of the study areas.

TABLE 2
Plant species found in the study areas.

| Family Name | Plant Name | Study Area | Plant Species |
|------------------|--|---------------|---------------|
| ADOXACEAE | <i>Sambucus</i> sp. | 2, 3 | Bush |
| APIACEAE | <i>Daucus carota</i> L. | 1 | Herbaceous |
| ARALIACEAE | <i>Hedera helix</i> L. | 2 | Ivy |
| ASPIDIACEAE | <i>Dryopteris filix-max</i> (L.) Schott | 3 | Herbaceous |
| ASTERACEAE | <i>Anthemis</i> sp. | 4 | Herbaceous |
| | <i>Anthemis tinctoria</i> L. var. <i>pallida</i> | 7 | Herbaceous |
| | <i>Eupatorium cannabinum</i> L. | 7, 8 | Herbaceous |
| | <i>Matricaria</i> sp. | 5 | Herbaceous |
| | <i>Petasites albus</i> (L.) Gaertner | 2 | Herbaceous |
| | <i>Lactuca saligna</i> L. | 2 | Herbaceous |
| | <i>Pulicaria dysenterica</i> (L.) Bernh. | 1 | Herbaceous |
| | <i>Conyza canadensis</i> (L.) Cronquist | 6, 7 | Herbaceous |
| | <i>Taraxacum</i> sp. | 2 | Herbaceous |
| | <i>Xanthium strumarium</i> L. | 3 | Herbaceous |
| BETULACEAE | <i>Carpinus betulus</i> L. | 2, 3 | Tree |
| | <i>Alnus glutinosa</i> subsp. <i>glutinosa</i> | 5 | Tree |
| | <i>Ostrya carpinifolia</i> Scop. | 5 | Tree |
| BORAGINACEAE | <i>Cynoglossum creticum</i> Miller | 2 | Herbaceous |
| | <i>Cynoglossum</i> sp. | 5 | Herbaceous |
| BUXACEAE | <i>Buxus sempervirens</i> L. | 6 | Bush |
| CONVOLVULACEAE | <i>Convolvulus cantabrica</i> L. | 1 | Herbaceous |
| CORNACEAE | <i>Cornus mas</i> L. | 1, 2, 3, 5, 7 | Shrub |
| | <i>Cornus sanguinea</i> L. | 2, 5 | Herbaceous |
| CORYLACEAE | <i>Corylus avellana</i> L. | 2, 4 | Shrub |
| CRASSULACEAE | <i>Sedum</i> sp. | 2 | Herbaceous |
| DENNSTAEDITACEAE | <i>Pteridium</i> sp. | 5 | Herbaceous |
| DIPSACACEAE | <i>Scabiosa atropurpurea</i> L. subsp. <i>maritima</i> (L.) Arc. | 1 | Herbaceous |
| ERICACEAE | <i>Erica carnea</i> L. | 1, 3 | Bush |
| | <i>Rhododendron ponticum</i> L. | 3, 5, 8 | Bush |
| EUPHORBIACEAE | <i>Euphorbia chamaesyce</i> L. | 1 | Herbaceous |
| EQUISETACEAE | <i>Equisetum</i> sp. | 7 | Bush |
| FABACEAE | <i>Medicago lupulina</i> L. | 2, 4 | Herbaceous |
| | <i>Melilotus officinalis</i> (L.) Desr. | 7 | Herbaceous |
| | <i>Psoralea bituminosa</i> L. | 7 | Herbaceous |
| | <i>Argyrolobium biebersteinii</i> P.W. Ball | 4 | Herbaceous |
| | <i>Robinia pseudoacacia</i> L. | 6, 8 | Tree |
| | <i>Lotus</i> sp. | 5, 8 | Herbaceous |
| | <i>Fagus orientalis</i> L. | 2, 8 | Tree |
| | <i>Castanea sativa</i> Miller | 2, 3, 5, 8 | Tree |

| | | | |
|------------------|--|---------|------------|
| | <i>Quercus</i> sp. | 1, 3 | Tree |
| JUGLANDACEAE | <i>Juglans regia</i> L. | 1 | Tree |
| LAMIACEAE | <i>Salvia</i> sp. | 1, 4 | Herbaceous |
| | <i>Clinopodium vulgare</i> L. | 2 | Herbaceous |
| | <i>Calamintha</i> sp. | 3 | Herbaceous |
| | <i>Menthaspicata</i> L. subsp. <i>tomentosa</i> (Briq.) Harley | 1 | Herbaceous |
| LAURACEAE | <i>Laurus nobilis</i> L. | 6, 7, 8 | Shrub |
| MAGNOLIACEAE | <i>Magnolia x soulangeana</i> Soul.–Bod. | 8 | Shrub |
| MORACEAE | <i>Ficus carica</i> L. | 7 | Tree |
| OLEACEAE | <i>Phillyrea latifolia</i> | 2 | Bush |
| OXALIDACEAE | <i>Oxalis corniculata</i> L. | 3 | Herbaceous |
| PHYTOLACCACEAE | <i>Phytolacca americana</i> L. | 2 | Shrub |
| PINACEAE | <i>Pinus nigra</i> L. | 1 | Tree |
| | <i>Pinus pinea</i> L. | 6 | Tree |
| | <i>Pinus sylvestris</i> L. | 4 | Tree |
| PLANTAGINACEAE | <i>Plantago lanceolata</i> L. | 1 | Bush |
| PLATANACEAE | <i>Platanus orientalis</i> L. | 1 | Tree |
| POLYGONACEAE | <i>Polygonum aviculare</i> L. | 2, 8 | Herbaceous |
| | <i>Polygonum persicaria</i> L. | 3 | Herbaceous |
| | <i>Rumex crispus</i> L. | 8 | Bush |
| RANUNCULACEAE | <i>Clematis vitalba</i> L. | 2, 6 | Bush |
| ROSACEAE | <i>Crataegus monogyna</i> Jacq. | 1, 3, 5 | Shrub |
| | <i>Malus</i> sp. | 1 | Tree |
| | <i>Rosa canina</i> L. | 4 | Bush |
| | <i>Rosa</i> sp. | 7 | Bush |
| | <i>Pyracantha coccinea</i> Roemer | 1 | Bush |
| | <i>Rubus</i> sp. | 3, 7 | Bush |
| SALICACEAE | <i>Populus alba</i> L. | 3, 6, 7 | Tree |
| | <i>Populus nigra</i> L. | 1 | Tree |
| | <i>Populus tremula</i> L. | 2 | Tree |
| | <i>Salix alba</i> L. | 3 | Tree |
| SCROPHULARIACEAE | <i>Verbascum</i> sp. | 5 | Bush |
| SIMAROUBACEAE | <i>Ailanthus altissima</i> (Miller) Swingle | 1 | Tree |
| SOLANACEAE | <i>Solanum</i> sp. | 1 | Bush |
| ULMACEAE | <i>Ulmus minor</i> Miller | 1, 5, 6 | Tree |
| VIOLACEAE | <i>Viola</i> sp. | 2 | Herbaceous |

VISUALIZATIONS OF RURAL HIGHWAY LANDSCAPE RECLAMATION AREAS (ALTERNATIVE DESIGN PATTERNS)

Fig. 3 illustrates the important differences among the 3-D simulations in the intensive use of

plants. In the “closed” option, the plants are tall and rough textured, the density is low in the “semi-open” option (which consists of small trees and plants with few branches), and the lowest plant intensity is the “open” option, where the plants are thin-textured ground cover.

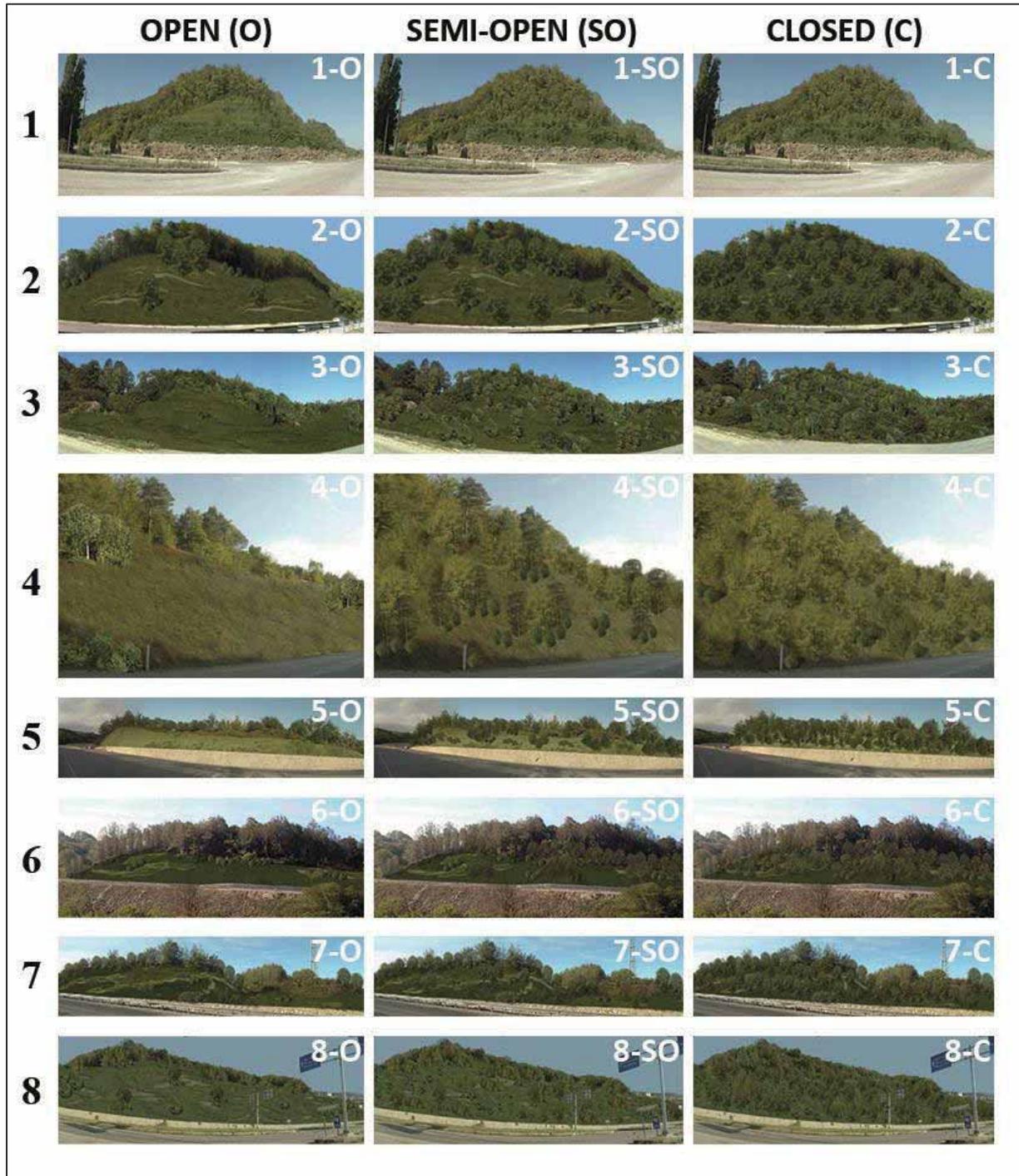


FIGURE 3
Vegetation management alternatives (3-D visualizations of rural Highway Landscape Reclamation Areas).

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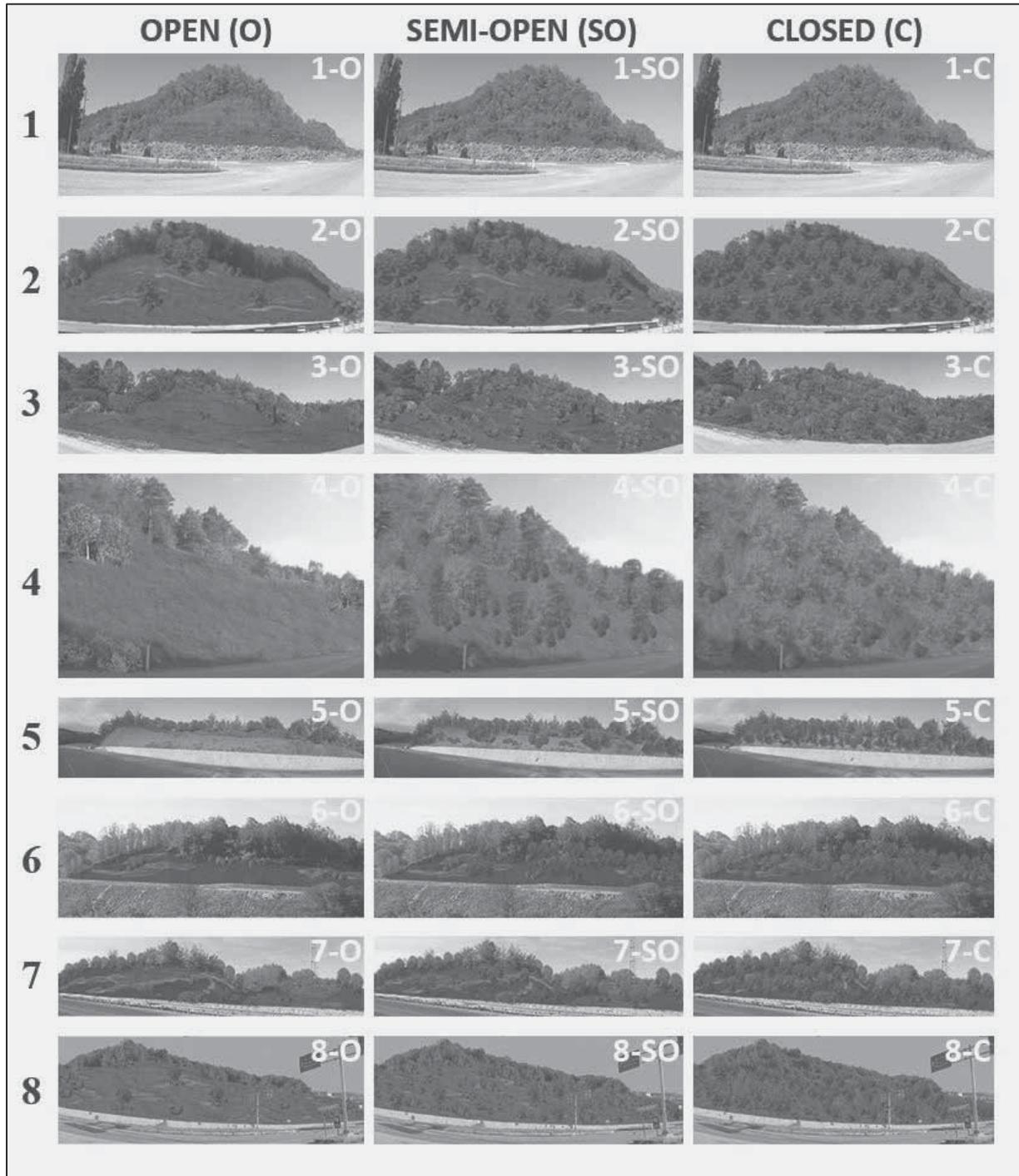


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Vegetation management alternatives (3-D visualizations of rural Highway Landscape Reclamation Areas).

RESPONDENT EVALUATIONS OF THE NEW HIGHWAY

Respondent Preferences. The socio-demographic characteristics of the respondents were assessed and correlated with each other. The results of the zero-order correlations are presented in Table 3. Gender was positively related to education, income, and occupation, but negatively related to survey group. The male respondents reported higher educational attainment and higher incomes. Age was negatively correlated with education, but positively correlated with income, occupation, and survey group. Age was negatively related to the use of the new Bartın-Amasra highway for work, using it at least a few times every day or week, and negative issues. Higher educated respondents preferred the highway more for watching the view of the sea and landscape, reported many negative aspects of the highway, and suggested planting designs that counter noise and visual pollution and the reformation of the damaged vegetation to mitigate the problems.

Income was positively related to using the new Bartın-Amasra highway more for work and because it is safer for everyday use. Income also was related to the respondents' beliefs that the hard rock quarries and concrete construction sites are negative aspects of the highway. Whereas the academic staff and public staff who used the highway for work believed that there is nothing negative about the highway, they stated that the visual pollution of hard rock quarries and concrete construction sites should be removed. Respondents who reported using the road about once a year reported enjoying the scenic spots and seasonal changes in plants. The respondents suggested planting design and stream remediation to offset the damage done to the dry and wet riverbeds and to counter the noise and visual pollution. The respondents stated that the negative effects of the highway could be reduced by planting designs and landscape reclamation.

A frequency analysis was performed to assess the differences among the types of users (academic staff, public staff, students, and laypersons) regarding landscape reclamation proposals. Table 4 presents the percentages regarding positive aspects, negative aspects, and solutions by group. Regarding the positive aspects, all of the groups except the students were most likely to choose "closed and open scenic views." The students ranked "scenic lookout points" first (26%) and "closed and open scenic views" second (23%). All the groups were similar in their ranking of the negative aspects as well, choosing "hard rock quarries and concrete

construction sites" as the most negative aspect of the highway. The negative attitudes toward the quarries and construction were reflected in their suggested solutions in that all of the groups were most likely to choose the removal of the visual pollution caused by hard rock quarries and concrete construction sites as their highest ranked solution.

Visual Quality Analysis Results. The respondents were asked to review the landscape reclamation proposals on the study sites. They reviewed proposals for open, semi-open, closed, and current status. Differences between the 12 descriptive pairs of terms used to differentiate between the alternatives were statistically significant ($n = 800$, $p < .01$). Table 5 shows the results of the analysis of variance. There are no statistically significant differences among the groups. Therefore Duncan test was applied to the study.

The Duncan test results are shown in Table 6. In the overall sample, the landscape reclamation proposals (open, semi-open and closed) and the images differed with respect to the descriptive pairs or terms' scores. Although the groups' responses to these questions were homogenous within each group (Table 6), all of the groups were most likely to be positive about designs using the "closed" option, which use the highest quantities of plants. The adjectives that were used in the evaluations included beautiful, attractive, regular, relaxing, safe, accessible, practical, visible, natural, varied, rich in terms of species, and sufficiently green. These alternatives were the most successful "closed" option was applied according to the Duncan test result. The photos of the current status of the highway were least positive in the evaluations by all of the groups, followed by their evaluations of open and semi-open vegetation management alternatives (Table 6).

DISCUSSION

INTEGRATING VISUAL PREFERENCES INTO LANDSCAPE RECLAMATION APPLICATIONS

The results of this study on the integration of visual preferences into landscape reclamation applications for designed alternative proposals suggest that complex patterns of vegetation are needed to strengthen structural and functional connectivity and people's visual preferences regarding landscape. Reclamation design alternatives were



based on open, semi-open, and closed landscape patterns.

Public participation in this study was accomplished during highway landscape reclamation work. In addition, as put forth by Blumentrath and Tveit [20], The European Landscape Convention stated that people’s landscape perceptions should be central to landscape planning and management. The results of this study are useful for the assessment of the visual quality of roads because the study adapted public landscape perceptions to highway landscaping issues. Highways are increasingly important in people’s daily exposure to landscapes and new

planning tools that comply with the requirements stated by the Convention are needed. According to the results of this study, respondents who used the new highway once a year appreciated its aesthetic features, such as its scenic points and seasonal flora transitions, whereas respondents who used the highway every day of the week or intensively during the week were more aware of the negative aspects of the highway. Thus, the group of respondents that more frequently used it responded more negatively to it. The results suggest that those who use the highway relatively more frequently are better positioned to identify the problems and provide suggestions for solving problems.

TABLE 3
Relationships between the socio-demographic properties of the Bartın-Amasra new highway users and the new highway.

| | Variable | 1b | 1c | 1d | 1e | 1f | 1g | 1h | 2a | 2b | 2c | 2d | 2e | 2f |
|------------------------------------|---------------------------|------|---------|--------|--------|--------|-------|---------|---------|---------|-------|---------|--------|---------|
| Sample characteristics | 1a) Gender | .101 | .117* | .211* | .265** | .084 | .047 | -.121* | -.108 | -.113 | -.068 | -.029 | -.034 | .010 |
| | 1b) Age | | -.158** | .538** | .516** | .039 | -.085 | .127* | -.125* | -.122* | -.076 | -.075 | .151** | -.070 |
| | 1c) Education | | | .223** | .027 | .214** | .123* | -.583** | .175** | -.102 | -.031 | -.024 | -.331* | .146* |
| | 1d) Income | | | | .738** | .164** | -.025 | -.170** | -.125* | -.221** | -.051 | -.150** | -.053 | -.044 |
| | 1e) Occupation | | | | | .043 | .027 | -.026 | -.165** | -.156** | -.031 | -.113 | .032 | -.060 |
| | 1f) Rural residence | | | | | | -.113 | -.153** | .123* | -.004 | -.018 | .036 | -.022 | -.030 |
| | 1g) Local residence | | | | | | | -.070 | .053 | .040 | -.016 | -.040 | -.101 | -.030 |
| | 1h) Survey group | | | | | | | | -.131* | .090 | -.060 | .034 | .249** | -.116* |
| Defining the Bartın-Amasra Highway | 2a) Uses | | | | | | | | | .028 | .052 | .089 | -.074 | .151** |
| | 2b) Frequency of use | | | | | | | | | | .121* | -.002 | .088 | .036 |
| | 2c) Most positive aspects | | | | | | | | | | | .013 | -.008 | .054 |
| | 2d) Most negative aspects | | | | | | | | | | | | .094 | |
| | 2e) Negative aspects | | | | | | | | | | | | | .371 |
| | 2f) Solutions | | | | | | | | | | | | | -.527** |

TABLE 4
Respondents' evaluations of the new Bartın-Amasra highway.

| | Percentages | | | |
|--|----------------------------|--------------------------|-----------------------|-------------------------|
| | Academic staff (n = 50) | Public staff (n = 50) | Students (n = 100) | Laypersons (n = 100) |
| <i>What are the positive aspects that attract you the most?</i> | | | | |
| Rural landscape character | 12 | 22 | 20 | 12 |
| Vegetation types | 10 | 14 | 6 | 15 |
| Closed and open scenic views | 36 | 36 | 23 | 32 |
| Rural settlements | 16 | 6 | 7 | 12 |
| Experiencing the seasonal changes of the plants | 20 | 20 | 17 | 4 |
| Scenic outlook points | 6 | 20 | 26 | 25 |
| <i>What are the negative aspects that attract you the most?</i> | | | | |
| Hard rock quarries and concrete construction sites | 38 | 50 | 31 | 26 |
| Areas that are subject to erosion | 20 | 24 | 8 | 19 |
| Damage to the topography as a result of excavation and digging works | 20 | 12 | 27 | 19 |
| Damage to the vegetation | 8 | 18 | 18 | 20 |
| Damage to the dry and wet river beds | 4 | 2 | 2 | 5 |
| Noise pollution and visual pollution | 10 | 12 | 14 | 11 |
| <i>What are your suggestions to solve these problems?</i> | | | | |
| Removal of the visual pollution caused by hard rock quarries and concrete construction sites | 44 | 64 | 46 | 50 |
| Planting design for erosion control | 14 | 22 | 15 | 17 |
| Landscape reclamation on roadside slopes | 24 | 8 | 21 | 16 |
| Renewal of the damaged vegetation | 6 | 12 | 10 | 10 |
| Carrying out river reclamation | 2 | 2 | 2 | 2 |
| Planting design against noise and visual pollution | 10 | 10 | 6 | 5 |

In this study, scenario techniques were used to simulate alternative rural highway landscape reclamation designs. The 3-D visualization method (e.g. [15, 33, 72-74]) has been successfully used in the past in a consultative planning approach to identify the preferences and perceptions of users through exposure to suggested alternative designs. Therefore, these results could be integrated into highway landscape reclamation applications. Our results found that respondents preferred the "closed" option among the simulations offered for the reclamation of the damaged natural areas due to stone quarries. As shown in Table 4, the aspects that the respondents reported as the most negative were the quarries and construction works. Thus, to improve people's perceptions, landscape reclamation applications should remove and/or decrease the ecological problems and visual pollution caused by these activities. In addition, activities should be pursued in line with the "closure" option to increase the likelihoods that the sites return to their original natural states and the

rural characteristics of the areas are maintained. The respondents' suggestions for mitigating damaged vegetation were planting designs that mitigate noise and visual pollution and landscape reclamation proposals on the roadside slopes.

The descriptive pairs of terms used in this study related to visual quality are in accord with those used by Blumentrath and Tveit [20] and Roth [75]. Blumentrath and Tveit [20] defined 12 visual characteristics: coherence, imageability, simplicity, visibility, maintenance, naturalness, integration, contrast, variety, aesthetics of flow, legibility, and orientation. The terms diversified, beautiful, and natural, defined by Roth [75] in an online visual landscape assessment study, are similar to the terms used in the present study. Table 6 demonstrates that during the landscape reclamation proposals being conducted on the highway at the time of the survey were not liked by the respondents.

TABLE 5
Analysis of variance test results of the differences among types of respondents regarding pairs of descriptive terms with respect to the new Bartın-Amasra highway.

| Descriptive pair of terms | User | Sum of squares | Sum of squares | F value |
|---------------------------|------|----------------|----------------|---------|
| Beautiful | A | 3897,545 | 1299,182 | 967,997 |
| | B | 4490,667 | 1496,889 | 908,990 |
| | C | 5841,446 | 1947,149 | 983,627 |
| | D | 6789,076 | 2263,025 | 1,209E3 |
| Attractive | A | 3919,597 | 1306,532 | 1,010E3 |
| | B | 4534,010 | 1511,337 | 935,634 |
| | C | 6082,964 | 2027,655 | 1,049E3 |
| | D | 6962,985 | 2320,995 | 1,185E3 |
| Regular | A | 3844,612 | 1281,337 | 901,780 |
| | B | 4480,285 | 1493,428 | 902,683 |
| | C | 6394,343 | 2131,448 | 1,052E3 |
| | D | 6886,172 | 2295,391 | 1,194E3 |
| Relaxing | A | 4055,047 | 1351,682 | 967,793 |
| | B | 4450,637 | 1483,546 | 870,794 |
| | C | 6539,576 | 2179,859 | 1,095E3 |
| | D | 7158,023 | 2386,008 | 1,281E3 |
| Safe | A | 3402,955 | 1134,318 | 779,394 |
| | B | 4170,465 | 1390,155 | 772,328 |
| | C | 6428,901 | 2142,967 | 1,130E3 |
| | D | 7007,821 | 2335,940 | 1,537E3 |
| Accessible | A | 2962,542 | 987,514 | 614,764 |
| | B | 4095,972 | 1365,324 | 756,154 |
| | C | 4130,428 | 1376,809 | 634,384 |
| | D | 4954,373 | 1651,458 | 838,609 |
| Practical | A | 3343,447 | 1114,482 | 753,217 |
| | B | 4130,392 | 1376,797 | 754,869 |
| | C | 5301,218 | 1767,073 | 862,795 |
| | D | 5622,421 | 1874,140 | 961,715 |
| Visible | A | 2747,152 | 915,717 | 536,114 |
| | B | 4069,035 | 1356,345 | 729,158 |
| | C | 3435,249 | 1145,083 | 465,968 |
| | D | 3339,023 | 1113,008 | 494,813 |
| Natural | A | 3274,365 | 1091,455 | 657,437 |
| | B | 4280,377 | 1426,792 | 777,690 |
| | C | 5453,478 | 1817,826 | 747,982 |
| | D | 6669,836 | 2223,279 | 1,072E3 |
| Varied | A | 4011,077 | 1337,026 | 987,505 |
| | B | 4384,605 | 1461,535 | 844,969 |
| | C | 6789,911 | 2263,304 | 1,046E3 |
| | D | 7592,628 | 2530,876 | 1,257E3 |
| Rich in terms of species | A | 4250,965 | 1416,988 | 1,026E3 |
| | B | 4516,295 | 1505,432 | 878,469 |
| | C | 7595,846 | 2531,949 | 1,250E3 |
| | D | 7949,956 | 2649,985 | 1,409E3 |
| Sufficient green | A | 4667,602 | 1555,867 | 1,146E3 |
| | B | 4642,372 | 1547,457 | 889,515 |
| | C | 8094,814 | 2698,271 | 1,277E3 |
| | D | 8734,211 | 2911,404 | 1,804E3 |

User type: A = Academic staff, B = Public staff, C = Students, D = Laypeople



TABLE 6
Duncan analysis values for the landscape reclamation proposals (open, semi-open, and closed) suggested for the new Bartın-Amasra highway and the descriptive pairs selected regarding the present status of the highway

| Adjective pairs | Projects ^a | n | Subset for alpha = 0.05 | | | | | | | | | | | | | | | |
|--------------------------|-----------------------|-----|-------------------------|---|---|---|--------------|---|---|---|----------|---|---|---|-----------|---|---|---|
| | | | Academic staff | | | | Public staff | | | | Students | | | | Laypeople | | | |
| | | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Beautiful | 1 | 300 | 2,0900 | | | | 1,7700 | | | | 2,2875 | | | | 2,3925 | | | |
| | 2 | 300 | 4,0975 | | | | 3,7550 | | | | 4,3938 | | | | 4,0588 | | | |
| | 3 | 300 | 5,2075 | | | | 5,0200 | | | | 5,1788 | | | | 5,2812 | | | |
| | 4 | 300 | 6,3150 | | | | 6,3100 | | | | 5,8962 | | | | 6,3000 | | | |
| Attractive | 1 | 300 | 2,0425 | | | | 1,7625 | | | | 2,1612 | | | | 2,1750 | | | |
| | 2 | 300 | 4,0625 | | | | 3,7425 | | | | 4,2438 | | | | 3,8325 | | | |
| | 3 | 300 | 5,2450 | | | | 5,0375 | | | | 5,1438 | | | | 5,1600 | | | |
| | 4 | 300 | 6,2475 | | | | 6,3175 | | | | 5,8238 | | | | 6,0975 | | | |
| Regular | 1 | 300 | 2,0300 | | | | 1,7625 | | | | 2,0925 | | | | 2,0638 | | | |
| | 2 | 300 | 4,0625 | | | | 3,7300 | | | | 4,3450 | | | | 3,8912 | | | |
| | 3 | 300 | 5,2450 | | | | 5,0500 | | | | 5,2275 | | | | 5,2050 | | | |
| | 4 | 300 | 6,1800 | | | | 6,2775 | | | | 5,8088 | | | | 5,9200 | | | |
| Relaxing | 1 | 300 | 1,9450 | | | | 1,7700 | | | | 2,0775 | | | | 2,1388 | | | |
| | 2 | 300 | 4,2000 | | | | 3,7500 | | | | 4,3938 | | | | 3,8300 | | | |
| | 3 | 300 | 5,2550 | | | | 5,0325 | | | | 5,1762 | | | | 5,2212 | | | |
| | 4 | 300 | 6,2275 | | | | 6,2800 | | | | 5,8762 | | | | 6,0912 | | | |
| Safe | 1 | 300 | 2,2000 | | | | 1,8575 | | | | 2,0350 | | | | 2,1938 | | | |
| | 2 | 300 | 4,2775 | | | | 3,7850 | | | | 4,3738 | | | | 4,0300 | | | |
| | 3 | 300 | 5,2625 | | | | 5,0600 | | | | 5,2288 | | | | 5,2350 | | | |
| | 4 | 300 | 6,1100 | | | | 6,2075 | | | | 5,7300 | | | | 6,1488 | | | |
| Access-ible | 1 | 300 | 2,4225 | | | | 1,8850 | | | | 2,4750 | | | | 2,3350 | | | |
| | 2 | 300 | 4,3725 | | | | 3,7775 | | | | 4,4812 | | | | 3,9862 | | | |
| | 3 | 300 | 5,2525 | | | | 5,0575 | | | | 5,0638 | | | | 5,0312 | | | |
| | 4 | 300 | 6,0850 | | | | 6,1925 | | | | 5,4088 | | | | 5,6088 | | | |
| Practical | 1 | 300 | 2,2025 | | | | 1,8850 | | | | 2,1612 | | | | 2,1588 | | | |
| | 2 | 300 | 4,2525 | | | | 3,7800 | | | | 4,3850 | | | | 3,9812 | | | |
| | 3 | 300 | 5,2475 | | | | 5,0925 | | | | 5,0788 | | | | 5,0688 | | | |
| | 4 | 300 | 6,0725 | | | | 6,2000 | | | | 5,5000 | | | | 5,6350 | | | |
| Visible | 1 | 300 | 2,6500 | | | | 1,9275 | | | | 2,9700 | | | | 2,8350 | | | |
| | 2 | 300 | 4,3775 | | | | 3,8100 | | | | 4,6075 | | | | 4,1062 | | | |
| | 3 | 300 | 5,4025 | | | | 5,0775 | | | | 5,2804 | | | | 4,9738 | | | |
| | 4 | 300 | 6,1425 | | | | 6,2250 | | | | 5,6862 | | | | 5,5462 | | | |
| Natural | 1 | 300 | 2,4800 | | | | 1,8850 | | | | 2,5875 | | | | 2,2962 | | | |
| | 2 | 300 | 4,3175 | | | | 3,7475 | | | | 4,4950 | | | | 3,9638 | | | |
| | 3 | 300 | 5,3775 | | | | 5,0950 | | | | 5,3338 | | | | 5,2788 | | | |
| | 4 | 300 | 6,3350 | | | | 6,2850 | | | | 6,0900 | | | | 6,1175 | | | |
| Varied | 1 | 300 | 2,2075 | | | | 1,8375 | | | | 2,2588 | | | | 2,1012 | | | |
| | 2 | 300 | 4,0725 | | | | 3,7050 | | | | 4,1975 | | | | 3,9150 | | | |
| | 3 | 300 | 5,4325 | | | | 5,0775 | | | | 5,3175 | | | | 5,3288 | | | |
| | 4 | 300 | 6,4300 | | | | 6,2900 | | | | 6,1450 | | | | 6,1638 | | | |
| Rich in terms of species | 1 | 300 | 2,1125 | | | | 1,8175 | | | | 2,0938 | | | | 2,0750 | | | |
| | 2 | 300 | 4,0175 | | | | 3,6750 | | | | 4,1212 | | | | 3,8712 | | | |
| | 3 | 300 | 5,4750 | | | | 5,0975 | | | | 5,3488 | | | | 5,3488 | | | |
| | 4 | 300 | 6,4350 | | | | 6,3300 | | | | 6,1900 | | | | 6,2312 | | | |
| Sufficient green | 1 | 300 | 2,0450 | | | | 1,7800 | | | | 2,0662 | | | | 2,1262 | | | |
| | 2 | 300 | 4,1475 | | | | 3,6925 | | | | 4,3562 | | | | 4,0500 | | | |
| | 3 | 300 | 5,6225 | | | | 5,1475 | | | | 5,4788 | | | | 5,5600 | | | |
| | 4 | 300 | 6,5725 | | | | 6,3450 | | | | 6,2962 | | | | 6,4925 | | | |

^a1 = Current, 2 = Open, 3 = Semi-open, 4 = Closed

NATURAL PLANT USE IN RURAL HIGHWAY LANDSCAPE RECLAMATION

The plants that were identified in the study area (Table 2) should be used in landscape reclamation applications and they should be widespread. The natural plants of the area have significant

ecological, aesthetic, functional, and economic importance to the feasible and economic landscaping of the areas that require landscape reclamation. The importance of indigenous flora was previously emphasized by Cengiz et al. [60] and Yılmaz and Irmak [76]. In addition, planting designs developed by using natural plant species

will contribute to the sustainability of the rural highway landscape. Lange [74] emphasized that the landscape contributes to the visual and ecological value of the area. As a result, landscape planners have new opportunities for improving the visual and ecological value of landscapes with the proposal of new structural elements.

CONCLUSION

In conclusion, this study contributes to our knowledge of sustainable regional development and helps to guide rural highway landscape reclamation proposals aimed at sustaining the rural quality of the new Bartın-Amasra highway. The results of this study show that users prefer the highway for its scenic properties (forest and sea as well as rural character). Because users prefer this highway's scenic attributes, landscape reclamation proposals will attract their attention.

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