Research Paper

Impact of Zeolite on Protein, Mg and Zn Content of Triticum aestivum L. Wheat in Contaminated Soils of War Zones

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ABSTRACT

Background: Nowadays, the use of unconventional weapons has made a serious damage to the environment. The use of zeolite is one of the solutions in soil pollution management. The aim of this study was to investigate the impact of natural zeolite on grain protein, Mg and Zn uptake in the wheat in contaminated soils by war weapons.

Methods: The experiment was carried out in factorial arrangement in a randomized complete design with three replications in a pot. The studied treatments were four levels of zeolite as zero, 0.5, 1.5 and 2.5% based on weight of soil and two types of soils including non-contaminated and contaminated soil by mines and war explosives. Contaminated and non-contaminated soil samples were taken from the southern region of Dehloran city.

Results: The results showed that the amount of grain protein and Mg in contaminated soils were significantly higher than non-contaminated soils. The use of zeolite in both soils caused a significant increase in either of them. In this study, the Zn concentration of grain in contaminated soils was higher than non-contaminated. In addition, the concentration of Zn was significantly decreased when zeolite consumption was increased.

Conclusion: The results showed that the use of zeolite in contaminated and non-contaminated soils had a positive effect in terms of grain protein and Mg. In addition, the use of zeolite can prevent the excessive increase in Zn due to war weapons and can be effective in plant and community health.

Keywords: Agriculture, Environment, Health Care, Protein, Soil pollution, Wheat, Zeolite

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1. Introduction

War is one of the causes of environmental pollution [1]. Pollution from mines and war explosives is estimated to be existed at 1100 km of the western and southwestern borders of Iran which is equal to 4.2 million hectares. Use of these lands in food production is necessary due to population growth. In these lands, over 16 million types of explosive mines and other ammunition have been used, of which the remainings are buried inside the soil [2]. According to the reports and researches on the soils of Khorramshahr, contamination with heavy metals caused by bombings and war weapons has been several times more than permissible concentration [3]. Unlike organic pollutants, heavy metals are not decomposed biologically or chemically and remain in the soil for a long time [4]. The materials left by the bombs change the acidity of the soil, and depending on the type of explosives, they make the soil to be acidic or alkaline [4].

Various materials such as clay minerals and zeolite have been used to reduce the pollution caused by heavy metals in the soil and prevent them to transfer to plants [5]. Zeolites are a group of crystallized hydrated aluminosilicates originated from the elements of groups I and II of the periodic table, especially sodium, potassium, magnesium, calcium, strontium and barium. Zeolites are found in both natural and artificial forms [6]. Due to their high cation exchange capacity (CEC) and the placement of some cations in their structure, they can also play a nourishing role and improve plant growth in addition to their soil remediation role. Selective uptake and controlled release of nutrients by zeolite helps the plant growth to be improved. It occurs by increasing in the long-term availability of nutrients if they are used correctly [7, 8]. The most obvious properties of zeolites are their conversion ability to dehydration, as well as their ability to balance the cations without changing the structure [6]. Their CEC varies between 100-300 cmol/kg, and has a high tendency to absorb cations in their three-dimensional structure [5]. During the processes of ion exchange, ions are forced to move through the zeolite mass and its canals. It has been reported that the added zeolite to soil increases the nutrients, such as nitrogen, phosphorus, potassium, iron, copper and zinc in plant tissues of soybean [9].

Zn and Mg exist in the soil and are absorbed through plant’s roots. They are the essential elements of plants. Absorption of these elements is affected by various factors such as soil acidity, texture, organic matter, etc. [10]. They are essential elements of humans as well. However, the human body cannot produce or store them. This is the reason that they must be provided through a permanent diet. Zn is involved in many processes in our body. It is responsible for the activity of more than 300 enzymes that helps metabolism, digestion, nerve function and many other processes. In addition, it is essential for growth and function of the immune cells. It is also essential for skin health, DNA synthesis and protein production so that development of the body depends on its role in cell growth and division [11]. Mg also plays a complementary role (as cofactor) in more than 300 enzymatic reactions in the human body. These enzymatic reactions are used for the production of proteins, muscle contraction, proper functioning of the nervous system, control of the blood’s sugar and pressure and production of cellular energy.

Mg is essential for cellular respiration as well. It is required for building bones and maintaining their health as well as transporting potassium and calcium ions into and out of cells. The ions transportation is necessary for conducting nervous messages, contracting the body’s muscles and maintaining a normal heart rate. Glucose metabolism takes place in the body during several chain reactions that require the presence of Mg [12]. It has been reported that adding the zeolite to uncontaminated soil increase the concentration of nutrients such as nitrogen, phosphorus, potassium, iron, copper and zinc in soybean plant tissues [9]. Damian and Damian conducted a research on the use of zeolite with organic matter in contaminated soils with lead, zinc, cadmium and copper. They reported that organic matter is effective in increasing the plant growth [13]. Because the plants access to food is increased in one hand, and the heavy metals are absorbed from the soil, thereby reducing their availability in the other hand. In addition, it has been suggested that the effects of heavy metals on plants in contaminated soils may be due to the binding of some heavy metal cations such as lead, silver and zinc to sulfhydril groups of proteins, which leads to degradation of protein structure or disruption of proteins [6].

Due to lands pollution by mines, war explosives, as well as use of these lands in food productions in one hand, and existence of huge resources of zeolite in Iran including Tabas, Semnan, Miyaneh, Saeen, Dej, Talha, Roodeen, Taleghan, Kerman and Zahedan regions in the other hand, it is, therefore, necessary to study the effect of this resources on the land in the regions. Thus, this study investigated the effect of zeolite on concentration of magnesium, zinc and protein percentage of wheat grain in contaminated lands.
2. Materials and Methods

This greenhouse study was carried out in factorial arrangement by a randomized complete design with three replications in a pot. The studied treatment was zeolite with values of zero, 0.5, 1.5 and 2.5% based on the weight soil (W/W). Two types of soil, including non-contaminated and contaminated soils by mines and war explosives were studied as well. In this experiment, Chamran wheat cultivar was used. Contaminated soil samples were taken from the southern region of Dehloran (border region of Iran and Iraq). The geographical location of the region has been provided in Figure 1.

In the region, a wide range of bombs and weapons were used during the war between Iraq and Iran. Therefore, according to the evidence, 50 infected points were sampled. Also, 50 soil samples were selected as non-contaminated samples. They were selected far from the terrestrial. Individual samples of both types of soil were mixed separately. After air drying, they were passed through a 2 mm sieve. Some physical and chemical properties of the studied soils have been presented in Table 1. The Electrical Conductivity (EC) of the soil saturation was measured using a conductivity meter [14]. Soil PH was measured by saturation method and a PH meter [14]. Soil texture was carried out using hydrometric method [15]. The CEC was measured by ammonium acetate replacement and a photoelectric flame photometer [16]. The exchangeable potassium was measured by a replacement procedure with one-normal ammonium acetate and a photoelectric flame photometer [14]. The absorbable phosphorus was measured using sodium bicarbonate and spectrophotometric readings [17]. Nitrogen was also measured by Kjeldahl method [16]. To determine the heavy metal concentrations, the digestion method using HNO₃, 30% oxygen dioxide and the Atomic Absorption device (Unicam 919AA model) was used [18].

In this study, the zeolite was prepared by Afrand Tosca Company. It was washed using distilled water and dried air through a 1 mm sieve. The mineralogy of zeolite was determined by X-ray diffraction method (i.e., the wavelengths between 0.2 and 10 nm). It should be mentioned that 75% of the zeolite was composed of pure clinoptilolite. Some properties of zeolite have been presented in Table 2.

The zeolite was mixed-up with contaminated and non-contaminated soils based on the desired treatment. It was then stored for one month for chemical reactions at constant temperature and humidity. Finally, it was added to the pots with the height of 40 cm and diameter of 20 cm. The amount of added soil moisture was so such that it was kept within the field capacity. The weight of soil was 10 kg in each pot. Within each pot, 10 seeds were planted. However, after planting, their number was reduced to 5. Because of rainfall in Ahvaz, the irrigation operations were carried out once a week. During the growing season, no inputs (e.g., pesticides and herbicides) were used to maintain the natural conditions of the soil. 150 days after planting, the plants were harvested. After milling and determination of grain nitrogen percentage by Kjeldahl method, the grain protein percentage was calculated using Equation 1 [6]:

1. \[ \text{Protein(\%)} = 6.25 \times \text{Nitrogen(\%)} \]

To determine the concentration of Mg in the grain, the normal 0.01 Versin solution was applied [19]. Also, the concentration of Zn was determined by digestion method using HNO₃ and 30% of oxygenated water.

Figure 1. Location of contaminated and non-contaminated soil samples
For this purpose, the Unicam 919AA Atomic Absorption device was used [20].

Data analysis was performed by SAS software, and Duncan method was used to compare the means at the 5% significance level.

3. Results and Discussion

Grain protein

The effect of zeolite and soil types on grain protein are shown in Table 3, which shows a significant difference in grain protein associated to zeolite consumption, soil type and interaction between the two treatments was observed.

The results showed that an increase in zeolite application in both contaminated and non-contaminated soils caused a significant increase in grain protein. In contaminated soil, the maximum protein of 13.6% was observed by using 2.5% zeolite while the minimum value of 10.8% was related to no value of zeolite. In non-contaminated soil the maximum protein value of 10.5% was observed under using 2.5% of zeolite while the minimum of 7.6% was under using no value of zeolite (Figure 2). The increase in grain protein under use of zeolite can be attributed to the amount of soil nitrogen. It has been reported that zeolite can release nitrogen into

Table 1. The physical and chemical properties of contaminated and non-contaminated soils

| Soil Properties | Unit | Contaminated Soil | Non-contaminated Soil |
|-----------------|------|-------------------|-----------------------|
| Fe   | ppm  | 9276.4            | 658.13                |
| Cu   | ppm  | 150.4             | 12.48                 |
| Mn   | ppm  | 156.8             | 11.46                 |
| Zn   | ppm  | 264.8             | 44.56                 |
| Pb   | ppm  | 274.5             | 25.48                 |
| Sn   | ppm  | 225.8             | 15.4                  |
| Ni   | ppm  | 132.8             | 25.78                 |
| Cd   | ppm  | 154.7             | 4.76                  |
| Co   | ppm  | 148.4             | 5.72                  |
| Cr   | ppm  | 152.75            | 18.3                  |
| Hg   | ppm  | 61.5              | 4.14                  |
| As   | ppm  | 58.92             | 0.233                 |
| N    | ppm  | 0.163             | 0.058                 |
| P    | ppm  | 47.4              | 4.85                  |
| K    | ppm  | 539.7             | 142.6                 |
| Mg   | ppm  | 12.1              | 10.6                  |
| CEC  | Cmol/kg | 13.64          | 9.52                 |
| EC   | dS/m | 4.83              | 3.15                  |
| Clay | %    | 29                | 22                    |
| Sand | %    | 41                | 41                    |
| pH   | --   | 6.63              | 7.56                  |

EC: Electrical Conductivity; CEC: cation exchange capacity.
the soil, thereby providing it to the plant through a selective and controlled way, which has been effective in increasing the grain protein [21]. In fact, zeolites can be used as a nitrogen fertilizer by providing nitrogen. The increase in protein can also be due to the presence of some elements such as magnesium, calcium, potassium and phosphorus in the cationic structure of zeolite, which play an effective role in production of the proteins (Table 2). Other reasons for the increase in wheat protein due to the zeolite are physical and chemical improvement of soil, increasing root penetration into the soil, and accordingly better absorption of nitrogen, potassium, phosphorus and other elements [9].

Also, it has been reported that the increase in grain protein in contaminated soils compared to non-contaminated soils can be attributed to the high concentrations of iron, manganese, zinc and copper in contaminated soils (Table 1). They are monitored by controlled use of zeolite [22].

**Mg concentration**

The result of analysis of variance has been provided in Table 3. It shows that the effects of zeolite, soil types and their interaction effect on grain Mg were significant. The results of mean comparison revealed that the zeolite had a positive effect on the grain Mg in both soils. In this study, the maximum grain Mg of 15 mg/kg in contaminated soil was obtained under use of 2.5% of zeolite. The minimum of 8.2 mg/kg was observed in non-contaminated soil with no values of

### Table 2. The chemical properties of zeolite

| Parameters | Units | Amounts |
|------------|-------|---------|
| pH         | -     | 8.45    |
| EC         | dS/m  | 0.097   |
| CEC        | Cmol/kg | 170    |
| SiO₂       | %     | 66.5    |
| Al₂O₃      | %     | 11.81   |
| Fe₂O₃      | %     | 1.3     |
| TiO₂       | %     | 0.21    |
| CaO        | %     | 3.11    |
| MgO        | %     | 0.72    |
| Na₂O       | %     | 0.5     |
| K₂O        | %     | 3.12    |
| P₂O₅       | %     | 0.01    |

EC: Electrical Conductivity; CEC: cation exchange capacity.

### Table 3. Results of analysis of variance for protein, Mg and Zn of wheat grain

| Source of Variance | df | Protein | Mg  | Zn  |
|--------------------|----|---------|-----|-----|
| Zeolite            | 3  | 26.37** | 32.04** | 295** |
| Soil               | 1  | 120.87** | 75.4** | 391** |
| Zeolite×Soil       | 3  | 2.73**  | 4.37*  | 32.2* |
| Error              | 16 | 0.186   | 6.11  | 14.9 |

CV%: coefficient of variation.

*; **: not significant; significant values at P=0.05 and P=0.01, respectively.
In this study, chemical treatments were designed to evaluate the effect of soil type and zeolite consumption on grain protein due to the zeolite and other elements contamination in soil. It was revealed that zeolite had a positive effect on the increase in grain protein in contaminated soils compared to the control. The reason was due to the zeolite’s high cation exchange capacity and increased magnesium concentration. The results showed that the maximum increase in grain protein was observed under a 3:1.5% zeolite consumption rate. It is because of the zeolite’s potential to release nitrogen and increase soil nitrogen. The P≤0.05 was considered as significant value.

The effect of soil type (b1: non-contaminated and b2: contaminated soil) and zeolite treatments (a1: zero, a2: 0.5%, b3:1.5%, b4:2.5%) on grain protein was observed under different soil types. The increase in grain protein was related to no zeolite application in contaminated soils. Also, the increase in grain protein was significant (Table 3). Other reasons for the increase in wheat protein have been increased in the soil stabilization effect of zeolite can be attributed to the zeolite in non-contaminated and contaminated soil (Fig 1). The p ≤ 0.05 was considered as significant value.

The effect of soil type (b1: non-contaminated and b2: contaminated) and zeolite treatments (a1: zero, a2: 0.5%, b1:1.5%, b2:2.5%) on grain mg/kg was observed under different soil types. The increase in grain mg/kg was related to no zeolite application in contaminated soils. Also, the increase in grain mg/kg was significant (Table 3). Other reasons for the increase in wheat protein have been increased in the soil stabilization effect of zeolite can be attributed to the zeolite in non-contaminated and contaminated soil (Fig 1). The p ≤ 0.05 was considered as significant value.

The effect of soil type (b1: non-contaminated and b2: contaminated soil) and zeolite treatments (a1: zero, a2: 0.5%, b1:1.5%, b2:2.5%) on grain Zn was observed under different soil types. The increase in grain Zn was related to no zeolite application in contaminated soils. Also, the increase in grain Zn was significant (Table 3). Other reasons for the increase in wheat protein have been increased in the soil stabilization effect of zeolite can be attributed to the zeolite in non-contaminated and contaminated soil (Fig 1). The p ≤ 0.05 was considered as significant value.
zeolite (Figure 3). The reason was due to the Mg in the structure of zeolite in the form of MgO. Indeed, as zeolite consumption increases, the Mg absorption, and consequently the concentration of Mg is increased in the grain (Table 2). Fathi in his study aiming the zeolite effect on essential elements of plant, found that the use of zeolite can increase the calcium and Mg in plant tissues. It was due to the presence of these elements in the structure of zeolite [18]. In this experiment, a significant difference was observed between contaminated and non-contaminated soil in term of grain Mg (Figure 3). In contaminated soil, the Mg concentration was 9.8 mg/kg under use of no value of zeolite. In non-contaminated soil, it was 8.2 mg/kg. Therefore, there was significantly higher Mg concentration in contaminated compared to non-contaminated soil (Table 1).

Zn concentration

The analysis of variance showed that the effect of soil types, zeolite and their interaction effect on Zn concentration of grain was statistically significant (Table 3). The analysis of mean comparisons showed that the Zn concentration has been significantly increased in contaminated compared to non-contaminated soils (Figure 4). However, as the zeolite was increased, the Zn was significantly decreased. The maximum Zn concentration of 27.8 mg/kg per grain was related to the contaminated soils in which no zeolite was applied. While, the minimum of 8.6 Mg/kg was observed under 2.5% use of zeolite in non-contaminated soils.

Fathi in his research revealed that the zeolite has a high potential effect on removal of heavy elements. The reason was because of high CEC. They also reported that the most important mechanism in decreasing of Zn was the process of zeolite ion exchange as well as its PH effect on Zn retention in the soil through the deposition of soluble phases [18]. It has been showed that the ions are forced to be moved through the zeolite mass and its channels during the ion exchange process. Similar results have been reported by Kumpiene et al. [23]. This is considered a positive point because the zeolite has a high ability to monitor and stabilize the element in contaminated places. In addition, it prevents the toxicity and excessive transfer to the food chain.

In this regard, Erdem et al. stated that zeolites reduced the Zn accumulation in contaminated soils [5]. Also, Garcia-sanchez et al. stated that the natural zeolite increases soil adsorption capacity for heavy metals. It also reduces the leaching rate of heavy metals through soil stabilization [24].

4. Conclusion

The results showed that the nitrogen and grain protein have been increased in the contaminated soils with weapons. It is because of the nitrogenous compounds of explosives structure. We also showed that the zeolite released nitrogen into the plant through a selective and controlled way, which was effective in increasing the grain protein. In addition, our results showed that the Mg and Zn concentration were higher in contaminated soils than non-contaminated soils. Due to presence of Mg in the structure of zeolite, its usage is effective in increasing soil and grain Mg. However, due to the process of ion exchange as well as soil pH, the zeolite usage leads to a decrease in Zn concentration in the soil and grain. Therefore, it can effectively be helpful for the plant and community health.

Ethical Considerations

Compliance with ethical guidelines

This article was a study with no human or animal sample. There were no ethical considerations to be considered in this research.

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Authors’ contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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