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# EVALUATION OF METALS AND METALLOIDS IN WHEAT (*TRITICUM AESTIVUM* L.) UNDER APPLICATION OF ZEOLITE AND COMPOSTED COW MANURE

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Abstract - Today, large parts of the world are exposed to heavy metal pollution. The highest contamination with heavy metals is found in industrial areas, around large cities and mines. This experiment was carried out in order to evaluate uptake and accumulation of metals and metalloids in wheat (*Triticum aestivum* L.) under zeolite and composted cow manure application. The experimental design was factorial according to a completely randomized design with three replications. To contaminate the soil metals and metalloids included lead, nickel and arsenic were mixed with the soil. In this experiment, four different amounts of zeolite and composted cow manure include (zero) (control), 0.5, 1 and 1.5 percent of the soil weight were investigated in soil contaminated. The concentration of lead, nickel, and arsenic were determined in different parts of the plant including root, stem and grain at final harvest stage. Results showed that the effect of zeolite, compost and interaction between zeolite and compost on concentration of lead, nickel and arsenic in root, stem and grain were significant. The maximum and minimum accumulation of lead, nickel and arsenic under application and non-application of treatments were in root and grain, respectively. In general, in the contaminated soils, application of zeolite and compost by 1.5% is effective in reducing the contamination below the standards level of food. So it can increase food safety and food security.

Keywords: soil pollution, food safety, wheat, zeolite.

## Introduction

Today, large parts of the world are exposed to heavy metal pollution (Musilova et al., 2016). Heavy metals are non-biodegradable and remain indefinitely in the environment, so removing or minimizing their concentration from farming systems is closely linked to the health of the community (Alves et al., 2016). The highest contamination with heavy metals is found in industrial areas, around large cities and mines (Puschenreiter et al., 2005). Although some heavy metals are necessary for human metabolism in very small amounts, their large amounts are toxic to humans (Jan et al., 2015). The routes of entry of heavy metals into the body include inhalation of polluted air, direct consumption of contaminated water and soil and consumption of food plants grown on metal contaminated soil (Bhagure and Mirgane 2011). Studies have shown that plants grown in metal-contaminated soil have a higher concentration of metal than on non-contaminated soil (Dowdy and Larson 1975). Therefore, plants grown in polluted environments can accumulate trace elements at high concentrations and act as a major route for the transfer of metals into the food chain, thereby putting consumers at serious risk (Alloway et al., 1990).

Wheat has the largest world's production among cereals and it is mainly consumed by humans (Kazemzadeh et al., 2013). Wheat is one of the main products and an integral part of the diet that plays a vital role in human growth by providing

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carbohydrates, proteins, minerals and micronutrients (Anita 2010). Wheat grain consumption is safe when the heavy metal accumulation is at the allowed level (Khan et al., 2008). When excessive concentrations of metals in the soil increase, they can cause various diseases in humans (Al-Othman et al., 2012).

Arsenic belongs to group 5 of the periodic table in four oxidation states in nature but is widely present in the form of $H_3AsO_3$ and $HAsO_3$. The permissible limit of arsenic in soil is 20 mg/kg and for crops is 0.2 mg/kg dry matter. Symptoms of arsenic toxicity in the plant include leaf wilting and reduced root and stem growth, root discoloration, leaf necrosis, and inhibition of water uptake and plant death (Azough et al., 2018). Lead is an element that is used in the composition and structure of war weapons (Tokhmehchian et al., 2020). Lead has a negative effect on respiration and photosynthesis at a concentration of less than 1 ppm. It is stated that the negative effect of lead is due to its effect on the electron transfer system (Fathi, 2013). High levels of lead cause plant poisoning, decrease in growth, yellowing of young leaves and reduce the absorption of essential elements such as iron, which reduces photosynthesis and intracellular activity (Pourrut et al., 2011). Nickel is one of the heavy metals that small amounts of them are essential for humans, but they are dangerous to human health at high concentrations (Baycu et al., 2006). The amount of nickel in nature is very low (Wuana and Okieimen 2011). It has been suggested that nickel can accumulate in the tissues of some plants especially in vegetables (Zwolak et al., 2019).

Zeolites are aluminosilicate with a scaffold structure that occupies large ions and water molecules in the cavities and whose mobility allows ion exchange and dehydration reversible reactions. These materials have been used in various sciences because of their unique properties (Li et al., 2016). For example, it is noted that the zeolites have high porosity for gas diffusion and inlet and outflow of liquids, especially water and the application of zeolite increases the efficiency of fertilizers and ultimately improves plant growth by preventing nutrient loss (Polat et al., 2004). High selectivity for a particular ion is a special advantage of zeolites that can be used to separate harmful ions from municipal, industrial and agricultural wastewater. The most important mechanism of heavy metal uptake by zeolites can be attributed to the ion exchange and subsequent deposition of insoluble phases (Dimirkou 2008). Zeolite powders have been reported to effectively inhibit the transport and accumulation of elements such as copper, lead, zinc and cadmium in the plant and prevent severe soil contamination by these elements (Beltcheva et al., 2015).

Studies have shown that increasing organic matter such as livestock fertilizers increase the microbial population (fungi and bacteria) (Das et al., 2017). Among the organic fertilizers, compost fertilizers play an important role in sustainable agriculture and the improvement of ecosystems due to increased soil aggregate stability, increased soil porosity, increased soil water storage capacity, increased soil nitrogen availability and improved soil rhizosphere (Abiven et al., 2009). Organic fertilizers such as compost, in addition to their nutritional role in plants, in many cases reduce the availability of heavy metals by increasing the uptake sites (Chirakkara and Reddy 2015). By studying the effect of compost application on cadmium concentration in shoots of clover showed that the application of compost resulted in a significant reduction of cadmium concentration in shoots (Chaab et al., 2016). It has been reported healthy compost to be one of the most important and key strategies to improve soil and plant health that can prevent the increase in contaminants from chemical fertilizer (Annabi et al., 2007).

Based on huge resources of zeolites in different parts of the world including in Iran and the role of them in sustainable agriculture and the improvement of crop systems, so the purpose of the study is to grow wheat on contaminated soils and use amendments to reduce heavy uptake and thus make wheat consumable.

## Materials and Methods

For the investigation of the uptake and accumulation of heavy metals in different organs of wheat (*Triticum aestivum* L.), a pot experiment was conducted at the Agricultural Research Station, Ahvaz University, Iran. Ahvaz is located in South-west of Khuzestan province. longitude, latitude, height sea level of Ahvaz are 48°,40’, 31°,20’ and 18 m, respectively. The climate is considered to be dry and semi-arid. The average annual rainfall is 213 mm and very irregular. In the months of June, July, August, September without rain and in September and May there is no effective rainfall. January is the coldest month (6.9 °C) and August is the warmest month (43.6 °C). In this experiment two factors were investigated. The Experimental design was a factorial according to a randomized complete block design (RCBD) with three replications. In this experiment, four different amounts of zeolite and composted cow manure include (zero) (control), 0.5, 1 and 1.5 percent of soil weight were investigated in soil contaminated to lead, nickel and arsenic. The percentage of zeolite and compost was based on previous research and their recommended dosage (Azough et al., 2017; Chaab et al., 2016). Plastic pots made of PVC (60 cm height and 20 cm diameter) with a weight of 10 kg soil were used. Pot soils were collected from different areas of the field and after mixing, a sample was sent to the laboratory for analysis. Physical and chemical characteristics of soil are shown in Table 1.

**Table 1.** Physical and chemical characteristics of soil. EC: Electro Conductivity; CEC: Cation Exchange Capacity; OC: Organic Matter.

| pH | EC dS/m | CEC Cmol/kg | K | P | As | Pb | Ni | N | OC | Silt | Clay | Sand | Texture |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mg/kg | | | | | % | | | | | |
| 7.5 | 3.15 | 9.52 | 142.6 | 4.58 | 0.23 | 25.5 | 25.7 | 0.058 | 0.17 | 37 | 22 | 41 | Loam |

In this experiment, the natural zeolite after receiving from Afrand Toska Company- Iran was washed by distilled water and after air drying with air passed through a 1 mm sieve. The characteristics of zeolite are shown in Table 2.

**Table 2**. Characteristics of natural zeolite (Azough et al., 2017).

| Parameter | Unit | Value |
|---|---|---|
| pH | - | 8.45 |
| EC | dS/m | 0.097 |
| CEC | Cmol/kg | 170 |
| $SiO_2$ | % | 66.5 |
| $Al_2O_3$ | % | 11.81 |
| $Fe_2O_3$ | % | 1.3 |
| $TiO_2$ | % | 0.21 |
| CaO | % | 3.11 |
| MgO | % | 0.72 |
| $Na_2O$ | % | 0.5 |
| $K_2O$ | % | 3.12 |
| $P_2O_5$ | % | 0.01 |
| (Drop due to combustion) LO | % | 10.06 |

Meanwhile, compost was prepared from cow's fertilizer. Zeolite powder and compost were added to the soil based on the type of treatment and then heavy metals including lead, nickel and arsenic based on 25% higher than agricultural lands contaminant standards i.e 93.7, 137.5 and 50 mg/kg were mixed with the soil, respectively (Totha, 2016). After mixing zeolite, compost and heavy metals, the soils were maintained separately in constant temperature and humidity for two weeks for chemical reactions and then were added to the pots. Chamran cultivar was used for wheat cultivation. In each pot, 10 seeds were planted and after germination, the seedlings were thinned to 5 plants per pot. Irrigation of pots was carried out according to rainfall and weather conditions and the soil moisture content and the pot's soil were maintained within the field capacity range. To maintain the natural conditions of the soil, no herbicides and insecticides and chemical fertilizer were used during the growth period. For determination of lead, nickel and arsenic, at the time of final harvest of plants, the underground and aerial organs of plants including root, stem and grain were sampled and after washing with distilled water the samples were dried for 48 hours in an oven at 60-70°C and then grind. The method of digestion with $HNO_3$, 30% oxygen dioxide and the atomic absorption device (unicam919AA model) were used for investigation of lead, nickel and arsenic concentrations of plant. The data collected was analyzed statistically by using the computer software "Mstatc". Duncan's multiple range tests at alpha level 5% was computed to compare the significant differences among means.

## Results and Discussion

### Lead concentration

The screening of lead investigated in root, stem and grain showed that the effect of zeolite, compost and interaction of zeolite and compost were significant (Table 3). The maximum lead in root, stem and grain were obtained under non-application of zeolite and compost (control) and the minimum was under 1.5% zeolite along with 1.5% compost. In this study, Pb reduction in root, stem and grain under 1.5% zeolite and compost were 93.7%, 93.1% and 96.1% as compared to control, respectively (Table 4). Also, results showed that the distribution of lead in different parts of the plant was not the same. The maximum accumulation of Pb was in root and the minimum was in grain (Table 4). Keller et al. (2005) stated that zeolites are used in the adsorption of heavy metals from water and soil due to their high selectivity to some cations. Shi et al. (2009) cited the reason for the decrease in lead uptake by converting available forms of lead into inaccessible forms under zeolite application. Also, Hasanabadi et al. (2015) suggested to the reduction of lead in annual Alfalfa in contaminated soil under zeolite application. Concerning the effect of compost application on heavy metal uptake, it has been suggested that the addition of organic matter to the soil reduces the availability of lead by increasing the adsorption sites in the solid part of soil (Chirakkara and Reddy 2015). The results of experiment showed that uptake and translocation of lead to the shoot depend on its amount in the soil and increasing of zeolite and compost in the soil, reduced the concentration of lead in the shoot. The results also showed that lead translocation from root to grain is low

**Table 3.** Mean square (MS) and standard deviation (SD) of Lead ( Pb) , Nickel (Ni) and Arsenic (As) concertation in different parts of wheat under application of zeolite and composted cow manure.* and **, significant at the P value of 0.05 and 0.01 probability level, respectively. CV indicate, coefficient of variation.

| Source of Variance | df | Pb | | | | | | Ni | | | | | | As | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Root | | Stem | | Grain | | Root | | Stem | | Grain | | Root | | Stem | | Grain | |
| | | MS | SD | MS | SD | MS | SD | MS | SD | MS | SD | MS | SD | MS | SD | MS | SD | MS | SD |
| Compost | 3 | $^{**}$6.125 | 2.47 | $^{**}$2.562 | 1.60 | $^{**}$0.022 | 0.14 | $^{**}$42.73 | 6.53 | $^{**}$423.57 | 20.5 | $^{**}$162.45 | 12.5 | $^{**}$51.44 | 7.15 | $^{**}$32.78 | 5.72 | $^{**}$0.021 | 0.14 |
| Zeolite | 3 | $^{*}$1.467 | 1.21 | $^{**}$0.203 | 0.45 | $^{**}$0.021 | 0.14 | $^{**}$3.74 | 1.93 | $^{**}$37.14 | 6.09 | $^{**}$34.73 | 5.89 | $^{**}$16.42 | 4.05 | $^{**}$8.72 | 2.95 | $^{**}$0.011 | 0.10 |
| Compost * Zeolite | 9 | 0.428$^{*}$ | 0.65 | $^{**}$0.027 | 0.16 | $^{**}$0.043 | 0.20 | $^{**}$0.17 | 0.42 | $^{**}$7.64 | 2.76 | $^{**}$11.63 | 3.41 | $^{**}$5.97 | 2.44 | $^{**}$4.32 | 3.07 | $^{**}$0.23 | 0.47 |
| Error | 32 | 0.359 | 0.59 | 0.203 | 0.45 | 0.002 | 0.04 | 0.04 | 0.20 | 2.33 | 1.52 | 0.441 | 0.66 | 0.10 | 0.31 | 0.08 | 0.38 | 0.0003 | 0.01 |
| CV% | | 13.01 | | 11.83 | | 9.65 | | 9.9 | | 8.09 | | 10.88 | | 11.94 | | 10.08 | | 12.72 | |

**Table 4.** Mean value and standard error of Lead (Pb), Nickel (Ni) and Arsenic (As) concertation in different parts of wheat under application of zeolite and composted cow manure. Means with different letters are significantly different at P = 0.05 probability level, using Duncan's Multiple Range Test.

| Treatments | | Means | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Compost (%) | Zeolite (%) | Pb (mg/kg) | | | Ni (mg/kg) | | | As (mg/kg) | | |
| | | Root | Stem | Grain | Root | Stem | Grain | Root | Stem | Grain |
| 0 | 0 | a 5.41±0.13 | a 1.433±0.02 | a 0.111±0.01 | a 21.40±3.01 | a 16.90±3.02 | a 6.963±0.64 | a 12.49±0.21 | a 8.437±0.13 | a 0.096±0.0006 |
| | 0.5 | b 3.63±0.09 | b 1.250±0.05 | ab 0.105±0.02 | b 15.30±2.02 | b 9.183±1.03 | b 2.537±0.23 | b 7.038±0.11 | b 3.695±0.09 | b 0.077±0.0004 |
| | 1 | bc 3.23±0.10 | c 1.027±0.05 | ab 0.100±0.01 | bc 14.42±2.03 | bc 8.347±1.00 | bc 2.000±0.12 | bc 6.741±0.09 | bc 3.367±0.08 | c 0.063±0.0005 |
| | 1.5 | 2.6±0.07 de | 0.936±0.02 c | b 0.097±0.01 | bcd 11.85±1.07 | def 6.500±0.95 | cde 1.470±0.09 | def 5.670±0.07 | cd 2.708±0.01 | de 0.044±0.0003 |
| 0.5 | 0 | cd 2.88±0.05 | c 0.92±0.08 | c 0.083±0.01 | bcd 11.93±2.02 | cd 7.140±0.85 | bcd 1.907±0.05 | cd 6.034±0.08 | cd 2.923±0.05 | d 0.046±0.0005 |
| | 0.5 | de 2.86±0.07 | 0.793±0.25 d | d 0.068±0.01 | cd 11.04±2.05 | de 6.730±0.55 | cde 1.580±0.07 | de 5.823±0.06 | cd 2.709±0.04 | e 0.032±0.0002 |
| | 1 | def 2.60±0.08 | e 0.596±0.03 | e 0.048±0.01 | de 10.31±1.95 | def 6.050±0.65 | cdef 1.230±0.05 | def 5.475±0.03 | de 2.464±0.03 | f 0.017±0.0002 |
| | 1.5 | efg 2.25±0.10 | ef 0.47±0.09 | ef 0.036±0.004 | def 9.073±1.03 | efg 5.417±0.46 | defg 1.101±0.01 | efg 5.225±0.03 | def 2.205±0.01 | fg 0.011±0.0003 |
| 1 | 0 | fg 2.15±0.11 | fg 0.363± 0.02 | fg 0.025±0.005 | efg 7.047±1.01 | fg 5.003±0.43 | efgh 0.896±0.03 | efg 5.181±0.02 | efg 1.829±0.009 | fg 0.009±0.0002 |
| | 0.5 | gh 2.03±0.09 | gh 0.330±0.02 | gh 0.020±0.006 | fgh 6.253±0.99 | gh 4.410±0.36 | efgh 0.626±0.02 | fgh 4.911±0.01 | fgh 1.613±0.006 | fg 0.007±0.0001 |
| | 1 | gh 1.83±0.02 | ghi 0.263±0.01 | gh 0.014±0.004 | ghi 5.393±0.55 | ghi 3.840±0.12 | fgh 0.296±0.07 | ghi 4.605±0.01 | gh 1.374±0.003 | fg 0.006±0.0001 |
| | 1.5 | hi 1.60±0.03 | hij 0.213±0.01 | gh 0.013±0.009 | ghi 4.757±0.67 | hij 2.243±0.09 | gh 0.191±0.03 | hij 4.673±0.02 | gh 1.213±0.003 | fg 0.005±0.0001 |
| 1.5 | 0 | ij 1.27±0.03 | ij 0.170±0.01 | gh 0.013±0.008 | hij 2.632±0.39 | ijk 2.367±0.06 | gh 0.068±0.006 | ijk 3.851±0.03 | h 1.006±0.002 | fg 0.003±0.0007 |
| | 0.5 | ij 1.13±0.04 | j 0.130±0.02 | h 0.010±0.003 | hij 2.803±0.43 | jk 2.153±0.04 | gh 0.070±0.005 | jk 3.3778±0.01 | h 0.951±0.001 | fg 0.033±0.0004 |
| | 1 | j 1.03±0.02 | j 0.113±0.01 | h 0.006±0.002 | ij 2.203±0.12 | kl 1.570±0.03 | gh 0.051±0.003 | k 3.397±0.02 | h 0.907±0.001 | g 0.002±0.0003 |
| | 1.5 | k 0.67±0.01 | j 0.090±0.01 | h 0.007±0.003 | j 0.396±0.04 | l 0.090±0.008 | h 0.0086±0.0006 | l 0.993±0.005 | i 0.0996±0.002 | g 0.001±0.0001 |

and depends on the amount of adsorption by plants and due to the percentage of zeolite and compost applied. Accumulation of lead in the roots relative to the grain is important because the roots prevent the transfer of lead to the grain and prevent the increase of grain contamination by lead. According to the National Institute of Standards and Industrial Research, the maximum amount of lead in wheat that does not cause human adverse effects is 0.15 mg/kg (Zafarzadeh et al., 2018). Therefore, it seems that in the contaminated soils, application of zeolite and compost by 1.5% is effective in reducing the contamination below standards level, especially in the grain.

### Nickel concentration

Results for nickel concentration in different parts of the plant showed that zeolite and compost inhibited significantly uptake and translocation of nickel in wheat (Table 3). The maximum concentration of nickel in root, stem and grain were obtained under non-application of zeolite and compost and the minimum was in 1.5% zeolite along with 1.5% compost. The reduction of nickel in root under application of 1.5% zeolite and compost were 98.18%, 99.9% in the stem and 99.8% in the grain (Table 4). Erdem (2004) in their research suggested to removal nickel under zeolite application due to its high cation exchange capacity and the deposition of insoluble phases. Boros-Lajszner (2017) also obtained similar results in oat under zeolite application. The results also showed that compost applications were effective in reducing plant nickel contamination. Singh et al. (2010) also suggested to the reduction of heavy metals in the sugar beet shoots under compost application. Liu et al. (2009) point to similar results regarding the effect of compost on the reduction of heavy metal concentrations in roots and shoots of wheat. Results of this experiment confirmed that compost and zeolite consumption decreased nickel concentration in the root. Meanwhile, the accumulation of nickel in the root prevents them from being transferred to the shoot. Comparison of nickel concentration in wheat samples with world standard of food (1.63 mg/kg) (Rast Manesh 2018) showed that the application of zeolite and compost can reduce nickel concentration especially in wheat grain and can be stored at minimized levels to consumption risk by entering the food chain.

### Arsenic concentration

In this research, the effect of zeolite, compost and interaction between zeolite and compost on the arsenic concentration of root, stem and grain were significant (Table 3). The maximum concentration of arsenic in root, stem and grain were obtained under non-application of zeolite and compost and the minimum was under application of 1.5% zeolite along with 1.5% compost (Table 4). Arsenic reduction in root, stem and grain under 1.5% zeolite and compost application as compared to non-application of them were 92%, 98.8% and 98.9%, respectively. Azough et al. (2018) also obtained similar results regarding the reduction of arsenic contamination in wheat under zeolite application conditions. The reduction of arsenic after zeolite application was because of alkaline pH and high cation exchange capacity of zeolite, which adsorbs and incorporates some metals into its network (Macedo-Miranda and Olguin 2007). It has been reported that arsenic to be strongly adsorbed on zeolite anionic sites by the ligand exchange phenomenon (Macedo-Miranda and Olguin 2007; Guanna and Evgeny 2018). Overall, the negative charge of the anions is counteracted by the cations in the building and the channels and thus can be replaced by arsenic ions. Results for compost application on arsenic uptake showed a reduction in different parts of the plant. Some researchers have suggested increasing adsorption sites and reduction of arsenic availability under organic fertilizer application (Chirakkara and Reddy 2015). Recently, it has been suggested that metal cations such as $Al^{+3}$, $Fe^{+3}$ and $Fe^{+2}$ act as a bridge between arsenic and organic matter application, which reduces the availability and mobility of arsenic (Silvetti et al., 2017). In this regard, Yassen et al., (2007) have also pointed to a reduction of arsenic concentrations and their detrimental effects under the application of organic fertilizers. The results also showed that the maximum arsenic was in root and then was in stem and grain and with increasing of zeolite and compost percentage in the soil the arsenic concentration in different parts of plant decreased. It has been stated that the maximum amount of arsenic in human food is 0.15 mg/kg (Esmaeili Sari 2004). Therefore, the zeolite and compost application can reduce the concentration of arsenic, especially in the grain below the permissible level which is closely linked to human health.

## Conclusion

In general, the results showed that the roots of wheat had a high ability to absorb lead, nickel and arsenic from the soil contaminated. The maximum accumulation of lead, nickel and arsenic were in root and then was in stem and grain, respectively. The results also showed that the application of zeolite and compost application in the soil contaminated can be useful in controlling and reducing their uptake in the plant. In this experiment, the maximum reduction of lead, nickel and arsenic was observed under 1.5% zeolite and compost application especially in grain and make wheat grain consumable. So it can increase food safety and food security.

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