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Effect of Clinoptilolite and Heavy Metal Application on Some Physiological Characteristics of Annual Alfalfa in Contaminated Soil

Tahereh Hasanabadi*, Shahram Lack*, Mohammad Reza Ardakani**, Hosein Ghafurian and Adel Modhej*******

**Department of Agronomy, College of Agriculture,*
Khouzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran
***Department of Agronomy and Plant Breeding,*
College of Agriculture, Karaj Branch, Islamic Azad University, Alborz, Iran
****Department of Environmental Chemistry,*
North Tehran Branch, Islamic Azad University, Tehran, Iran
*****Department of Agronomy, College of Agriculture,*
Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

(Corresponding author: Shahram Lack)
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ABSTRACT. Heavy metal pollution in air and agricultural soils is one of the most important ecological problems in the whole world. In this experiment, the effect of zeolite adsorbents in five levels and two heavy metals (lead and cadmium, each one in four levels) was tested on heavy metals absorption of *Medicago scutellata* L. The experiment was conducted in factorial in the form of a randomized complete design with three replications and two treatments, in 2013, at the Agriculture and Natural Recourse Research Center of Islamic Azad University, Karaj, Iran. Results indicated that the best biomass was related to applying the highest rate of zeolite and increased 65% compared with the control. Results of this study indicated that zeolite was able to decrease the amount of uptake and transmission of lead and cadmium in plant and with decreasing the harmful effects of these elements cause to increase the growth traits, protein and uptake of nutrient in plant. So, zeolite can be used in order to decrease heavy metals uptake such as lead and cadmium and also improvement of growth of plants in polluted areas.

Keyword: cadmium, lead, *Medicago scutellata* L., yield, zeolite.

INTRODUCTION

The release of heavy metals into the environment is a potential threat to water and soil quality as well as to plant, animal and human health. As heavy metal elements cannot be destroyed chemically, therefore, approaches to treat metal-contaminated waters and soils must aim at methods to reduce the availability of polluting heavy metals, e.g. by precipitation of insoluble compounds, sorption to solids or extraction. Heavy metals, especially those acting as soft Lewis acids like Hg, Pb, and Cd, are known to have a high affinity to sulfur (Nooney *et al.,* 2001). Many toxic heavy metals have been discharged into the environment as industrial wastes, causing serious soil and water pollution (Lin *et al.,* 2002). Pb^{+2}, Cu^{+2}, Fe^{+3}, and Cr^{+3} are especially common metals that tend to accumulate in organisms, causing numerous diseases and disorders (Inglezakis *et al.,* 2003). They are also common groundwater contaminants at industrial and military installations. Numerous processes exist for removing dissolved heavy metals, including ion exchange, precipitation, phytoextraction, ultrafiltration, reverse osmosis, and electrodialysis (Applegate, 1984; Schnoor, 1997). Heavy metals are considered to be the main sources of pollution in the environment, since they have a significant effect on its ecological quality. Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plant species (Peralta, 2000). Among toxic metals, lead and cadmium appear to be the most dangerous to the environment (Shinggu *et al.,* 2007). Lead and cadmium cause toxicity and environmental impact, although this toxicity depends on the concentration and environmental parameters.

The fact that zeolite exchangeable ions are relatively innocuous (sodium, calcium and potassium ions) makes them particularly suitable for removing undesirable heavy metal ions from industrial effluent waters. One of the earliest applications of a natural zeolite was in removal and purification of cesium and strontium radioisotopes (Hafez *et al.,* 1978). Zeolites are a group of highly crystalline hydrated aluminosilicates minerals, that when dehydrated, develop a porous structure with minimum pore diameters of between 0.3 to 1.0 nm.

All zeolites are considered molecular sieve, materials that can selectively absorb molecules based on their size (PeresCaballero *et al.* 2008). This characteristic enables zeolite to act as a suitable substitute to remove toxic cations (Arellano *et al.,* 1995: Inglezakis *et al.,* 2002). Among more than 40 natural zeolites species, clinoptilolite is the most abundant zeolite in soils and sediments (Ming and Dixon, 1987) and seems to be the most efficient ion exchange and ion selective material (Nava *et al.,* 1995) for removing and stabilizing heavy metals (Echeverria *et al.,* 1998). Several authors have reported the effect of clinoptilolite application on plant yield improvement (Castaldi *et al.,* 2005; Baikova and Semekhina, 1996; Loboda, 1999), decrease in fertilizer requirement (Loboda, 1999), immobilization of heavy metals in contaminated soils (Oste *et al.,* 2002; Rehakova *et al.,* 2004), and reduction of heavy metals uptake by plant roots (Chen *et al.,* 2000; Gworeke, 1992).

MATERIALS AND METHODS

This research was conducted in three different and supplementary experiments in 2013. The experiments were conducted in factorial in the form of a completely randomized design with three replications, under greenhouse conditions. First of all, soil samples were analyzed in laboratory to make sure they are lead and cadmium free. Then, soil lumps were grinded and passed through a 4 mm sieve. The soil was polluted with the pollutants at higher rates than the allowed limit. The polluted pots were kept at this position for 30 day to fix the chemical condition of soil and reach a uniform soil mixture.

Then, treatments were applied to the pots and seeds of *Medicago scutellata* L. were cultivated. To evaluate the effect of treatments on the plant, sampling was conducted at the beginning of flowering stage. At this time, the whole plant was harvested, washed with deionized water and dried in 70°C oven for plant analysis and measurement of heavy metals in plant tissue. The properties of the used zeolite are listed in Table 1. This experiment was conducted in three replications with two factors. The first factor was natural zeolite with nanopores in five levels including 0, 30, 60, 90 and 120 g zeolite / 21000 g soil (the full capacity of pots was 30 kg). The size of nanoporous of this zeolite was 0-3 mm. The second factor was the heavy metal pollutants in four levels including (1) a pollutant-free control, (2) lead in the form of 99% lead nitrate at the rate of 400 mg lead in one kg dry soil, (3) cadmium at the rate of 8 mg cadmium in one kg dry soil, and (4) cadmium + lead. Nearly 3 kg sand was poured at the bottom of each pot for drainage. Irrigation was also conducted from the bottom of pots. Total N was determined by a micro-Kjeldahl method (Sparks, 1996). The root length (cm) was measured as described by Tennant (1975). Finally, analysis of variance was conducted using SAS and means were compared according to the Duncan's multiple range test at $P \leq 0.05$.

RESULT AND DISCUSSION

Analysis of variance of the measured traits is given in Tables 2. Mean comparison is also represented in figures 1 to 7. Application of natural zeolite significantly ($P \leq 0.01$) increased protein content, protein yield, biomass, root dry weight and root length.

Table 1: Chemical analysis of the natural zeolite used in this study.

Component	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O
Content (%)	63.74	11.7	1.65	1.18	1.83	0.51	2.44

Table 2: Analysis of variance of the effect of treatments on the measured traits.

		MS				
SOV	**df**	**Biomass**	**Protein yield**	**Protein content** shoot	**Root dry weight**	**Root length**
Pb	1	0.01ns	0.34ns	4.63**	0.22**	2.04ns
Cd	1	0.17**	0.52**	1.22*	0.22**	17.76**
Zeolite	4	0.34**	0.213**	3.18**	0.33**	11.55*
Pb*Cd	1	0.000ns	0.002ns	0.3314ns	0.00ns	0.15**
Pb*Ac	4	0.02ns	0.05ns	7.24**	0.02**	0.33ns
Cd*Ac	4	0.01ns	0.0042ns	0.1567ns	0.022*	16.78**
Z*Pb*Cd	4	0.48*	0.554**	6.654*	0.00ns	11.33*
Error	40	0.01	0.002	0.007	0.0001	0.08
Cv%		11.4	9.9	9.7	13.2	10.61

Ns, not significant; *, 5%; **, 1%.

The highest biomass and root dry weight was achieved when the highest rate of zeolite was applied ($Z_{120}Pb_0Cd_{80}$); indicating that zeolite is an efficient adsorbent for reducing heavy metals translocation to plant tissue in polluted soils. Results indicated that the lowest biomass and root dry weight was related to ($Z_0Pb_{400}Cd_{80}$; Fig. 1, 2, 4. and 5).

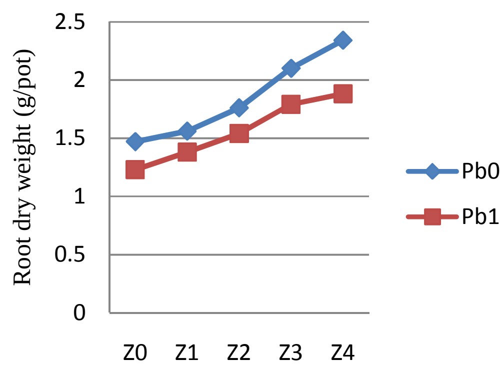

Fig. 1. Effect of zeolite and cadmium on root dry weight

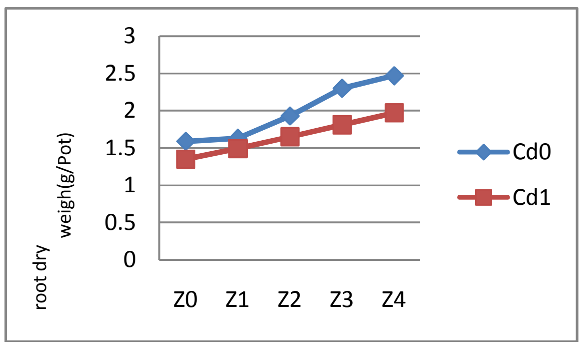

Fig. 2. Effect of zeolite and lead on root dry weight.

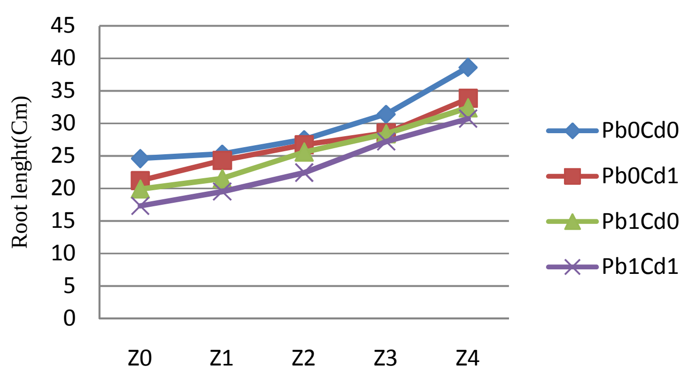

Fig. 3. Effect of zeolite, lead and cadmium on Root length.

The reducing trend of Pb and Cd concentration in alfalfa root and biomass was also observed in the interaction of Pb × Cd; application of higher zeolite rates in treatments containing both Pb and Cd resulted in higher reduction of Pb and Cd in plant root and biomass compared with the treatments containing only Pb and Cd. Zeolite ameliorated the adverse effect of Cd and Pb contamination and decreased the reduction rate of dry weight when compared to treatments that no zeolite was added.

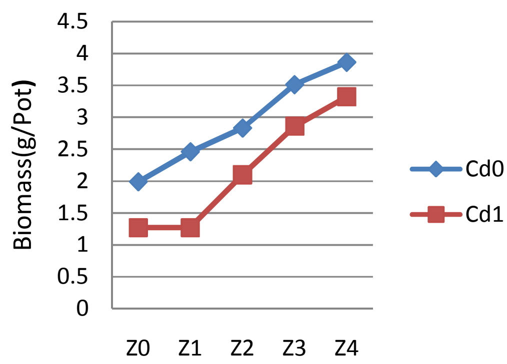

Fig. 4. Effect of zeolite and cadmium on Biomass.

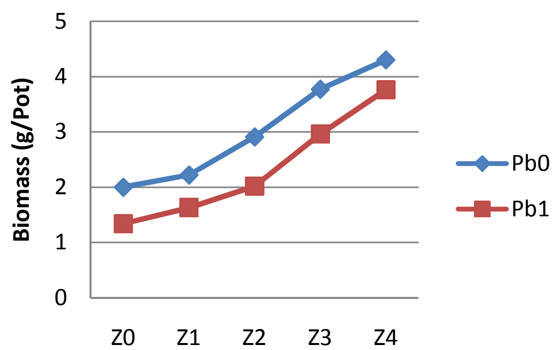

Fig. 5. Effect of zeolite and lead on biomass.

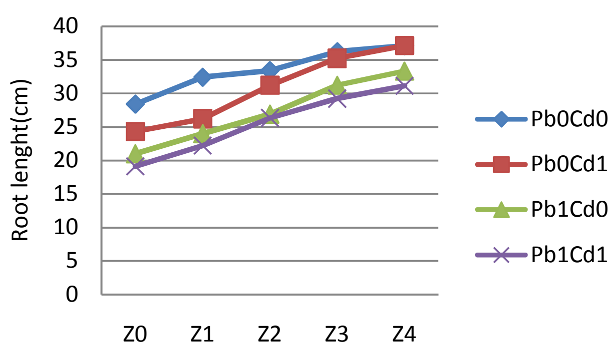

Fig. 6. Effect of zeolite, lead and cadmium on content of protein.

Absorption and retention capacity for major nutrient ions such as K^+ and $NH^{4\ +}$ released from fertilizer, maintenance of adequate water supply and a slow release of micronutrient might be the reason behind this increase. Rehakova *et al.* (2004) reported that between plants grown in contaminated soil, those treated with zeolite had higher growth parameters. Zeolite combined with normal fertilizer greatly enhanced growth and yield of peach and grape trees (Burriesci *et al.*, 1984). In fact bio-available fraction of heavy metals reduces in the presence of zeolite (Chen *et al.*, 2000). Increasing the application rate of zeolite reduced Pb and Cd content in plant root and shoot; this reducing trend was more noticeable in 90 and 120 mg zeolite/kg soil rates. Also using 120 mg zeolite per kg soil produced the highest content of protein and root length. Application of zeolite in all levels except for the control significantly increased traits compared with the control but there were no significant differences between 90 and 120 mg zeolite per kg soil treatment (Fig. 3 and 6).

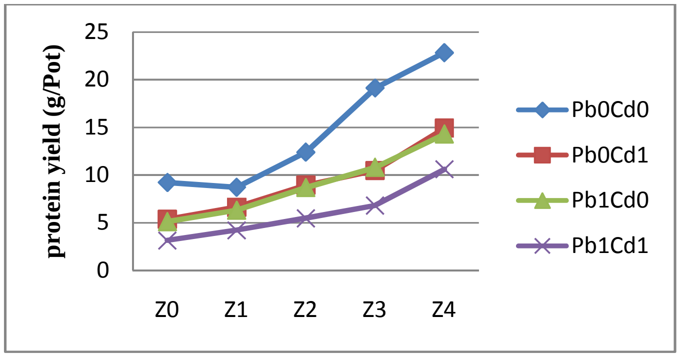

Fig. 7. Effect of zeolite, lead and cadmium on protein yield.

Results showed that the highest protein yield was achieved in $Z_{120}Pb_0Cd_0$ and the lowest was achieved in $Z_0Pb_{400}Cd_{80}$ (Fig. 7). In all zeolite treatment levels reduced the concentration of Pb and Cd in soil compared with the zeolite-free control as a result increased biomass, protein and protein yield. Yasuda *et al.* (1995) reported that the application of zeolite improved nitrogen efficiency in soil by about 16 to 22%. Furthermore, zeolite reduced the leaching of ammonium and nitrate by up to 86 to 99% from the soil. Trinchera *et al.* (2010) reported that secondary roots and the proliferation of root hairs in maize increased with both micronized and granular clinoptilolite substrates where zeolite particles adhere to the root surface, and this result is related to the enhanced solubilization of organic matter and nutrient availability. Shi *et al.* (2009) reported that zeolite fixes heavy metals in soil and prevent them from being absorbed by plants in three stages: at first, the dissolved zeolite produces an alkaline environment in soil, resulting in the deposition of insoluble phases. The newly formed phase contains the heavy metals as the main component. Then, enhancement of alkalinity improves the adsorption of metals by the complex levels. The surface of minerals has positive charge in low pH; when the pH increases, it changes to negative charge which increases cations adsorption in stable complexes with negative bases.

The enhancement of protein content as the result of zeolite application may be attributed to the enhanced absorption of protein and nitrogen peripheral. Moreover, the enhancement of soil moisture improves root system development and promotes the absorption and translocation of nitrogen containing compounds. The especial structure of zeolites such as high porosity, improves soil aeration in long term conditions which is important for plant growth and soil microbial activity. Applying zeolite-like compounds to soil prevents the loss of nutrient from soil through leaching and run-off and results in the enhancement of fertilizers use efficiency, improved nutrient uptake, enhanced water holding capacity and consequently increased plant yield.

CONCLUSION

Results of the present study can be used for predicting the efficiency of zeolite application for the remediation of contaminated soils. Our findings indicated a general negative influence of Cd and Pb contamination on growth parameters but in contrast zeolite could ameliorate the adverse effects of heavy metals such as Pb and Cd and improve the overall growth responses.

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