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Zeolite Ameliorates the Adverse Effect of Cadmium Contamination on Growth and Nodulation of Soybean Plant (*Glycine max* L.)

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ABSTRACT

In present study effect of cadmium (Cd) contamination and Zeolite presence in growth media on soybean plants (*Glycine max* L.) was evaluated. The experiment was conducted as a 3x3 factorial design in a green house with 3 replications. Different levels of zeoilte (0, 2 and 5 g kg^{-1} soil) and Cd (0, 10 and 25 mg kg^{-1} soil) were added and mixed with soil thoroughly. The seeds of soybean plants cv. 'Williams' were inoculated. Results indicated that Cd contamination reduced shoot and root dry weight, number and weight of nodules and also N, P, K, Fe, Cu and Mn content in plant tissues in contrast, zeolite application had promoting influence on shoot and root dry weight in both cases of Cd presence and absence. It also mitigates the negative impact of Cd contamination on mineral elements content of shoots. Application of zeolite reduced the Cd accumulation within plants. Our findings demonstrated a general negative effect of Cd presence in growth media of plants and a beneficial and promoting influence of zeloite application on plant growth. In fact zeolite has the ability of reducing the adverse effects of Cd contamination.

Key Words: Cd, dry weight, shoot and root, concentration, nodulation, soybean.
Running Title: mitigating effect of Zeolite

INTRODUCTION

Cd is a non-essential element that can be highly phytotoxic. However, average concentration of Cd in soil and plant can be quite low. This element has deleterious impacts on soil quality and mineral nutrition cycle .Of all toxic heavy metals, Cd ranks the highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain. The presence of excessive amounts of Cd in soil commonly elicits many stress symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism and may thus strongly reduce biomass production (John et al. 2008).

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 (Peres-Caballero 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). Of 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). Yield increase resulted from clinoptilolite application observed by several authors (Castaldi et al. 2005 , Baikova and Semekhina 1996 , Loboda 1999), decrease in fertilizer requirements (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) are also reported. The objective of this study was to evaluate the effects of zeolite application on soybean plants grown on Cd contaminated soil in greenhouse conditions.

MATERIALS AND METHODS

The soil used was collected from horizon which is situated at Bajgah Experimental Station of Agriculture College, Shiraz University, Shiraz, Iran. This soil is classified as fine, mixed, mesic, Fluventic Haploxerepts. The physicochemical soil properties were measured by experimental lab method. The zeolite used in this research was obtained from Anzimite Company that its chemical composition is presented in Table 1. Basal fertilizer applied were Urea, Monophosphat Potassium, Fe-EDDHA, Manganeze Sulfate, Zinc Sulfate and Copper Sulfate according to soil analysis. Different levels of of zelite (0, 2 and 5 g zeolite kg^{-1} soil) and Cd

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(0, 10 and 25 mg kg^{-1} soil) were added and mixed with the soils thoroughly. The seeds of soybean cv. 'Williams' were inoculated with *Rhizobium japonicum* then 6 seeds were sown 2 to 3 cm depth into the 3 kg pots, with 3 retained in each pot after thinning. The experiment was conducted as a 3x3 factorial design with 3 replications in a greenhouse with natural light and temperatures of 14±2° C during nights and 18±2° C during days. Plants were harvested 8 weeks after sowing by cutting the shoots (stems and leaves at the soil surface, roots then separated from the soil. At harvest, seedlings were clipped above the cotyledonary node. The roots of each plant were washed and carefully rinsed with water, then nodules were harvested, oven dried at 70°C for 48 h, and weighed to determine the total nodule dry weight per plant. Shoots and roots were washed with tap water rinsed with distilled water, weighed and then dried at 70°c for 48h, and the dry weight was measured. Plant material including shoots and roots were ground using an agate ball mill, and in order to chemical analysis were transferred to laboratory. Nitrogen was measured by Kjeldahl method, phosphorus by Bray-2 and potassium by flame photometer. Dried plant residues were ashed at 550°C in oven, 1g of plant ash was dissolved in 5ml hydrochloric acid (2N), and then passed through Wattman filter paper No.5. A final 50ml of solution was obtained using distilled water. Afterwards the concentration of Fe, Mn, Zn and Cd was determined using a Shimadzo AA-670 atomic absorption spectophotometer (Sparks 1996). Data was statistically analyzed using MSTATC software and means were compared using Duncan's test.

Table 1. Chemical composition of zeolite used in this study

Minerals	quantity	quantity
Potassium	6.52 % K	7.85 % K_2O
Sodium	0.69 % Na	0.93 % Na_2O
Strontium	1.15 % Sr	1.35 % SrO
Calcium	0.06 % Ca	0.08 % CaO
Magnesium	0.16 % Mg	0.27 % MgO
Manganese	0.02 % Mn	0.03 % MnO
Aluminum	6.22 % Al	11.74 % Al_2O_3

RESULTS

Data present in Table 2, show the relation between Cd-contamination and shoot and root dry weight. At maximum level of Cd treatment (25 mg/kg), the shoot dry weight was significantly reduced (48%). Application of zeolite had positive influence on shoot and root dry weight in both cases of Cd presence and absence. There was no significant difference between applications of 2 or 5g/kg zeolite.

According to data in Table 3, plants treated with Cd, had lower N and K concentration in their shoot (an 11% and 38% reduction respectively). In contrast the phosphorus concentration in shoots increased from 4.14 to 5.77g/kg. Application of zeolite ameliorated the inhibitory effect of Cd on accumulation of N and K as concentration of these two elements increased from 0.93% and 1.66% in control plants to 1.40% and 2.24% respectively, in treatments to which 5g/kg zeolite was added. The similar augmenting holds for P concentration as it increased from 4.50 g/kg to 6.03 g/kg in plants to which 5g/kg zeolite was applied.

Cd application increased shoot and root Cd concentrations. Diminution of Cd concentration occurred after zeolite application, as it has indicated in figures 1 and 2. Concentration of Cd in roots was 20 folds higher than above ground parts.

A reduction occurred in Fe, Zn, Cu and Mn concentration of soybean shoots treated with Cd. A diminution of Fe, Zn, and Mn concentration was observed in roots as well. Oppositely, zeolite application increased Fe, Zn, Cu and Mn concentration in shoot and root parts (Tables 4 and 5). Highest concentration level of these micro elements was observed in plants that were treated with 5g/kg zeolite.

Data present in Table 6, indicate that Cd-application in all concentrations reduced weight and number of nodules in roots, in contrast zeolite treatments increased both parameters significantly.

Table 2. Effect of Cd and zeoilte on shoot and root dry weight (g plant $^{-1}$)

Cd level (mg Cd/kg soil)	Zeolite level (g zelolite/kg soil)			
	0	2	5	**Mean**
		Shoot		
0	1.99 bc	2.13 ab	2.23 a	**2.12 A**
10	1.44 e	1.69 d	1.85 cd	**1.65 B**
25	1.02 f	1.23 ef	1.39 e	**1.21 C**
Mean	**1.48 B**	**1.69 A**	**1.82 A**	
		Root		
0	0.90 cde	1.02 bcd	1.23 abc	**1.05 AB**
10	074 de	1.51 a	1.37 ab	**1.21 A**
25	0.53 e	0.82 cde	1.17 abcd	**0.84 B**
Mean	**0.72 B**	**1.11 A**	**1.26 A**	

Means followed by the same letters in each column and each row (small letter for means and capital letters for means of columns and rows) are not significantly different at $P \leq 0.05$.

Table 3. N, P and K concentrations of shoots in plants treated with zeolite and Cd.

Cd level (mg Cd/kg soil))	Zeolite level (g zeolite/kg soil)			
	0	2	5	**Mean**
		N (%)		
0	1.08 cd	1.12cd	1.39 ab	**1.19 A**
10	0.97 de	1.21bcd	1.52 a	**1.23 A**
25	0.76 e	1.09 cd	1.30abc	**1.05 B**
Mean	**0.93 C**	**1.14 B**	**1.40 A**	
		P (g/Kg Soil)		
0	3.54 b	3.56 b	5.32 ab	**4.14 B**
10	4.54 ab	4.61 ab	6.25 a	**5.13AB**
25	5.43 ab	5.36 ab	6.52 a	**5.77 A**
Mean	**4.50 B**	**4.51 B**	**6.03 A**	
		K(%)		
0	1.94 abc	2.33 ab	2.89 a	**2.39 A**
10	1.85 bc	1.67 bc	2.29 ab	**1.94AB**
25	1.20 c	1.70 bc	1.55 bc	**1.48 B**
Mean	**1.66 B**	**1.90 AB**	**2.24 A**	

Means followed by the same letters in each column and each row (small letter for means and capital letters for means of columns and rows) are not significantly different at $P \leq 0.05$.

Table 4. Average shoot concentration of Fe, Zn, Cu and Mn (mg/kg DW) in soybean plants treated with zeolite and Cd

Cd level (mg Cd/kg soil)	Zeolite level (g zeolite/kg soil) 0	2	5	**Mean**
		Fe(mg/Kg DW)		
0	58.10 a	60 a	59.05 a	**59.05 A**
10	56.92 a	54.12 a	55.82 a	**55.62 A**
25	31.32 b	32.64b	33 b	**32.32 B**
Mean	**48.78A**	**48.92A**	**49.29 A**	
		Zn(mg/Kg DW)		
0	23.32 ab	23.25ab	26.33 a	**24.30 A**
10	20.25 b	18.59 b	22.72ab	**20.52 B**
25	19.62 b	18.26 b	18.89b	**18.92 B**
Mean	**21.06 A**	**20.03 A**	**22.64 A**	
		Cu(mg/Kg DW)		
0	23.62 bc	25.41ab	27.14 a	**25.39 A**
10	21.31de	22.98cd	24.40bc	**22.90 B**
25	20.20 e	22.59cd	23.40bcd	**22.06 B**
Mean	**21.71 C**	**23.66 B**	**24.98 A**	
		Mn(mg/Kg DW)		
0	102.9 c	113.1b	129 a	**115 A**
10	84.99de	91.65 d	101.3 c	**92.63 B**
25	74.61f	79.99 ef	88.65 de	**81.08 C**
Mean	**87.52 C**	**94.93 B**	**106.3 A**	

Means followed by the same letters in each column and each row (small letter for means and capital letters for means of columns and rows) are not significantly different at $P \leq 0.05$.

Table 5. Average root concentration of Fe, Zn, Cu and Mn (mg/kg DW) in soybean plants treated with zeolite and Cd.

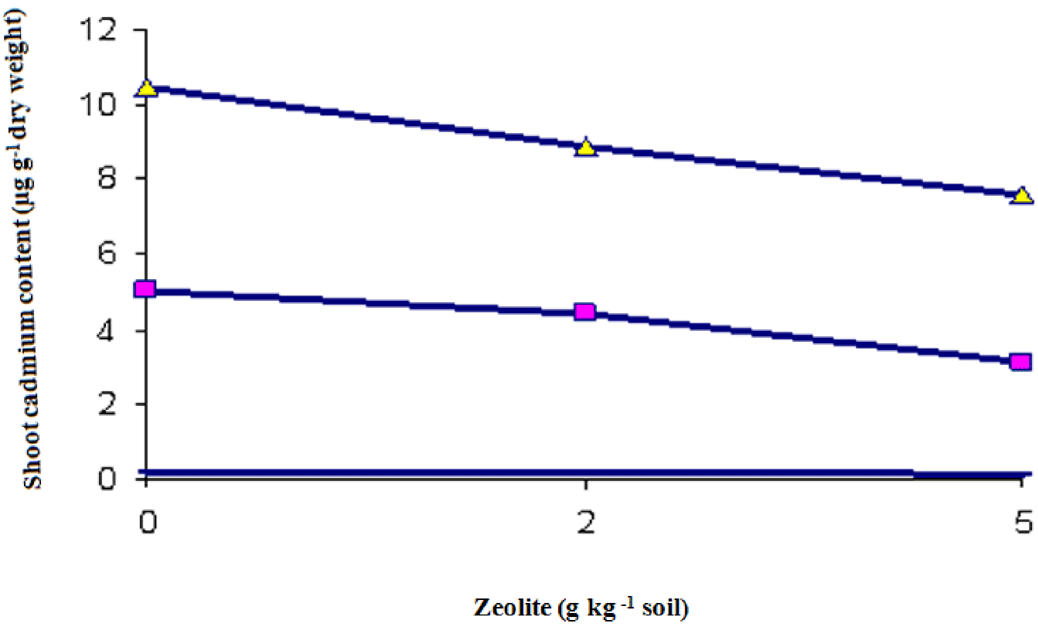
Cd level (mg Cd/kg soil)	Zeolite level (g zelolite/kg soil) 0	2	5	**Mean**
		Fe(mg/Kg DW)		
0	577.8 a	566.6 a	578.6 a	**574.3 A**
10	494.6ab	550.6ab	524.6 ab	**529.3 A**
25	406.6ab	367.9 b	373.3 b	**382.6 B**
Mean	**493 A**	**495.1 A**	**498.2 A**	
		Zn(mg/Kg DW)		
0	69.35 ab	73.94 a	76.78 a	**73.36 A**
10	65.29 ab	60.22 b	74.78 a	**66.76 AB**
25	64.98 ab	60.59 b	57.84 b	**61.14 B**
Mean	**66.54 A**	**64.92 A**	**69.80 A**	
		Cu(mg/Kg DW)		
0	103.9 ab	106.9 a	108.1 a	**106.3 A**
10	95.50 ab	101.8 ab	104.6 ab	**100.6 A**
25	93.29 b	106.2 ab	104.3 ab	**101.3 A**
Mean	**97.57 B**	**105 A**	**105.7 A**	
		Mn(mg/Kg DW)		
0	20.40 cd	31.98 c	40.98 a	**34.12 A**
10	27.24 de	29.63 cd	36.95 b	**31.27 B**
25	25.97 e	26.79 de	36.91 b	**29.89 B**
Mean	**27.54 C**	**29.47 B**	**38.28 A**	

Means followed by the same letters in each column and each row (small letter for means and capital letters for means of columns and rows) are not significantly different at $P \leq 0.05$.

Table 6. Dry weight and nodules number of roots in soybean plants treated with zeolite and Cd.

Cd level (mg Cd/kg soil)	Zeolite level (g zelolite/kg soil) 0	2	5	**Mean**
		Nod number		
0	37.33 b	40 b	48.33 a	**41.89 A**
10	24.67d	29.67 cd	34.67 bc	**29.67 B**
25	13.33 e	17 e	17.33 e	**15.89 C**
Mean	**25.11 B**	**28.89 B**	**33.44 A**	
		Nod dry weight (mg)		
0	109.1 cd	138.6 b	170.5 a	**139.4 A**
10	86.58 d	103.2 cd	112 c	**100.6 B**
25	42.48 e	54.84 e	60.97 e	**52.76 C**
Mean	**79.41 C**	**98.92 B**	**114.52 A**	

Means followed by the same letters in each column and each row (small letter for means and capital letters for means of columns and rows) are not significantly different at $P \leq 0.05$.



Δ Cd= 25 mg Cd/kg soil   y = -1.45x + 11.87   R2 = 0.9964
□ Cd= 10 mg Cd/kg soil y = -0.955x + 6.0967 R2 = 0.9536
- Cd= 0 mg Cd/kg soil y = -0.04x + 0.2533   R2 = 0.9796

Figure 1. Zeolite application effect on Cd concentration of shoots

ΔCd= 25 mg Cd/kg soil y = -28.1x + 212.97 R2 = 0.9809
□ Cd= 10 mg Cd/kg soil y = -17.485x + 107.79 R2 = 0.995
- Cd= 0 mg Cd/kg soil y = -0.36x + 5.6367 R2 = 0.0886

Figure 2. Zeolite application effect on Cd concentration of roots

DISCUSSION

Shoot dry weight of plants reduced to almost half as Cd application rate increased to higher levels in the absence of zeolite, Khan and Khan (1983) reported similar results. Zeolite ameliorated the adverse effect of Cd contamination and decreased the reduction rate of dry weight when compared to treatments that no zeolite was added. 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. As 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).

Our data indicated an inhibitory effect of Cd contamination on N and K uptake and an ameliorating impact of zeolite on this negative influence of Cd presence. In tomato plants treated with zeolite, total yield increased about 50%. It has been suggested that presence of Fe and K, released from zeolite had beneficial and promoting influence on tomato plants growth and yield (Valente et al. 1982). Supapron et al (2002) demonstrated that application of 125 and 250 kg/ha zeolite caused a 16% increase of soil nitrogen content also similar increase was observed in soil phosphorus content. Natural zeolite has been used for a long time in Japan to improve ammonium retention and therefore better nitrogen management (Dwyor and Dyer 1984).

Cd presence in growth media of plants caused a significant increase in Cd concentration accumulated within plants, also Jalil et al (1994) reported that, adding 0, 0.5 and 0.1 µM $CdCl_2$ to fertigation solution of three wheat cultivars, increased shoot and root Cd concentration in all cultivars and treatments significantly. Castaldi et al. (2005) reported zeolite can induce an increase of heavy metals fraction in soils, and decrease heavy metals uptake. Zeolite reduced the extractability of Cd in soil and significantly Changes in extractability and metal sequential fractions indicated that available form of Cd in soil can be transformed into unexchangeable forms after zeolite application (Chen et al. 2000). Results of another study on lettuce carried out by Gworek (1992) demonstrated up to 86% reduction of Cd accumulation in leaves after zeolite application. Similar results were obtained by Chlopecka and Adriano (1997) on maize. Rebedea and Lepp (1994) used synthetic zeolite to evaluate metal uptake by plants under field and greenhouse conditions. The pot experiement showed that zeolites significantly reduced metal uptake in polluted soils. Zeolites as natural cation exchangers are suitable substitutes to remove toxic cations (Inglezakis et al. 2002). The root Cd concentration was 20 folds higher than shoot sections because most of absorbed Cd remains in root parts (Zornoza et al 2002, Castaldi et al 2005).

Our results indicated that Cd treatment had negative influence on uptake of Fe, Zn, Cu and Mn, this was in accordance with observation made by Jalil et al (1994) who reported that a reduction in Mn and Zn in wheat root and shoot and also Cu and Fe decrease in shoot occurred when plants were treated by 0.1, 0.5 and 2 μM Cd. Zeolite ameliorated inhibitory effect of Cd treatment on micronutrient uptake. Also previously conducted investigations showed similar positive effect of zeolite in this respect. Rehakova et al (2004) observed the lowest content of heavy metals in plants treated by zeolite in contaminated soil. Their results imply that natural zeolite is effective in improving soil properties, also the intake of Cd from soil decrease significantly. The results of Cd application on micro and macro element uptake can be variable depending on conditions and cultivars. Synergistic (Smith and Breman 1983) and antagonistic responses (Abdel-Sabur et al. 1988, Bjerre and Schierup 1985) have been reported.

Data presented in Table 7, show negative effect of Cd contamination on weight and number of root nodules in contrast zeolite application had promoting effect on both these features regardless of Cd presence or absence in growth media of plants. Toxicity of Cd and/or inhibitory effect of this heavy metal on micro nutrients and also N and K uptake may be two reasons for this decrease, zeolite can act as a barrier against these negative characteristics of Cd contamination and mitigate the adverse influence of Cd in growth media.Our findings indicated a general negative influence of Cd contamination on growth parameters but in contrast zeolite could ameliorate adverse effects of heavy metals such as Cd and improve overall growth responses. However, since studies focused on effects of Cd- contamination and zeolite application and also interaction between them are relatively rare, further investigations seem necessary.

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