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SHORT NOTE [NOTA CORTA]

EFFECTS OF ZEOLITE AND CADMIUM ON GROWTH AND CHEMICAL COMPOSITION OF SOYBEAN (*Glycine max* L.)

[EFECTO DE LA ZEOLITA Y CADMIO SOBRE EL CRECIMIENTO Y COMPOSICIÓN QUÍMICA DE LA SOYA (*Glycine max* L.)]

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SUMMARY

There are areas in the world which are polluted by trace metals some of which may not be degraded by biotic process. Some of these metals might enter into surface and/or underground water resources thus causing serious human and animal health problems. In recent years, natural amendments, such as the use of zeolite, have been widely used to address trace metals contamination. In the present study the effect of zeolite on the growth and nodulation of soybean (*Glycine max* L.) was evaluated. Treatments consisted on factorial combination of three levels of zeolite (0, 2 and 5 g kg^{-1}) and three levels of cadmium (0, 10 and 50 mg kg^{-1}). Cadmium application significantly decreased shoot and root dry weight while its concentration in plant parts was increased. In addition, cadmium application decreased number and dry weight of nodules, and N, K, and Mn concentrations. On the other hand, zeolite application markedly increased number and dry weight of nodules and N, P, K concentrations in shoot, Mn and Cu concentrations in shoot and root. The results from the present study can be used for predicting the efficiency of zeolite application for the remediation of contaminated soils.

Key words: Zeolite; cadmium toxicity; soybean growth.

RESUMEN

Existen areas que se encuentran contaminadas por metales traza y algunos de ellos no son degradados por procesos bioticos. Algunos de estos metales pudieran ingresar a la corteza y/o los recursos acuíferos causando problemas serios de salud humana y animal. En los años recientes la zeolita ha sido empleada como recurso para reparar la contaminación por estos metales. En el presente estudio se evaluó el efecto de la zeolita sobre el crecimiento y nodulación de la soya (*Glycine may* L.). Se empleó un diseño factorial con tres niveles de zeolita (0, 2 y 5 g kg^{-1}) y tres niveles de cadmio (0, 10 y 50 mg kg^{-1}). La aplicación del cadmio redujo de manera significativa el peso seco de los tallos y raíces, al mismo tiempo se incrementó su concentración en las plantas. Adicionalmente, el cadmio redujó el número y peso seco de los nodulos y las concentraciones de N, K y Mn. Por otro lado, la zeolita incremento el número y peso seco de los nodulos y las concentraciones de N, P y K en los tallos, Mn y Cu en los tallos y raíces. Los resultados sugieren que la zeolita puede ser empleada para remediar suelos contaminados.

Palabras clave: Zeolita; toxicidad por cadmio; soya.

INTRODUCTION

Environmental pollution with trace metal is a major problem for human health and environmental quality (Chen *et al*., 1996; Ma and Rao, 1997). Cadmium accumulation in plant has a positive correlation with Cd availability in plants and plant growth inhibition (Greger and Bertell, 1992). In spite of its toxicity, cadmium is widely used in several industrial processes including metallurgical alloying, ceramic manufacturing, electroplating, textile printing and in production of inorganic pigments (Shanbleh and Kharabsheh, 1996). Three major reclamation techniques can be used for metal-contaminated soils which include biological, agriculture engineering, and physicochemical treatments. The biotreatment method is carried out via planting, harvesting and eventually removing non-edible crops in contaminated soils. The problem with this method is that it is time-consuming and fails to prevent pollution expansion (Lin *et al*., 1998). Deep plowing is an agricultural method that disturbs the field and results in the contamination of groundwater in future (Lin *et al*., 1998). Physicochemical technique such as the use of natural clay minerals is another method to extract, stabilize and solidify trace metals (Colella *et al*., 1995).

According to traditional definition, zeolites are hydrated aluminosilicates of alkaline and alkaline-earth minerals (Akbar *et al*., 1999). Their structure is made up of a framework of -$[SiO_4]^{-4}$ and $[AlO_4]^{-5}$ tetrahedron linked to each other's corners by sharing oxygen atoms. The substitution of Si^{+4} by Al^{+3} in tetrahedral sites results in more negative charges and a high cation exchange capacity (Akbar *et al*., 1999; Aleksiev *et al*., 2000). Zeolites, as natural cation exchangers, are suitable substitutes to remove toxic cations (Arellano *et al*., 1995; Mondale *et al*., 1995; Inglezakis *et al*., 2002). Zeolite application enhance plant yield (Castaldi *et al*., 2005), decrease the amount of fertilizer usage, trace metal immobilization (Oste *et al*., 2002a and Rehakova *et al*., 2004) and uptake (Chen *et al*., 1996; Gworek, 1992).

The aim of this study was to investigate the effects of zeolite on Cd toxicity, growth, chemical composition and nodulation of soybean plants.

MATERIAL AND METHODS

This experiment was conducted in a greenhouse in the Department of Horticulture, Shiraz University. Top soil (0-30 cm) layer of Ramjerdi's soil (Fine, mixed, mesic, Fluventic Haploxerepts) was used in this experiment. Soil physicochemical properties were determined using standard methods (Page, Miller, and Keeney 1982). The soil was air dried, crushed and passed through a 2-mm sieve prior to analysis and potting. Treatments consisted of three levels of Zeolite (0, 2 and 5 g zeolite kg^{-1} soil) and three cadmium (Cd) rates (0, 10 and 25 mg Cd kg^{-1} soil) arranged in a factorial manner in a Completely Randomized Design (CRD) with three replicates. The zeolite used in this research was collected from the Firoozkouh mine, northeast of Firouzkooh township (Table 1). Pots were filled with 3-kg air-dried soil thoroughly mixed with the appropriate amounts of zeolite and cadmium. The pots were uniformly fertilized with 50 mg N kg^{-1} as $CO(NH_2)_2$ (1/2 before planting and 1/2 three weeks after planting), 25 mg P kg^{-1} as KH_2PO_4, 5 mg Fe kg^{-1} as Fe EDDHA, 5 mg Zn kg^{-1} as $ZnSO_4.7H_2O$, 5 mg Mn kg^{-1} as $MnSO_4$ and 2.5 mg Cu kg^{-1} as $CuSO_4$. The mixture was subjected to three cycles of the same air dry/ rewetting procedure in order to achieve equilibrium. After preparing the soil, the soybean seeds were inoculated with *Bradyrhizobiumn japonicum* before planting. Six seeds of soybean cv. Williams were sown in each pot and seedlings were thinned to three after 10 days. The pots were irrigated with distilled water to keep soil moisture near the field capacity (FC). Plants were maintained in greenhouse under natural light (>800µmol $m^{-2}.s^{-1}$) at a day temperature of 20±2 °C and night temperature of 11±2 °C and a RH of about 55±5%. Plants were harvested after 8 weeks, plant parts were separated and dried at 65 °C and weighed. The ground plant samples were dry-ashed at 550°C , and analysis were performed in order to determine K by flame photometer, P by using the yellow ammonium-molybdate methods (Murphy and Riley 1962) and Fe, Mn, Zn, Cu and Cd by atomic absorbtion spectrophotometer. Total N was determined by a micro-Kjeldahl method (Sparks 1996). The number and dry weight of root nodules were also determined.

Measured variables included shoot dry weight (SDW), root dry weight (RDW), and Fe, Mn, Zn, Cu and Cd concentrations. The number and dry weight of root nodules were assessed to analyze the variance. Data were analyzed statistically by using MSTATC and Excel software packages.

Table 1. Chemical composition of Firoozkouh zeolite used in the experiment*

Element	%
SiO_2	67.24
Al_2O_3	11.7
Fe_2O_3	0.58
TiO_2	0.42
CaO	3.04
MgO	1.16
Na_2O	1.19
K_2O	1.48
P_2O	No
LOI[a]	13.47
Total	100.43

[a] Loss on ignition
*Faghihian and Kazemian, 1999.

RESULTS AND DISCUSSION

Application of Cd at25 mg Cd kg^{-1} soil resulted in a significant reductionon SDW which was 75% lower than the control treatment (Fig. 1). Zhang *et al*. (2002) reported that addition of 1 mg l^{-1} of Cd to the media significantly reduced SDW and RDW. On the other hand, zeolite application significantly increased SDW and RDW in contrast with Cd effects. Rehakova *et al*. (2004) showed that growing certain agricultural plants in contaminated soils increased with varying dosage of natural zeolite. They noted that the reasons of growth improvement were due to the increased availability of essential nutrient elements such as K, Mg, Ca, NH_4, and micronutrients in presence of zeolite.

Given that the water was maintained at field capacity in all pots, the addition of the zeolite presumably improved plant growth due to increased nutrient availability or decreased Cd toxicity. In our study Cd concentration of root and shoot markedly increased by application of cadmium and strongly decreased as zeolite application rates were increased (Fig. 1).

The highest concentration of Cd in shoot (10.47 $\mu g\ g^{-1}$ dry weight) and root (182.6 $\mu g\ g^{-1}$ dry weight) were obtained when 25 mg Cd kg^{-1} soil was added. Jalil *et al*. (1994) showed that addition of 2µM Cd from $CdCl_2$ source into nutrient solution increased plant parts Cd concentrations in three wheat varieties. In our study the concentration of Cd in roots was 20 times higher than that of shoots. Zornoza *et al.* (2002) and Castaldi *et al*. (2005) noted that more Cd accumulated in roots than shoots by application of this element. Castaldi *et al*. (2005) also reported that the concentration of Cd in the roots and above ground biomass of white lupin plants grown under zeolite-soil composition were 72% and 48% lower than the control sample, respectively.

In addition, Cd application decreased N and K and increased P concentrations (table 2). It is expected that zeolite application reduce N, P and K concentrations due to its dilution effect, however in this study zeolite increased their concentrations from 0.93, 2.50 and 1.66 in control pots to 1.40, 4.30 and 2.24 in 5g zeolite kg^{-1} soil treatment, respectively. Perez-Caballero *et al* (2008) reported the same results. The explanation for this response could be found on the chemical composition of the used zeolite in table 1. Supapron *et al*. (2002) found that zeolite application increased P and total N concentrations of soils.

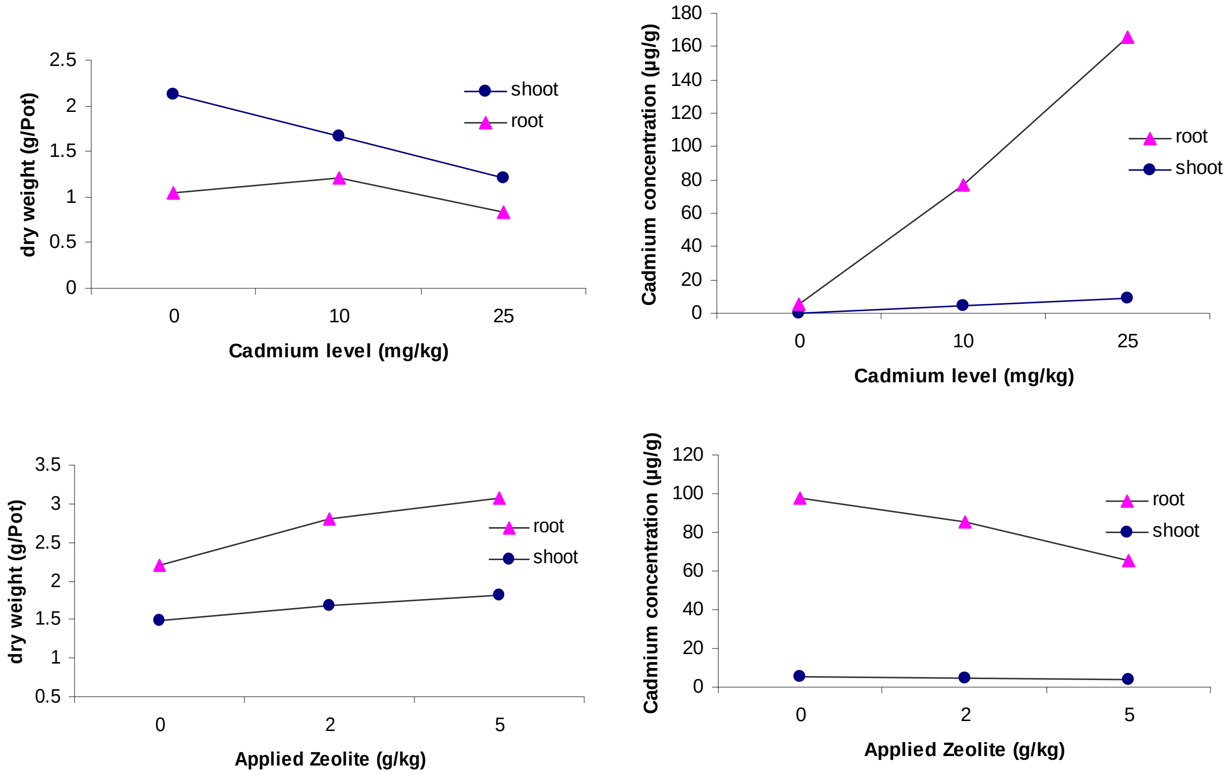

Figure 1. Effects of zeolite and Cd levels on the dry weight (g/Pot) and Cd concentration (µg/g) of soybean plants.

Table 2. Effects of Zeolite and Cd treatments on the N, P and K concentrations in the soybean shoots (g Pot^{-1}).

Cadmium level (mg kg^{-1})	Applied Zeolite (g kg^{-1}) 0	2	5	Mean
		N (%)		
0	1.08cd	1.12cd	1.39ab	1.19A
10	0.97de	1.21bcd	1.52a	1.23A
25	0.76e	1.09cd	1.30abc	1.05B
Mean	0.93C	1.14B	1.40A	
		P (g kg^{-1})		
0	1.54b	1.56b	3.32ab	2.14B
10	2.54ab	2.61ab	4.25a	3.13AB
25	3.43ab	3.36ab	4.52a	3.77A
Mean	2.50B	2.51B	4.03A	
		K (%)		
0	1.94abc	2.33ab	2.89a	2.39A
10	1.85bc	1.67bc	2.29ab	1.94AB
25	1.20c	1.70bc	1.55bc	1.48B
Mean	1.66B	1.90AB	2.24A	

For each parameter, means followed by the same letters in each column and in each row (capital letter) are not significantly different at $P \leq 0.05$.

Cd application significantly increased Fe, Zn and Cu concentrations of shoots.On the other hand, Cd application markedly decreased Fe and Mn concentrations of roots (Tables 3 and 4). Jalil *et al.* (1994) observed that Cd application led to a reduction of Mn and Zn concentrations in plants and increased Cu and Fe concentrations of shoot in wheat plants. Zeolite application significantly increased Cu and Mn concentrations of shoot and root. The highest concentrations of these elements obtained under 5g zeolite kg^{-1} treatment. Gworek (1992) reported that addition of 1% zeolite (based on weight) in lettuce plants reduced Cd concentration by 86%. Chlopecka and Adriano (1997) reported that application of 4g zeolite kg-1 soil significantly decreased Cd uptake by barley and corn. Application of Cd at all the rates led to a reduction in number and dry weight of root nodules. The highest number and dry weight of root nodules were obtained when zeolite was applied at 5g zeolite kg^{-1} soil. Addition of 5g zeolite kg^{-1} soil without Cd resulted in the highest number (48.33) and dry weight (170.5 mg pot^{-1}) of root nodules (Figure 2).

Addition of zeolite increased SDW, N, P, K, Mn and Cu concentrations and the number and dry weight of root nodules in the absence of Cd application. In addition, the highest value for these parameters were obtained when zeolite was applied at 5g zeolite kg^{-1} soil. Zeolite had positive effects on growth and chemical composition of soybean plants. In addition, soybean growth was improved by the addition of zeolite. It is suggested that available Cd (but no total Cd) strongly affected soybean growth. One of the reasons for Cd toxicity in plant is due to the interaction between Cd and essential nutrient elements (Ramos *et al.*, 2002).

Table 3. Effects of Zeolite and Cd treatments on the Fe, Zn, Cu and Mn concentrations in the soybean shoots (mg kg^{-1} dry weight).

Cadmium level (mg kg^{-1})	Applied Zeolite (g kg^{-1}) 0	2	5	Mean
		Fe		
0	31.32b	32.64b	33b	32.32B
10	56.92a	54.12a	55.82a	55.62A
25	58.1a	60a	59.05a	59.05A
Mean	48.78A	48.92A	49.29A	
		Zn		
0	19.62b	18.26b	18.89b	18.92B
10	20.25b	18.59b	22.72ab	20.52B
25	23.32ab	23.25ab	26.33a	24.30A
Mean	21.06A	20.03A	22.64A	
		Cu		
0	20.20e	22.59cd	23.40bcd	22.06B
10	21.31de	22.98cd	24.40bc	22.90B
25	23.62bc	25.41ab	27.14a	25.39A
Mean	21.71C	23.66B	24.98A	
		Mn		
0	102.9c	111.1b	129a	115A
10	84.99de	91.65d	101.3c	92.63B
25	74.61f	79.99ef	88.65de	81.08C
Mean	87.52C	94.93B	106.3A	

For each parameter, means followed by the same letters in each column and in each row (capital letter) are not significantly different at $P \leq 0.05$.

There were many contradictory reports about Cd and its effect on nutrients uptake and concentration. For example, the presence of Cd has increased (Smith and Brenman, 1983) or decreased (Abdel-Sabour *et al.* 1988) Zn uptake. Moreover, addition of Cd into growing media has increased (Sela *et al.*, 1988) or decreased (Bjerre *et al.*, 1985; Khan and Khan, 1983) Fe uptake and concentration in plant parts. Similarly, there were synergistic (Khan and Khan, 1983; Zornoza *et al.*, 2002) or antagonistic (Bjerre *et al.*, 1985) correlation between Cd and other nutrient elements concentrations such as Zn, Cu, and K. Smith and Brenman (1983) reported a synergistic relationship between Zn and Cd concentrations in plants. However Abdel-Sabour *et al.* (1988) showed an antagonistic correlation between Zn and Cd.

Table 4. Effects of Zeolite and Cd treatments on the Fe, Zn, Cu and Mn concentrations in the soybean roots (mg kg^{-1} dry weight).

Cadmium level (mg kg^{-1})	Applied Zeolite (g kg^{-1}) 0	2	5	Mean
		Fe		
0	577.8a	566.6a	578.6a	574.3A
10	496.6Ab	550.6ab	542.6ab	529.3A
25	406.6Ab	367.9b	373.3b	382.6B
Mean	493A	495.1A	498.2A	
		Zn		
0	64.98ab	60.59b	57.84b	61.14B
10	65.29ab	60.22b	74.78a	66.76AB
25	69.35ab	73.94a	76.78a	73.36A
Mean	66.54A	64.92A	69.80A	
		Cu		
0	103.9ab	106.9a	108.1a	106.3A
10	95.50ab	101.8ab	104.6ab	100.6A
25	93.29b	106.2ab	104.3ab	101.3A
Mean	97.57B	105A	105.7A	
		Mn		
0	29.40cd	31.98c	40.98a	34.12A
10	27.24de	29.63cd	36.95b	31.27B
25	25.97e	26.79de	36.91b	29.89B
Mean	27.54C	29.47B	38.28A	

For each parameter, means followed by the same letters in each column and in each row (capital letter) are not significantly different at $P \leq 0.05$.

CONCLUSION

The highest level of zeolite and lowest level of Cd resulted in maximum SDW (2.23 g pot^{-1}) of soybean plants. The results from the present study can be used for predicting the efficiency of zeolite application for the remediation of contaminated soils.

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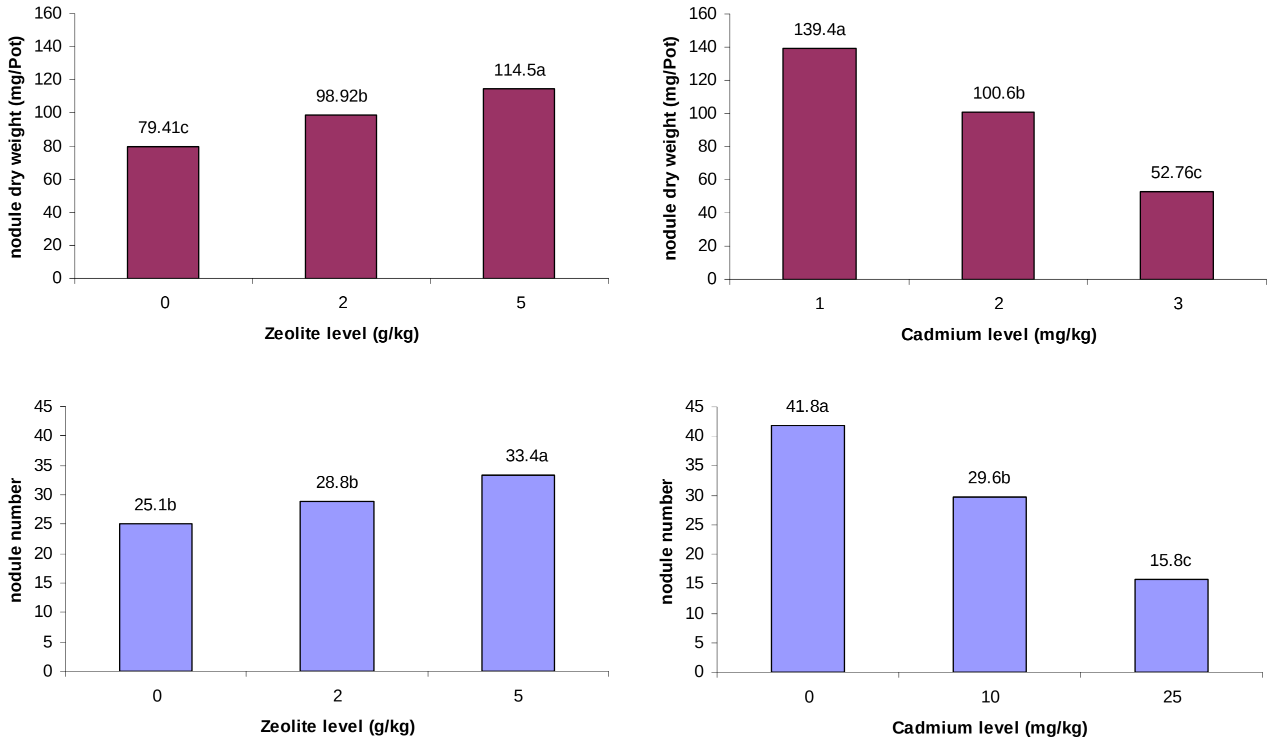

Figure 2. Effects of Zeolite and Cd treatments on the root nodule number and dry weight (mg Pot^{-1}). Bars with the same letter do not differ significantly (*P*= 0.05).

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