Int. J. Environ. Res., 4(3):525-532,Summer 2010
ISSN: 1735-6865

Study of the Enhanced Phytoextraction of Cadmium in a Calcareous Soil

Motesharezadeh, B.*, Savaghebi-Firoozabadi Gh. R., Mirseyed Hosseini, H. and Alikhani, H. A.

Department of Soil Science Eng., University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran

Received 25 Jun. 2009; Revised 5 Feb. 2010; Accepted 15 March 2010

ABSTRACT: Heavy metals such as cadmium mainly enter in the environment and ecosystem as a result of human activities. This study was conducted in order to evaluate the ability of bacterial inoculants to enhance efficiency of phytoextraction in a calcareous soil. Three plants (*Amaranthus retroflexus, Helianthus annus* and *Medicago sativa*), along with three levels of inoculants and four levels of Cd were evaluated. The experiment design was factorial with three replications. The ANOVA results showed that application of inoculants led to significant increase ($P<0.01$) of cadmium concentration in plant's root and shoot.Furthermore, as cadmium concentration increased in different treatments, fresh weight of plant's root and shoots decreased in all treatments but, weight reduction of plant's organs was less in treatments with inoculants application. The amaranthus highest amount of absorption, translocation of Cd to shoot, Translocation factor (TF) and Cd phytoextraction. Thus, amaranthus among studied plants is more appropriate for phytoextraction of cadmium.

Key word: Phytoextraction, Cadmium, Soil pollution, Inoculants, Translocation factor

INTRODUCTION

In recent years, heavy metal contamination in soil has become a major problem which their accumulation in plants can directly or indirectly influence animals and humans (Pal *et al.*, 2006). The highest amount of cadmium enter into the soil through application of sewage sludge and waste in agricultural soils and also as a result of industrial activities such as dye making, rubber making, production of fertilizer from phosphate rock , automobile fuel and metal melting industry (Ansari & Malik, 2007; pal *et al.*, 2006). Phytoextraction serves as one of the phytoremediation technologies and it is the technique of using plants for decontamination of the environment. Soil remediation by phytoextraction method has many advantages and disadvantages (Erakhrumen, 2007; Ghosh & Singh, 2005). Low biomass production in hyper-accumulator plants and susceptibility of other plant' root to high metal concentration lead to extension of researches on use of microorganisms in order to develop application of phytoextraction and to make this method economical (Glick, 2003; Ansari & Malik, 2007).

In the present study, effect of the application of inoculants of two strains, resistant to heavy metals was tested on cadmium absorption in greenhouse experiment with amaranthus, sunflower and alfalfa. The objective was to provide a practical solution for decreasing and diminishing of heavy metal contamination from different sources such as fertilizers, industries and mines to agricultural lands using native plant growth promoting rhizobacteria (PGPR) separated from soil around lead and zinc mines in Haft Emarat, Arak, at central province of Iran.

*Corresponding author E-mail: moteshare@ut.ac.ir

MATERIALS & METHODS

First, composite soil sample was collected from depth of 0-30 cm from the Campus of Agriculture and Natural Resources of University of Tehran located in Karaj with coordinates of latitude of northern $35^0 48' 35''$ and longitude of eastern $50^0 58' 18''$ and 1315.5 meters above sea level.The soil was classified as *Xeric Haplocambids, Fine Loamy, Mixed, Super Active thermic*. Samples were air dried and passed through 2-mm sieve and mixed uniformly. Physical and chemical properties and concentrations of elements in samples were measured: Measurements of the soil N was done by Kjeldal method, (Bremner, 1996), available phosphorus by Olsen Method (Kuo, 1996), available Potassium by normal acetate ammonium method (Hemke and Sparks, 1996). Measurements of the soil pH was done on saturated extract (Thomas, 1996) and electrical conductivity by Rhoades method (1996), Equal

Calcium Carbonate by Bouyoucos method (Bouyoucos, 1962), organic carbon percentage by Walkly Black (Nelson and Sommers, 1982) and texture of the soil by hydrometric method (Bouyoucos, 1962) and cation exchange capacity by Bower method (Sumner and Miller, 1996). Available concentration of cadmium, lead and zinc were extracted by DTPA method (Linsay and Norvell, 1978) and measured by Atomic Absorption Spectrometery (AAS). Results are given in Table 1. The methods for bacteria isolation and purification and their PGPR characteristics have been reported previous in Moteshare Zadeh *et al.* (2008). The effects of three levels of inoculants [control (B0), *Bacillus mycoides* M1 (B1), *Micrococcus roseus* M2 (B2)] and four levels of Cd [control (Cd0), Cd50, Cd100 and Cd200 mg/kg $CdCl_2.H_2O$] on three plant cultivars (*Amaranthus retroflexus* , *Helianthus annus* and *Medicago sativa*), were studied under controlled conditions in a factorial experiment with randomized complete block design (RCBD) with three replicates of each treatment. After 70 days, at the beginning of reproductive period, shoot and root of sunflower and aramanthus and after three cuts of alfalfa (mean of three cuts in calculations and comparing it with other plants) in alfalfa, plant material was washed with distilled water and fresh weight determined, then dried in oven at 70°c and dry weight was recorded. Then, samples were milled and extract was prepared by dry ash, and concentration of cadmium, iron, copper, zinc, and manganese were measured using ICP- OES, CAP-6500 model. (Madejon *et al*., 2003). In order to assess amount of metal transfer from root to shoot, translocation factor was determined by dividing metal concentration at shoot by its concentration at root (Marchiol *et al*., 2004). The analysis of variance of the data was done in a factorial design with random blocks basic design with three replications by means of SAS software. The comparison of means was done with LSD test at 1% level and also figures were drawn by Excel software.

RESULTS & DISCUSSION

The soil used in greenhouse test (Table 1) was selected based on metal toxicity limits and study of sources and doubling concentration of each treatment in comparison with the previous treatment, contaminated with cadmium at rates (0, 50, 100 and 200 mg Cd /kg) (Alloway, 1990; Purohit and Agrrawal, 2006). Results of the analysis showed that the intended soil has suitable physical and chemical properties for greenhouse culture and no heavy metal contamination was induced due to the treatments.Results of cadmium test for three plants: amaranthus, sunflower and alfalfa: The results of triplet effects of bacterium, cadmium level and plant in root and shoot are illustrated in figs. 1 to 14.

Strains of *bacillus mycoides* M1 (B1), among two strains used at this study, was individually indentified resistant to four metals: lead, zinc, cadmium and nickel (Moteshare Zadeh *et al*., 2008). According to Yan- de *et al*., (2007), multiple metal resistance (MMR) have more effects in bacteria than the resistance to one metal, thereby it is possible that in treatments with application of this inoculants, better results will be achieved in terms of plant growth and phytoextraction. In a similar study at India (Malik & Jaiswal, 2000), 45 Pseudomonas strains were separated from soils of contaminated lands with industrial sewage and also non-contaminated lands, and their biochemical and morphological characteristics were determined. The research's results of these researchers showed that 80% of strains were resistant to copper, %73 resistant to cadmium, 71% to zinc and % 48.8 to mercury. The applied concentration in the study were 3.12 to 3200 mg/ml. Aleem *et al*., (2003), in the study on non-contaminated soils and soils of grain fields which were irrigated by industrial sewage for a long time, 57 strains of *Azotobacter chroococcum* isolated from rhizospheric soil and assessed their chemical and su-

Table 1. Physical and chemical properties of soil before adding cadmium

Characteristic	Quantity	Characteristic	Quantity
Soil texture	Loam	Total N (%)	0.08
Clay (%)	25.00	Available P (mg/kg)	17.10
Silt (%)	36.00	Available K (mg/kg)	247.00
Sand (%)	39.00	SO_4(meq/l)	40.60
pH	7.90	Fe(mg/kg)*	4.28
EC(dS/m)	4.31	Cu(mg/kg)*	4.061
%$CaCO_3$	8.90	Mn(mg/kg)*	8.244
%OC	0.84	Zn(mg/kg)*	0.812
%SP	35.6	Pb(mg/kg)*	2.023
CEC(Cmol/Kg)	26.00	Cd(mg/kg)*	0.10

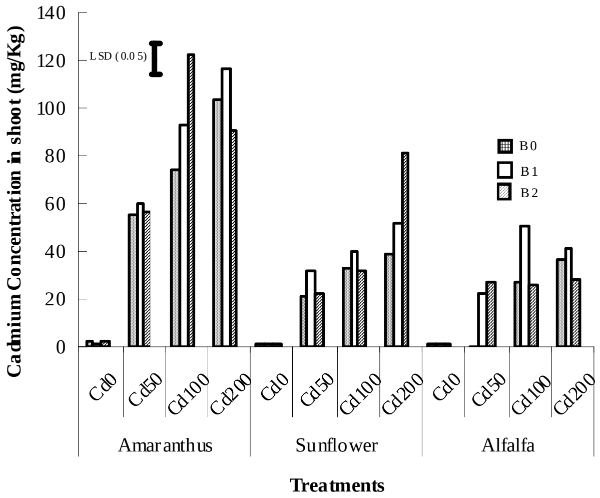

Fig. 1. The effect of treatments on the Cd concentration in different treatments of three plants

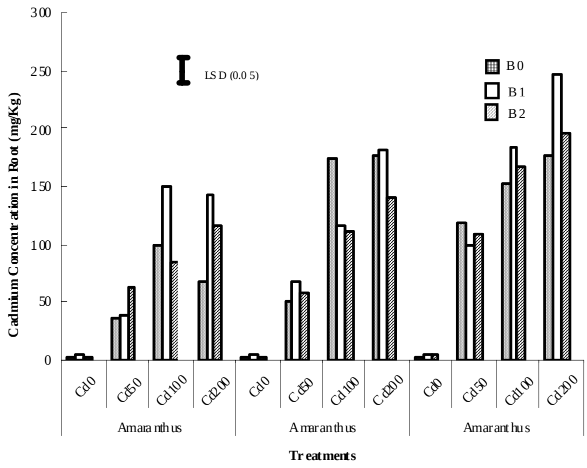

Fig. 2. The effect of treatments on the Cd concentration in different treatments of three plants

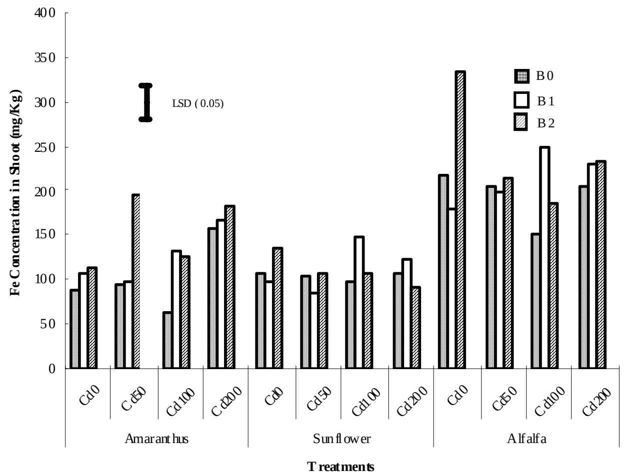

Fig . 3. The effect of treatments on the Fe concentration in different treatments of three plants

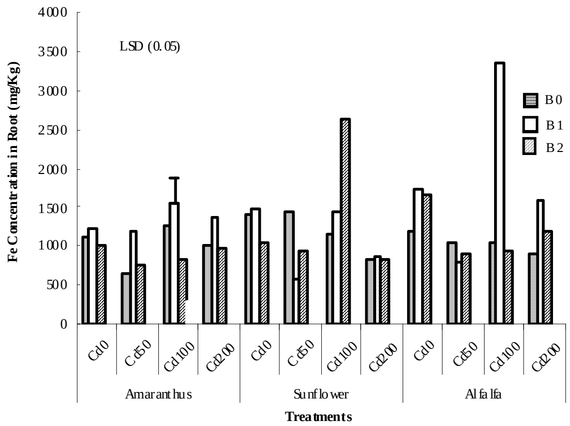

Fig. 4. The effect of treatments on the Fe concentration in different treatments of three plants

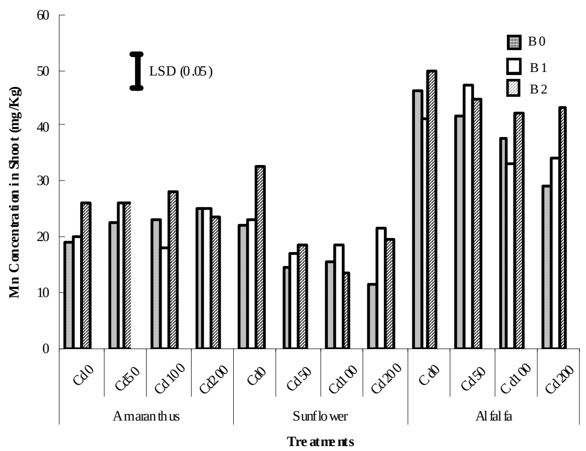

Fig. 5. The effect of treatments on the Mn concentration in different treatments of three plants

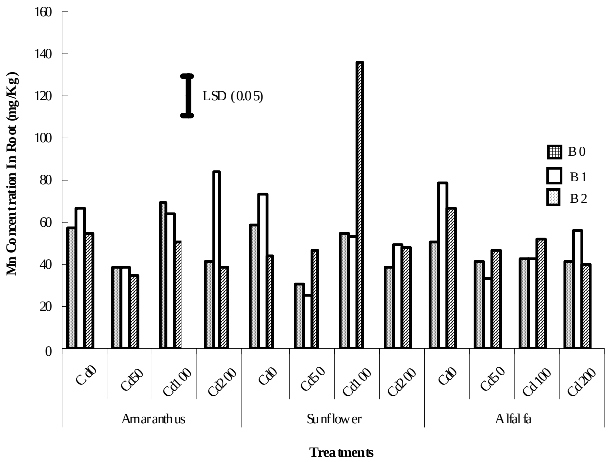

Fig. 6. The effect of treatments on the Mn concentration in different treatments of three plants

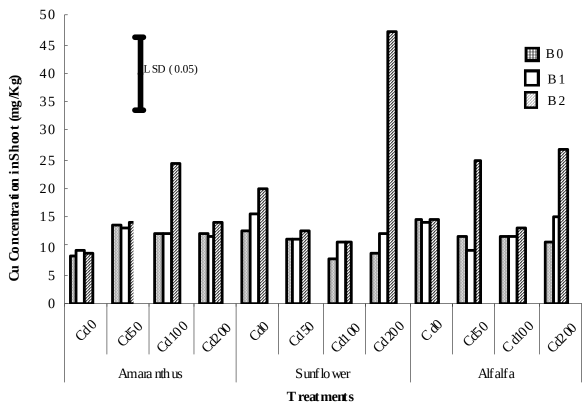

Fig. 7. The effect of treatments on the Cu concentration in different treatments of three plants

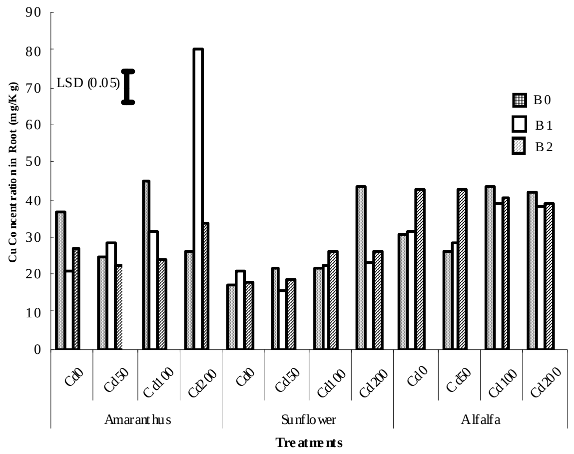

Fig. 8. The effect of treatments on the Cu concentration in different treatments of three plants

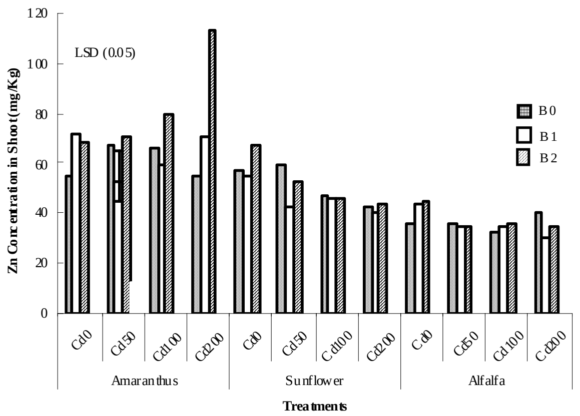

Fig. 9. The effect of treatments on the Zn concentration in different treatments of three plants

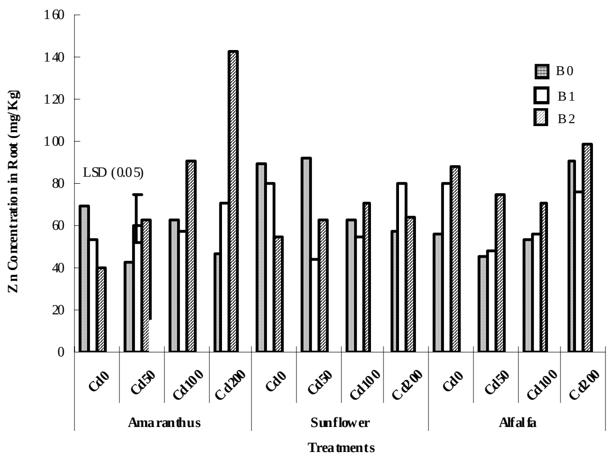

Fig. 10. The effect of treatments on the Zn concentration in different treatments of three plants

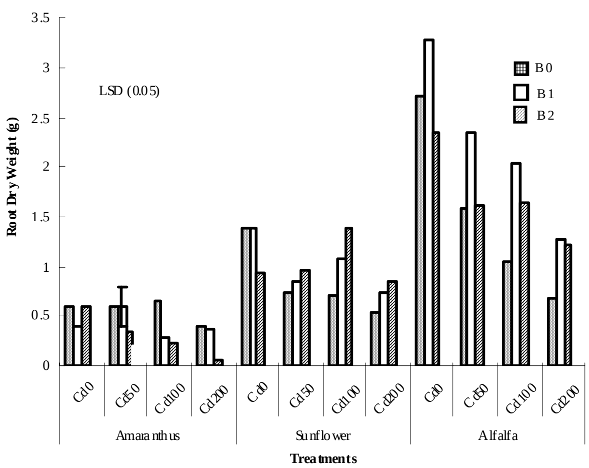

Fig. 11. The effect of treatments on the Root Dry Weight in different treatments of three plants

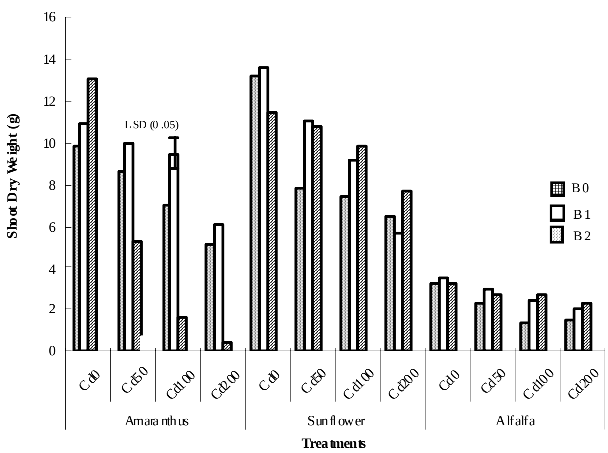

Fig. 12. The effect of treatments on the Shoot Dry Weight in different treatments of three plants

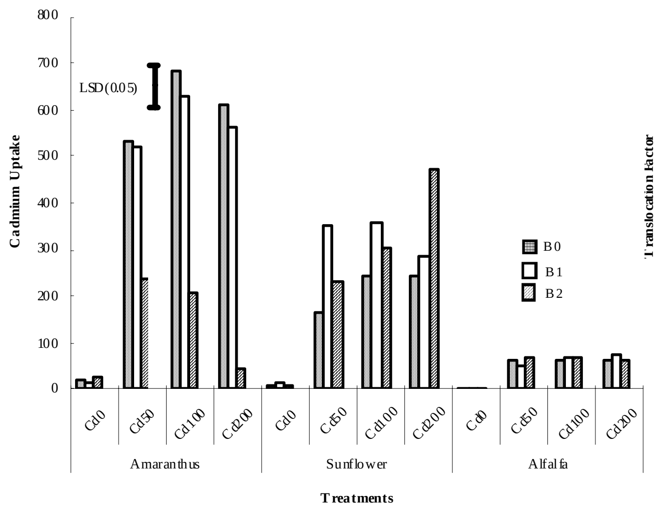

Fig. 13. The effect of treatments on Cadmium Uptake (μ g/Pot) in different treatments of three plants

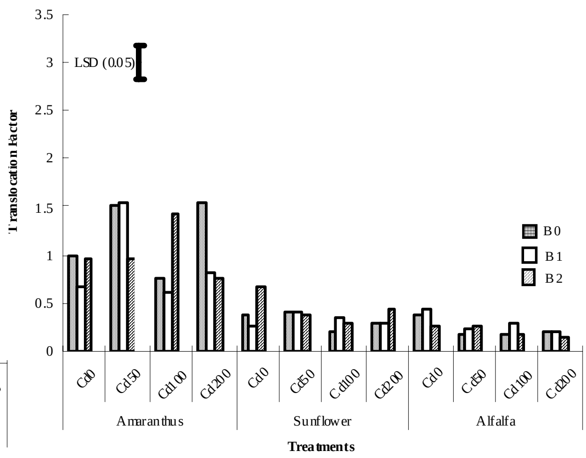

Fig. 14. The effect of treatments on the Translocation Factor in different treatments of three plants

perficial characteristics. Among 36 identified strains from rhizosphere of wheat and %94.4 strains were resistant to lead and mercury and %63.8, %77.5 and %86.1 resistant to zinc, Cr^{+6}, Cr^{+3}. According to obtained results, this researchers declared that identified bacteria from soil irrigated by industrial sewage had significant difference in terms of resistance to metal, with non-contaminated soil. According to the report of Hada and Sizemone (1981), although bacterium in non-contaminated area may be compatible to high concentration of metal, but evidences indicated that there were more resistant strains in contaminated area than in non-contaminated area. Also, it was reported that amount of metal in soil was associated with the rate of bacterium resistance to metal. The gram positive and negative bacteria could be resistant to heavy metals (Siler & Misra, 1998).

With regard to results provided in fig. 2. application of inoculants led to cadmium concentration increase in roots of three plants also, iron concentration have increased in similar treatments (figure 3). Glick (2003) declared that growth stimuli bacterium can effect on plant growth both directly and indirectly. In the direct effect, plant growth and consequently absorption and nutrition synthesis improvement will increase. Growth stimuli bacterium may stabilize nitrogen of atmosphere and increase availability of this element for plants or by siderosphore production, increase the solubility of iron in soil and facilitate iron absorption by plants. The synthesis of different plant hormone such as axing, considered as one method for stimulation and influence on plant growth by growth stimuli bacterium. By synthesis of ACC Deaminase enzyme, tension ethylene decrease and plant growth will stimulate. Moreover, in the indirect stimulation, useful bacteria prevent the influence of phyto-pathogens (Glick, 2003). This researcher expressed that high heavy metal concentration have negative influence on plant growth by generating two problems: tension ethylene and limited iron concentration. The growth stimuli bacteria have useful characteristics such as ability of ACC deaminase enzyme production and siderophore production which can help to hind's plant growth in confronting with high heavy metal concentration. The close identification and analysis of these characteristics can be useful for understanding of heavy metal phytoextraction and phytoremediation mechanisms. Kuffnet *et al*., (2008) studied on effects of rhizosphere bacteria on absorption and metal concentration in willows and identified 10 strains from contaminated soils of lead mine. Among identified bacteria, bacteria of six type were as follow: pseudomonas, agromyces, streptomyces, flavobacterium, servatia and janthinobacterium. Among strains, four strains (from pseudomonase, serratia type) and two strains (from streptomyces type) have the ability of siderophore production and three strains (two from janthinobacterium type and one from serratia type) have the ability of auxin production. Also none of the ten strains have the ability of ACC deaminase enzyme production. The resistance of these bacteria to zinc, lead and cadmium were assessed too. Yan-de *et. al*., (2007), provided the list of growth stimuli strains and their effects on plant including control of phytopathogen, growth and nutrition absorption improvement, resistant once to cadmium, zinc, copper, lead, nickel, cobalt, chrome contamination, root production and also resistant once to salinity and drought tension, however, rhizobacteria in addition to metal transmission from soil to plant can increase phytoremediation efficiency by effects on soil pH, iron supply through siderophore production, phosphorus solubility increase, effects on phytopathogen, resistant once to salinity and drought tension, and indole acetic acid (IAA) production.

According to figure 1, shoot highest cadmium concentration and also the most absorption were observed for amaranthus. It seems that, the result of inoculants application was not identical in three plants and amaranthus did not significantly respond to inoculants application. In terms of root cadmium concentration, the most amounts were observed at alfalfa and with B1 inoculants consumption. Also, the highest root and shoot iron concentration was observed at alfalfa and the same inoculants. As a whole, the wet weight of root was observed for alfalfa, the wet weight of shoot for sunflower and the most transmission factor and cadmium absorption for amaranthus. According to these findings, applicability of plants with high biomass production and inoculants effects on growth promotion and plant development was proved. Other researchers including Madejon *et al.*, (2003) and Cheng (2003) and Mathe- Gasper and Anton (2005) have reported the same findings. According to study of Madejon *et al.*, (2003), shoot biomass and root of sunflower were significantly less in contaminated soil than non contaminated soil. But, this effect was not important at maturation, of the product. Oil production was more in contaminated soil. These researchers declared that toxic effect of arsenic, cadmium, copper, lead, thallium was not observed at plants of the soil around mine. In this study, the potential phytoremediation of sunflower was affirmed, and with regard to its lower potential for phytoextraction, there were suggestion on sunflower application for soil protection and oil production for industrial consumption. According to results of this study, total cadmium concentration at sunflower was 125 mg/kg, which including 80 mg/kg at shoot, 13 mg/kg at root and 25 mg/kg at seeds. According to Cheng's report (2003), heavy metal accumulation and distribution in plant depends on different elements such as: environmental elements as plant species, type of element, chemical form, bioavailability, oxidation- reduction potential, pH, cation exchange capacity, solute oxygen, and temperature and root distribution. Song et al., (1996), by assessing heavy metal concentration such as cadmium, zinc, lead in espionage organ, has illustrated the relation between metal amount in espionage edible organ and forms of these metal in soil as several equations. These researchers expressed the process of metal accumulation as follow: cadmium> zinc> lead. Products that research's focus on them due to their high biomass production include: sunflower, cotton, oil seed, corn, Indian mustard, cereal (Vassiller *et al.*, 2002). The extent of cadmium concentration at corn in phytoremediation was mentioned as 25 to 150 mg/kg of shoot dry material.

CONCLUSION

Based on these results, plant type was effective in phytoextraction efficiency increase and also, inoculants application was useful and effective in phytoextraction efficiency increase. For conformation of the results we can point to the reports of Lasat (2002), Glick (2003), Aleem *et al.*, (2003), Yan- de *et al.*, (2007), Ansari and Malik (2007) and Kuffner *et al.*,(2008) which deals with the effect of growth stimuli bacteria through indirect and direct effect on root growth, root growth stimulation through siderophore production (Fig. 3,4) and phosphorous and iron supply and other nutrients (Fig. 7,9,10), control of heavy metal stress through ACC deaminase enzyme production and tension ethylene decrease, ability of auxin (IAA) production, control of salinity and drought stress, absorption stimulation and heavy metal transmission from rhizosphere environment to plant and pathogen element effect decrease and also declares the useful effects of plant growth stimuli bacteria and its synergistic effect with plant in Green remediation.

Alfalfa, as a whole, is considered as an appropriate plant for stabilization of pollutant metals and preserving their distribution, due to its high potential in biomass production. Also, there was no significant risk in terms of its shoot metal concentration. The same results mentioned for sunflower too. So we can successfully use this plant for phytoremediation technology with plant stabilization method. Amaranthus can be considered as a potential option for removing of pollutants and land remediation, due to its resistance to weather conditions and also its ability in metal accumulation and transmission from root to shoot by phytoextraction mechanism and low risk of nutrition in food cycle. As mentioned above, contaminated soil phytoextraction is a relatively new and developing technology with special advantages. Economical use of native and cultivated plants for phytoremediation of moderately polluted soils with heavy metals should be approached with more research and field evaluations.

REFERENCES

Aleem, A., Isar, J. and Malik, A. (2003). Impact of long-term application of industrial wastewater on the emergence of resistance traits in Azotobacter chroococcum isolated from rhizospheric soil. Bioresource Technol., **86**, 7-13.

Alexander, D. B. and Zuberer, D. A. (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soils, **12**, 39-45.

Alloway, B. J. (1990). Heavy metals in soils. John Wiley & Sons, Inc . New York. USA.

Ansari, M. I. and Malik, A. (2007). Biosorption of nickel and cadmium by metal resistant bacterial isilates from agricultural soil irrigated with industrial wastewater. Bioresource Technol., **98**, 3149-3153.

Bent, E., Tuzun. S., Chanway, C. P. and Enebak, S. (2001). Alterations in plant growth and in root hormone levels of iodgepole pines inoculated with rhizobacteria. Can. J. Microbiol., **47**, 793-800.

Bremner, J. M. (1996). Nitrogen-total. P. 1085-1122. In Sparks, D.L. *et al*., Method of soil analysis. Published by: Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Bingham, F. T., Pereyea, F. J., and Jarrell, W. M. (1986). Metal toxicity to agricultural crops. Metal Ions Biol. Syst., **20**, 119-156.

Bouyoucos, C. J. (1962). Hydrometer method improved for making particle size analysis of soil. Agron. J., **54**, 464-465.

Cheng, S. (2003). Heavy metals in plants and phytoremediation. Environ. Sci. and Pollut. Res., **10 (5),** 335-340.

Erakhrumen, A. and Agbontalor, A.(2007). Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educational Research and Reviews, **7,** 151-156.

Foy, C. D., Chaney, R. L. and White, M. C. (1978). The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol., **29 (1)**, 511-566.

Ghosh, M. and Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of its byproducts. Appied Ecology and Environmental Research, **3 (1)**, 1-18.

Glass.D. J. (2000). Economic potential of phytoremediation. P. 15-31. *In* I. Raskin and B. D. Ensley (ed.) Pytoremediation of toxic metals. John Wiley & Sons, New York.

Glick, B. R. (2003). Phytoremediation: Synergistic use of plants and bacteria to clean up the environment. Biotechnology Advances, **21**, 383-393.

Hada, H. S. and Sizemore, R. K. (1981). Incidence of plasmids in marine vibrio spp.isolated from an oil field in the north western gulf of mexico. Applied and Environmental Microbiology, **41**, 199-202.

Hemke, P. H. and Spark, D. L. (1996). Potassium. 551-574. In Sparks, D.L. *et al.*, Method of soil analysis. Published by: Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Holt, J. G., Kreig, N. R. Sneath, P. H. A. Staley, J. T. and Williams, S. T. (1994). Bergeys manual of determinative bacteriology, Ninth ed. Baltimore, Maryland: Williams and Wilkins.

Kuffner, M., Puschenreiter, M., Wieshammer, G., Gorfer, M. and Sessitsch, A. (2008). Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows, Plant Soil, **304**, 35-44.

Kuo, S. (1996). Phosphorus. P. 869-920. In Sparks, D.L. *et al*., Method of soil analysis. Published by: Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Lasat, M. M. (2002). Phytoextraction of toxic metal: A review of biological mechanisms, J. Environ. Qual. **31**, 109-120.

Lindsay, W. L. and Norvell, W. A. (1978). Development of DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J., **42**, 421-428.

Lombi *, E.*, Zhao, F. J., Dunham, S. J. and McGrath, S. P. *(2001)*. Phytoremediation of heavy metal–contaminated soils, natural hyperaccumulation versus chemically enhanced phytoextraction. *J. Environ. Qual.*, **30**, 1919-1926.

Madejon, P., Murillo, J. M., Maranon, T., Cabrera, F. and Soriano, M. A. (2003). Trace element and nutrient accumulation in sunflower plants two years after the Aznalcollar mine spill. The Science of the total Environment, **307**, 239-257.

Marchiol, L., Assolari, S., Sacco, P. and Zerbi, G. (2004). Phytoextraction of heavy metals by canola and radish grown on multicontaminated soil. Environ. Pollut., **132**, 21-27.

Mathe- Gaspar, G. and Anton, A. (2005). Phytoremediation study: Factors influencing heavy metal uptake of plants, Proceeding of the 8th Hungarian congress on plant physiology and the 6th Hungarian congress on photosynthesis. Acta Biologica Szegediensis, **49(1-2),** 69-70.

Nelson, D. W. and Sommers, L. E. (1982). Total carbon, organic carbon, and organic mater.p. 539-580.In A. L. Page(ed), methods of soil analysis. Part 2. 2nd ed. Chemical and microbiological properties. Agronomy monograph no.9. SSSA and ASA, Madison,WI.

Nelson, R. E. (1982). Carbonate and gypsum. P. 181-196. In A.L. Page (ed), Methods of soil analysis. Part 2. 2nd ed. Chemical and microbiological properties. Agronomy monograph no.9. SSSA and ASA. Madison, WI.

Pal, M., Horvath, E., Janda, T., Paldi, E. and Szalai, G. (2006). Physiological changes and defense mechanisms induced by cadmium stress in maize, J. Plant Nutr. Soil Sci., **169**, 239-246.

Penroz, D. M. and Glick, B. R. (2001). Levels of ACC and related compounds in exudates and extracts of canola seeds treated with ACC-deaminase- containing plant growth promoting bacteria. Can. J. Microbiol. **47**, 368-372.

Prasad, M.N.V. (2004). Heavy metal stress in plants, Second Ed. Norosa Publishing House. USA.

Purohit, S. S. and Agrrawal, A. K. (2006). Environmental Pollution. Agrobios Pub. India.

Reddy, G. B., Ed Wenda, F. and Aldridge, D. (1986). Seasonal changes in bacterial numbers and plant nutrients in point and non-point source ponds. Environ. Pollut. (Series A) **40**, 359-367.

Rhoades, J. D. (1996). Electrical conductivity and total dissolved solids. P. 417-436. In Sparks, D. L. *et al*., Method of soil analysis. Published by: Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Silver, S. and Misra, T. K. (1998). Plasmid mediated heavy metal resistance. Annual Review of Microbiology, **42**, 717-743.

Singh, A. and Ward, O. P. (2004). Applied Bioremediation and phytoremediation, Springer-Verlag, Berlin Heidelberg, Germany.

Song, F., Guo, Y. and Liu, X. (1996). Effect of compound pollution with Cadmium, Zinc and Lead on Spinach in brown earth. Agro-environmental protection, **15 (1)**, 9-14.

Steffens, J. C. (1990). The heavy metal-binding peptides of plants. Ann. Rev. Plant Physiol.Plant Mol. Biol., **41**, 553-575.

Sumner, M. E. and Milker, W. P. (1996). Cation exchange capacity and exchange coefficients. P. 1201-1230. In Sparks, D.L. *et al*., Method of soil analysis. Published by: Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Thomas, G. W. (1996). Soil pH and soil acidity. P. 475-490. In Sparks, D.L. *et al*., Method of soil analysis. Published by: Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Vander Lelie D., Schwitzguebel, J. P., Glass, D. J., Vangronsveld, J. and Baker, A. (2001). Assessing phytoremediations progress in the United States and Europe. Environ. Sci. Technol. **35**, 447-452.

Vassilev, A., Vangronsveld, J. and Yordanov, I. (2002). Cadmium phytoremediation: Present state, Biological back grounds and research needs, Bul. G. J. Plant Physiol., **28 (3-4)**, 68-95.

Wei, W., Jiang, J., Wang, X. L. and Yang, S. S. (2004). Isolation salt-sensitive mutants from sinorhizobium meliloti and characterization of genes involved in salt tolerance. Letters in Applied Microbiology, **39**, 278-283.

Yan-de, J., Zhen-li, H. and Xiao, Y. (2007). Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J. Zhejiang Univ. Sci., **8 (3)**, 197-207.