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Phytoextraction of Nickel, Lead and Cadmium from Metals Contaminated Soils Using Different Field Crops and EDTA

Mamdouh A. Eissa, Mohamed F. Ghoneim, Galal A. El-Gharably and Mohamed Abd El-Razek

Department of Soils and Water, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

Abstract: The success of phytoextraction depends upon the identification of suitable plant species that can tolerate and accumulate heavy metals and produce large amounts of plant martial. Field crops like corn (*Zea mays*), wheat (*Triticum asaetivum*), sunflower (*Helianthus annuus*), barley (*Hordeum vulgaris*), cotton (*Gossypium barbadense*) and faba bean (*Vicia faba*) were used in many researches for phytoremediation. Two pot experiments were conducted out to study the possibility of using these plants in phytoextraction. The first experiment was conducted using cadmium (Cd) at levels (control, 10 and 20 mg/kg), nickel (Ni) at levels (control, 150 and 300 mg/kg) and lead (Pb) at levels (control, 150 and 300 mg/kg). The second experiment was accomplished to study the effect of ethylene diamine tetra acetic acid (EDTA) on plants growth and heavy metals uptake. Roots and shoots growth of the studied plants decreased significantly ($P<0.05$) as affected by heavy metal and EDTA applied to soil. The roots of the studied plants were more sensitive to heavy metals stress and EDTA than the shoots, where the high levels of the studied heavy metals reduced the growth of roots by 44-47% and shoots by 35-37 %. Although the application of EDTA to soil increased the heavy metals uptake by the studied plants, the roots and shoots weights decreased by more than 25% as a result of EDTA treatment. Based on our results, it may be stated that the studied field crops had low ability to tolerate and accumulate heavy metals from contaminated soils even though the using of EDTA, therefore they not recommended to use in phytoextraction of heavy metals from contaminated agricultural soils.

Key words: Phytoremediation · Field Crops · Heavy metals · Contaminated soils · Ni · Pb · Cd

INTRODUCTION

Heavy metal pollution of soil is a significant environmental problem. It has negative impact on human health and agriculture. The clean up of metal-contaminated soils by traditional physicochemical methods is both very costly and destructive to the normal properties of the soil [1]. In contrast, phytoremediation, that use of green plants to decontaminate the metals out the soil is an emerging technique with advantages of being in situ, cost effective and environmentally sustainable [2, 3]. However, the success of phytoextraction depends upon the identification of suitable plant species that tolerate and accumulate heavy metals and produce large amounts of biomass using established agricultural techniques. Plants for phytoextraction, i.e. metal removal from the soil, should have the following characteristics: (i) tolerant to high levels of the metal, (ii) accumulate reasonably high levels of the metal, (iii) rapid growth rate, (iv) produce reasonably high biomass in the field and (v) profuse root system [4].

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as (i) uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents; (ii) efflux of heavy metals from cytoplasm to extranuclear compartments including vacuoles; (iii) complexation of heavy metal ions inside the cell by various substances, for example, organic acids, amino acids, phytochelatins and metallothioneins; (iv) accumulation of osmolytes and osmoprotectants and induction of antioxidative enzymes (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures [5]. The major problem hindering plant remediation efficiency is that some of the metals are immobile in soils and their availability and phytoextraction

Corresponding Author: Mamdouh A. Eissa, Department of Soils and Water, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.

rates are limited by solubility and diffusion to the root surface. Ethylene diaminetetraacetic acid (EDTA) is often found to be the most effective chelating agent [6, 7], which considerably enhances the accumulation of metals in the above ground parts of plants because it develops a metalchelate complex which enhances its mobility within the plant by increasing its transport from roots to aerial parts [8, 9].

This study aims to: i) study the growth of some field crops (corn (*Zea mays*), wheat (*Triticum asaetivum*), sunflower (*Helianthus annuus*), barley (*Hordeum vulgaris*), cotton (*Gossypium barbadense*) and faba bean (*Vicia faba*)) in contaminated soils ii) show the ability of these plants to accumulate heavy metals in the shoot. iii) explore the role of EDTA in plant growth and heavy metals uptake.

MATERIALS AND METHODS

Soil Characterization: Soil samples were collected from Ellwan, Assiut, Egypt where the soils have been irrigated with sewage waste water for more than 50 years. The samples were air-dried and sieved with a 2-mm diameter sieve for further analysis. Particles-size distribution of the soils was performed using the pipette method that was described by Jackson [10]. Soil pH was measured using a digital pH meter in a 1:1 suspension of soil-to-water ratio. Organic matter content of the soil was determined using the dichromate oxidation method described by Wakley and Black [10]. Total carbonates in the soil were estimated gasometrically using calcimeter method and calculated as $CaCO_3$ [11].The electrical conductivity (EC) was estimated in 1:1 soil to water extract using the salt bridge method [12]. DTPA-extractable metal: Cd, Ni and Pb were extracted from the studied soil samples using a 0.005M DTPA (diethylen triamine penta acetic acid) solution buffered at pH 7.3 as described by Lindsay and Norvell [13]. For determination of total heavy metals: The method of Baker and Amacher [14], which involved the digestion of samples in a mixture of HF-HNO_3-$HClO_4$-H_2SO_4 in Teflon beakers placed on a hot plate, was also employed to analyze the total contents of Cd, Cr, Cu, Ni, Pb and Zn.

Greenhouse Experiments: The soil was spiked with Cd, Ni and Pb in the form of sulphate.The soil was allowed to undergo tow cycles of saturation with de-ionized water and air drying process, each lasting one week. Tow pots experiment were conducted out, The first to study the effect of levels of heavy metals (Cd, Pb and Ni) on plant growth and concentration of these elements in the studied plants (corn, sunflower, cotton, wheat, barely and bean).The levels of Cd were 0, 10 and 20, Pb were 0,150 and 300 and Ni were 0,150 and 300 mg/kg soil. The second excrement was conducted to study the effect of EDTA on plant growth and heavy metals uptake. The soil in the second experiment was spiked with a mixture of Cd, Pb and Ni then the total heavy metals content was determined. Cd concentration after spiking was 20, Pb was 300 and Ni was 300 mg/kg soil. For EDTA treatment, 100 mL of 120 mM EDTA (as Na_2-EDTA salt) were applied to the surface of the soil in the pots of this treatment. Plastic pots were filled with 5 kg of the studied soil samples. Four plants of wheat and barely and tow plants of corn, cotton and bean were left in each pot. Pots were carefully watered as needed and fertilized as need with NPK fertilize mixture (0.5 g/pot) containing N: P: K=1: 0.4: 0.8.

Plant Analysis: Plants were left in the pots for 70 day after the sewing. At the end of the experiment the plant sample were collected, washed with tap water twice and rinse with distilled water before being separated into shoot and root and oven-dried (70° C) to a constant weight. Dried roots and shoots were ground and submitted to the acid-digestion using a 2:1 HNO_3:$HClO_4$ acid mixture. The digests were analyzed for heavy metals (Ni, Pb and Cd) by atomic absorption spectrophotometer (AAS).

Statistical Analysis: The experimental design of this experiment was Randomized Complete Blocks Design (RCBD) with three replicates. The collected data were statically analyzed using MSTAT computer program as described by Michigan State University [15]. Means were compared by using least significant difference (LSD) at 5% level of probability as described by Gomez and Gomez [16].

RESULTS AND DISCUSSION

Properties for the Studied Soil: Table 1: Some chemical and physical properties of the studied soil The physical and chemical characteristics as well as the concentrations of available and total heavy metals of the soils under the study are presented in Table 1. These soils were classified as a Typic Torripsamments. The DTPA-extractable Pb, Ni and Cd in the collected soil samples were 3.24, 3.78 and 0.09.The total metal content of the collected soil samples were 115, 123 and 1.05 mg/kg Pb, Ni and Cd respectively. The permissible limits of the heavy metals in the soil are

Table 1: Some chemical and physical properties of the

Property	
Particle size distribution	
Clay (%)	10
Silt (%)	23
Sand (%)	67
Texture	Sand Loam
$CaCO_3$ (%)	7.8
pH (1:1)	7.25
Organic matter (%)	2.55
EC (1:1 dS/m)	1.8
DTPA-extractable metals (mg/kg)	
Pb	3.24
Ni	3.78
Cd	0.09
Total metals (mg/kg)	
Pb	115
Ni	123
Cd	1. 05

100 mg Pb/kg and 100 mg Ni /kg, (as reported by Kabata-Pendias and Pendias [17] and (0.1-0.5 mg Cd/kg) (as reported by Scheffer and Schachtschabel [18].

Effect of Heavy Metals Stress on the Growth of the Studied Field Crops: The data presented in Table 2, 3 and 4 show the growth of the studied plants as affected by different Cd, Pb and Ni treatments. There are significant differences between the studied plants in their tolerance to Cd, Pb and Ni treatments.

Added Cd to soil significantly affected the weight of roots and shoots of all the studied plants. Increasing the level of Cd in soil decreased the dry matter of roots and shoots of all the studied plants. Comparison the growth of the studied plants in control and those in the soil with the high level of Cd, it is clear that there is a decrease in growth of roots and shoots by 44% and 35%respictivilly. Muramoto *et al*. [19] reported that root and shoot weights of rice were reduced by 32% and 21% when 100 mg Cd kg^{-1} added to soil. It is known that cadmium reduced the dry mater production [20, 21]. Cadmium (Cd), being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis and is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health [22].

Table 3 showed that there are significant effects for Pb treatments in the roots and shoots of the studied plants. Pb treatments reduced the growth of all the studied plants. The growth of roots and shoots in the high level of Pb decreased by 45 and 37% respectively compared with control. Decreasing dry mater as affected by Pb treatment was observed by Liu *et al*.[23]. For plants, although Pb is not considered as an essential element, its absorption and accumulation in different parts takes place frequently and its accumulation increases with increase in exogenous Pb levels [24, 25]. Once entered in plant, it detrimentally influences plant growth resulting in reduced leaf area and it also inhibits activities of many enzymes [26, 27].

Data presented in Table 4 showed a significant effect for Ni treatments in the growth of all the studied plants. Increasing the level of Ni in soil decreased the growth of all the plants. The root and shoot of the plants grown in the high level of Ni decreased by 47 and 35 % respectively compared with the control. In general, heavy metals severely inhibit root growth [28] and this is in the case of Ni [29].

Increased Ni in the soil increased its concentration in plants and reduced dry mater production [30, 31]. The seed germination, root and shoot growth were found significantly affected by these metals at higher concentration, [31]. Nickel is an essential micronutrient for higher plants [32]. However, Ni at sufficiently high levels may be toxic to plants [33]. Excess Ni can affect physiological/biochemical process like decreasing leaf chlorophyll contents [34] and leaf photosynthetic and transpiration activities [35] and impairing membrane permeability associated with enhanced extracellular peroxidase activity [36]. And reduced translocation of nutrient from root to shoot [37].

Concentrations of Heavy Metals in the Roots and Shoots of the Studied Field Crops: Table 2, 3 and 4 shows the effect of Cd, Pb and Ni treatments in the concentration of Cd, Pb and Ni in root and shoot of the studied plants. Shoots of the studied plants had the lowest values of Cd and the roots had the highest values. The highest value of Cd in the root and shoot was found in sunflower and corn. Comparisons of the Cd concentration in the studied plants show that there is a clear effect for the Cd treatments on its concentration in the root and shoot. Increased the level of Cd in soil increased the Cd concentration in the roots and shoots. Treatments of cadmium increased concentration of this element in plant [20-21]. Most of Cd in the plants stored in the root and similar results were found by Chaturvedi [38] and Zadeh [20]

As shown in table 3 increased the level of Pb in soil increased the concentration of Pb in the roots and shoots. The roots of all studied plants contained higher Pb

Table 2: Effect of Cd treatments on dry mater production (g/pots) and Cd concentration in plants (mg/kg)

	Roots			Shoots		
Crop	Cd 0	Cd 10	Cd 20	Cd 0	Cd 10	Cd 20
			dry mater production (g/pots)			
corn	3.77	3.10	2.27	22.33	16.00	13.00
sunflower	3.77	3.10	2.17	21.67	18.33	13.00
cotton	2.27	1.43	1.20	14.00	11.00	11.33
wheat	3.53	2.80	2.13	17.67	17.00	10.67
barley	3.60	3.80	2.10	14.00	14.00	12.67
bean	3.70	2.20	1.60	17.33	14.00	8.00
mean	3.44	2.74	1.91	17.83	15.06	11.44
LSD_C	0.19			1.93		
LSD_L	0.13			1.36		
			Cd concentration in plants (mg/kg)			
corn	0.60	16.01	22.52	0.05	8.00	9.35
sunflower	0.60	10.83	23.88	0.04	8.69	9.23
cotton	0.60	16.75	19.22	0.05	7.15	7.08
wheat	0.70	16.75	21.08	0.04	6.83	8.52
barley	0.73	14.42	16.19	0.06	5.50	7.07
bean	0.67	16.59	19.30	0.08	7.17	8.11
mean	0.65	15.23	20.37	0.05	7.22	8.23
LSD_C	ns			0.96		
LSD_L	2.31			0.68		

LSD is least significant difference at 5% and C refers to crops, L is the Cd levels

Table 3: Effect of Pb treatments on dry mater production (g/pot) and Pb concentrations in plants (mg/kg)

	Roots			Shoots		
Crop	Pb 0	Pb150	Pb300	Pb 0	Pb150	Pb300
dry mater production (g/ pots)						
corn	3.67	3.07	2.73	22.33	19.00	13.67
sunflower	3.60	2.67	1.90	21.67	16.67	12.67
cotton	2.47	1.23	1.43	15.33	12.00	10.33
wheat	3.57	2.80	1.77	17.67	15.00	13.33
barley	3.70	3.00	1.80	18.00	13.67	10.67
bean	3.43	2.20	1.60	17.00	12.67	9.33
mean	3.41	2.49	1.87	18.67	14.83	11.67
LSD_C	0.26			1.83		
LSD_L	0.18			1.29		
			Pb concentrations in plants (mg/kg)			
corn	14.07	33.73	50.07	6.29	9.91	14.41
sunflower	26.17	40.13	47.34	6.26	11.08	15.81
cotton	12.74	19.41	33.00	7.03	10.02	13.71
wheat	16.08	32.41	42.40	6.03	8.08	14.01
barley	19.06	35.03	41.33	7.00	11.02	13.36
bean	15.52	34.11	47.10	6.05	8.01	15.01
mean	17.27	32.47	43.55	6.44	9.69	14.38
LSD_C	4.92			ns		
LSD_L	3.48			0.93		

LSD is least significant difference at 5% and C refers to crops, L is the Pb levels

Table 4: Effect of Ni treatments on dry mater production (g/pot) and Ni concentrations in plants (mg/kg)

	Roots			Shoots		
Crop	Ni 0	Ni150	Ni 300	Ni 0	Ni 150	Ni 300
dry mater production (g/pots)						
corn	3.80	3.10	2.23	21.00	15.00	12.33
sunflower	3.67	2.80	1.90	22.00	17.00	11.00
cotton	2.73	2.43	1.33	14.33	12.67	12.33
wheat	3.87	2.80	1.90	18.33	14.33	13.00
barley	3.87	2.80	2.07	18.00	12.00	10.67
bean	3.43	2.20	1.83	17.33	16.00	12.33
mean	3.56	2.69	1.88	18.50	14.50	11.94
LSD $_C$	0.23			1.59		
LSD $_L$	0.17			1.12		
Ni concentration in plants (mg/kg)						
corn	22.04	35.04	51.37	9.07	12.10	14.85
sunflower	16.04	27.04	55.04	7.02	10.52	14.22
cotton	21.85	35.37	46.04	8.29	11.71	16.08
wheat	13.70	33.80	47.97	9.04	8.00	15.77
barley	20.63	35.17	46.02	5.00	9.07	13.70
bean	19.04	35.71	46.04	8.03	11.70	16.04
mean	18.88	33.67	48.75	7.74	10.52	15.11
LSD $_C$	3.13			1.07		
LSD $_L$	2.22			0.77		

LSD is least significant difference at 5% and C refers to crops, L is the Ni levels

concentrations compared with the shoots. Pb concentration in plants increased by increasing Pb level in soil and most of Pb accumulated in the root and this result also was found by John *et al*.[21] and Liu *et al* [23].

Concentration of Ni in the studied plants affected significant by the Ni treatment as shown in table 4. Increasing the level of Ni in soil increased the concentration of Ni in the roots and shoots of the plants. The roots contained more Ni than shoots. Similar results were found by Giordan *et al*.[30].

The highest value of Pb concentration was found in the roots of corn plants. Comparison the Pb concentration in the shoot of studied plants showed that sunflower had the highest value of Pb. The highest value of Ni concentration observed in the root of sunflower followed by corn. These tow plants were used for phytoremediation in many research due to their high biomass production [39-41].

Effect of EDTA on Dry Mater Production of the Studied Plants: Treated the soil with EDTA affected significantly in decreasing the weight of root and shoot of al the studied plants. There are significant differences in dry mater production of the studied plants as shown in Table 5. Treated the soil with EDTA decreased the weight of root and shoot compared with control. In general the root weight decreased by 25% as a result of EDTA treatment. The root of corn was more tolerate than the other crops.

The shoot weight decreased by 23% after adding EDTA to the soil. It is known that EDTA decreased the dry mater production. Reduction of yield production as affected by EDTA was observed by Chen and Cutright [39]. The root and shoot of sunflower reduced as a result of treated the soil with EDTA [41-42].

Possibility of Using Field Crops for Phytoremediation: The success of phytoextraction process depends upon both shoot biomass and shoots metal concentration. The remediation factor (RF) was calculated to asses the ability of studied plants in phytiextraction. This parameter shows the real ability of the plant in remediation soils from the contaminated. the remediation factor (RF) was calculated as the percentage of the element removed by the plant dry above ground biomass from the total metal content in the soil [43]. RF value of the studied plants ranged between (0.01- 0.38). The lowest values of RF were observed in control treatment but the added EDTA increased the RF for all studied plants as shown in Table 6. The ability of all the studied plants in heavy metals removal was very low. If any of these plants was used for phytoremediation the

Table 5: Effect of EDTA on dry mater production of the plants (g/pot)

	Root		Shoot	
	Control	EDTA	Control	EDTA
corn	2.93	2.43	14.30	10.67
sunflower	3.03	2.40	13.93	10.40
cotton	2.23	1.57	10.63	8.17
wheat	2.53	1.60	13.45	10.63
barley	2.17	1.53	14.23	9.733
bean	2.43	1.93	14.03	12.11
mean	2.56	1.91	13.43	10.28
LSD_C	0.27		2.10	
LSD_T	0.16		1.21	

LSD is least significant difference at 5% and C refers to crops, T is the EDTA treatment

Table 6: Remediation factor of the tested plants

	Cd		Pb		Ni	
Crop	Control	EDTA	Control	EDTA	Control	EDTA
corn	0.19	0.38	0.02	0.03	0.02	0.04
sunflower	0.18	0.35	0.01	0.03	0.02	0.03
cotton	0.13	0.24	0.01	0.02	0.01	0.02
wheat	0.17	0.28	0.01	0.03	0.02	0.03
barley	0.16	0.34	0.01	0.02	0.01	0.03
bean	0.20	0.33	0.02	0.03	0.02	0.03

remediation processes will need a very long time. Similar results were found by (Chen and Cutright [39], Meers *et al*. [40] Usman and Mohamed [41].

These results suggest that negligible amounts of these heavy metals could be removed in comparison to the total metal in the soil. Therefore, under our experimental conditions, overall extraction of heavy metals from contaminated soil was considered too low for these plants to consider for practical application of phytoextraction.

CONCLUSIONS

In general the growth of the studied plants decreased significantly by increasing the levels of heavy metals in soils. Under the stress of heavy metal the biomass production of all the studied plants was decreased. But the reduction in dry mater production was less than 50%. The roots of the studied plants were more sensitive to the heavy metal compared to the shoots. The ability of these plants to tolerate the high levels of heavy metals in the soil is very low as well as their ability for heavy metal accumulation in the shoots and roots. The highest values of the studied heavy metals were observed in corn and sunflower tissues. Using these plants in phytoremediation depend on their highly ability to produce large amount of the dry matter in short time, but when they grown on contaminated soils they will be lost that advantage. The using of chelating agent to enhance the accumulation of heavy metals resulted in reduction of dry mater production of all the studied plants. The ability of all the studied plants in heavy metals removal was very low. If any of these plants was used for phytoremediation the remediation processes will need a very long time. The researches in the field of phytoremediation should be concentrated in the other plants that have a real ability to tolerate and accumulate heavy metals.

REFERENCES

1. Tandy, S., R. Schulin and B. Nowack, 2006. Uptake of metals during chelate-assissted phytoextraction with EDDS related to the solubilized metal concentration. Environmental Science and Technology, 40: 2753-2758.
2. Lasat, M.M., 2002. Phytoextraction of toxic metals: a review of biological mechanisms. Journal of Environmental Quality, 31: 109-120.
3. He, Z.L., X.E. Yang and P.J. Stoffella, 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology, 19: 125-140.
4. Baker, A.J., S.P. McGrath, R.D. Reeves and J.A. Smith, 2000. Metal hyperaccumulator plants: A review of the ecology and, physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Ban˜ uelos G and Vangronsveld J (Eds), Phytoremediation of contaminated soil and water (pp: 85-107). LewisPublisher, Boca Raton, FL, USA.
5. Cho, M., A.N. Chardonnens and K.J. Dietz, 2003. Differential heavy metal tolerance of *Arabidopsis halleri* and *Arabidopsis thaliana*: a leaf slice test. New Phytol., 158: 287-293.
6. Blaylock, M.J., D.E. Salt, S. Dushenkov, C.D. Gussman, Y. Kapulnik, B.D. Ensley and I. Raskin, 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol., 31: 860-865.
7. Huang, H., T. Li, S. Tian, D.K. Gupta, X. Zhang and H. Yang, 2008. Role of EDTA in alleviating lead toxicity in accumulator species of Sedum alfredii. Bioresour. Technol., 99: 6088-6096.

8. Turgut, C., M.K. Pepe and J.T. Cutright, 2004. The effect of EDTA and citric acid on phytoremediation of Cd, Cr and Ni from soil using Helianthus annuus L. Environ. Pollut., 131(1): 147-154.
9. Zhuang, P., Q.W. Yang, H.B. Wang and W.S. Shu, 2007. Phytoextraction of heavy metals by eight plant species in the field. Water Air Soil Pollut. 184: 235-242.
10. Jackson, M.L., 1970. Soil chemical analysis. Prentic-Hall, Inc. Engle wood Cliffs, NJ, USA.
11. Nelson, R.E., 1982. Carbonate and gypsum. P. 181-198 In A.L. Page, R.H. Miller and D.R. Keeney. Methods of soil analysis, part 2. Chemical and microbiological properties 2nd edition. Soil Sci. Soc. Am. Inc. Madison, WI, USA.
12. Rhoades, J.D., 1982. Soluble salts. P.167-180 In A.L. Page, R.H. Miller and D.R. Keeney. Methods of soil analysis, part 2. Chemical and microbiological properties 2nd edition. Soil Sci. Soc. Am. Inc. Madison, WI, USA.
13. Lindsay, W. L. and W. A. Norvell, 1969. Equilibrium relationship of Zn^{+2}, Fe^{+3}, Ca^{+2} and H^{+} with EDTA and DTPA in soils.Soil Sci. Soc. Am. Proc., 33: 62-68.
14. Baker, D.E. and M.C. Amacher, 1982, 'Nickel, Copper, Zinc and Cadmium', in A. L. Page *et al*. (eds), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed. Agron. Monogr. Vol. 9, ASA and SSSA, Madison, WI, U.S.A. pp: 323-336.
15. Michigan State University. 1983. MSTAT-C Micro-Computer Statistical Program, Version 2. Michigan State University, East Lansing, USA.
16. Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agriculture Research. A Wiley-Inter Science Publication. John Wiley Sons, Inc, New York, USA.
17. Kabata-Pendias, A. and H. Pendias, 2001. Trace Elements in Soils And Plants, CRC Press, London.
18. Scheffer, F. and P. Schachtschabel, 2002. Lehrbuch der Bodenkunde. Auflage, vol. 15. Spektrum Akademischer Verlag, Heidelberg, Berlin.
19. Muramoto, S., H. Nishizaki and I. Aoyama, 1990. The critical levels and the maximum metal uptake for wheat and rice plants when applying metal oxides to soil. J. Environ Sci Health, Part B. 25(2): 273-280.
20. Zadeh, B.M., G. Savaghebi-Firozabadi, H. Alikhani and H. Hosseini, 2008. Effect of Sunflower and Amaranthus Culture and Application of Inoculants on Phytoremediation of the Soils Contaminated with Cadmium. American-Eurasian J. Agric. and Environ. Sci., 4(1): 93-103.
21. John, R.P., K. Ahmadb, S. Gadgila and Sharmab, 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. International Journal of Plant Production, 3 (3): 150-177.
22. Di Toppi S.L. and R. Gabrielli, 1999. Response to cadmium in higher plants. Environ. Exp. Bot., 41: 105-130.
23. Liu, W., O. Zhou, Y. Zhang and S. Wei, 2010. Lead accumulation in different Chinese cabbage cultivars and screening for pollution-safe cultivars Journal of Environmental Management, 91: 781-788.
24. Singh, R.P., S. Dabas, A. Chaudhary and R. Maheshwari, 1998. Effect of lead on nitrate reeducates activity and alleviation of lead toxicity by inorganic salts and 6-benzylaminopurine. Biol. Plant. 40(3): 399-404.
25. Zhu, Y.G., L. Wang, Z.J. Wang, P. Christie and J.N.B. Bell, 2007. China steps up its efforts in research and development to combat environmental pollution. Environ. Pollut. 147: 301-302.
26. Reddy, A.M., S.G. Kumar, J. Gottimukkala, S.Thimmanaik and C. Sudhakar, 2005. Lead induced changes in antioxidant metabolism of horsegram (Macrotyloma uniflorum (Lam.) Verdc.) and bengalgram (Cicer arietinum L.). Chemosphere, 60: 97-104.
27. Islam, E., D. Liu, T. Li, X. Yang, X. Jin, Q. Mahmooda, S. Tian and J. Li, 2008. Effect of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of Elsholtzia argyi. J. Hazard. Mater., 154: 914-926.
28. Punz, W.F. and H. Sieghardt, 1993. The response of roots of herbaceous plant species to heavy metals. Environ. Exp. Bot., 33: 85-98.
30. Giordani, C., S. Cecchis and C. Zanchi, 2005. Phytoremediation of Soil Polluted by Nickel Using Agricultural Crops Environmental Management. 36(5): 675-681.
31. Chhotu, D., J. Madhusudan and H. Fulekar, 2008. phytoremediation the application of vermicompost to remove zinc,cadmium,copper,nickel and lead by sunflower Environmental Engineering and Management Journal. 5: 547-558.
32. Brown, P.H., R.M. Welch and E.E. Cary, 1987. Nickel: A micronutrient essential for higher plants. Plant Physiol., 85: 801-803.
34. Piccini, D.F. and E. Malavolta, 1992. Effect of nickel on two common bean cultivare. J. Plant Nutr., 15: 2343-2356.

33. Bingham, F.T., F.J. Peryea and W.M. Jarrell, 1986. Metal toxicity to agricultural crops. Metal Ions. Biol. Syst., 20: 119-156.
35. Jones, M.D. and T.C. Hutchinson, 1988. Nickel toxicity in mycorrhizal birch seedlings infected with Lactarius rufus or Scleroderma flavidum I. Effects on growth, photosynthesis, respiration and transpiration. New Phytol., 108: 451-459.
36. Pandolfini, T., R. Gabbrielli and C. Comparini, 1992. Nickel toxicity and peroxidase activity in seedlings of Triticum aestivum L. Plant, Cell and Environ., 15: 719-725.
37. Yang, X., V.C. Baligar, D.C. Martens and R.B. Clark, 1996. Plant tolerance to nickel toxicity: II nickel effects on influx and transport of mineral nutrients in four plant species. J.Plant Nutration, 19(2): 265-279
38. Chaturvedi, I., 2004.Phytotoxicity of cadmium and its effect on two genotypes of Brassica juncea L. Emir. J. Agric. Sci., 16(2): 01-08.
39. Chen, H. and T. Cutright, 2001. EDTA and HEDTA effects on Cd, Cr and Ni uptake by Helianthus annuus. Chemosphere, 45: 21-28.
40. Meers, E., A. Ruttens, M. Hopgood, E. Lesage and F.M.G. Tack, 2005. Potential of Brassic rapa, Cannabis sativa, Helianthus annuus and Zea mays for phytoextraction of heavy metals from calcareous dredged sediment derived Soils Chemosphere, 61: 561-572.
41. Usman, A. and H.M. Mohamed, 2009. Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metal by corn and sunflower. Chemosphere, 76: 893-899.
42. Azhar, A., M. Ashraf, M. Hussain and R. Ahmed, 2009. EDTA-Induced improvement in growth and water relations of (*Helianthus annus* L.) Plant growen in lead contaminated medium. Pak. J. Bot., 41(6): 3065-3074.
43. Neugschwandtner, R.W., P. Tlustos, M. Komarek and J. Szakova, 2008. Phytoextractionof Pb and Cd from a contaminated agricultural soil using different EDTA application regimes: laboratory versus field scale measures of efficiency. Geoderma, 144: 446-454.