Research Journal of Agriculture and Biological Sciences, 3(5): 370-376, 2007

Phytoremediation Potential of Crop and Wild Plants for Multi-metal Contaminated Soils

[1]Reda Abou-Shanab, [2]Nevine Ghanem, [2]Khaled Ghanem and [3]Abdu Al-Kolaibe

[1]Department of Environmental Biotechnology, Mubarak City for Scientific
Research and Technology Applications, Borg El Arab, P.O. 21934, Alexandria, Egypt.
[2]Botany Department, Faculty of Science, University of Alexandria, Egypt.
[3]Microbiology Department, Faculty of Science, University of Taiz, Yemen.

Abstract: A pot experiment was conducted to compare the growth and metal accumulation of *Zea mays, Sorghum bicolor, Helianthus annuus, Conyza discoridies* and *Cynodon dactylon* were grown in four different soils containing moderate to high amounts of heavy metals. Shoot and root biomass of plant species was significantly smaller in the metal rich soil than in the low metal soils. Mostly metal concentrations in plant tissues were positively related to their total and/ or water extractable metal in soils. Total uptake of Cr in the shoot of *C. dactylon* was about 39, 8, 6 and 4 times higher than Cr concentrations in the shoots of *H. annuus*, *S. bicolor*, *Z. mays* and *C. discoridies*, respectively when grown on Cr- rich soil. Most of metal accumulated in *Z. mays* and *S. bicolor* found in roots, which are difficult to harvest. Translocation of metal in these plants from root to shoot was restricted. Therefore, *Z. mays* and *S. bicolor* were more suitable for phytostabilization of metal contaminated soils. *Conyza discoridies*, alternatively, accumulated higher amounts of metals in their shoots. This suggest that *C. discoridies* was the best species for phytoremediation of Zn, Cu and Pb.

Keywords: Phytoremediation, Heavy metals, *Zea mays*, Sorghum bicolor, *Helianthus annuus*, Conyza discoridies, *Cynodon dactylon*

INTRODUCTION

The continued industrialization of countries has led to extensive environmental problems. A wide variety of chemicals have been detected in soil, water, and air[1,2]. Heavy metals pose a critical concern to human health and the environment due to their common occurrence as an contaminant, low solubility in biota, and the classification of several heavy metals as carcinogenic and mutagenic[3]. Remediation of soils contaminated with toxic metals is particularly challenging. Unlike organic compounds, metals cannot be degraded, and the cleanup usually requires physical or chemical removal[4].

A promising, relatively new technology for heavy metal contaminated sites is phytoremediation. Phytoremediation is the use of plants to remove organic and/ or inorganic contaminants from soil (phytoextraction), uptake and conversion into non-toxic forms (phytovolatilization), or stabilization of an inorganic into a less soluble form (phytostabilization). These technologies have attracted attention in recent years for the low cost of implementation and environmental benefits. Moreover, the technology is likely to be more acceptable to the public than other traditional methods[5,6,7].

A few plant species are able to survive and reproduce on soils heavily contaminated with Zn, Cu, Pb, Cd, Ni, Cr, and As[8]. Such species are divided into two main groups: the so-called pseudometallophytes that grow on both contaminated and non-contaminated soils, and the absolute metallophytes that grow only on metal-contaminated and naturally metal-rich soil[8]. Depending on plant species, metal tolerance may result from two basic strategies: metal exclusion and metal accumulation[9,10]. The exclusion strategy, comprising avoidance of metal uptake and restriction of metal transport to the shoots[11], is usually used by pseudometallophytes. The accumulation strategy caused high uptake of metal and storage in vacuoles to prevent metal toxicity. The extreme level of metal tolerance in vascular plants is called hyperaccumulation. Hyperaccumulators are defined as higher plant species whose shoots contain > 100 mg Cd kg^{-1}, > 1000 mg Ni, Pb, and Cu kg^{-1}or > 10 000 mg Zn an Mn kg^{-1} (dry wt.) when grown in metal-rich soils [12,13]. The capacity to specifically accumulate high amounts of metals in shoots makes hyperaccumulators suitable for

Corresponding author: Reda Abou-Shanab, Department of Environmental Biotechnology, Mubarak City for Scientific Research and Technology Applications, Borg El Arab, P.O. 21934, Alexandria, Egypt.
Tel.: 002 03 459 4168 / 002 03 459 3420/ 0128963380
Fax: 002 03 459 3423 or 2034593407 E. Mail: redaabushanab@yahoo.com

phytoremediation purposes. However, various practical drawbacks can reduce the applicability of phytoremediation[14]. Crops with both a high metal uptake capacity and a high biomass production are needed to extract metals from soils within a reasonable time frame[15].

Therefore, the objectives of this study were to compare the uptakes of heavy metals by crop and indigenous plants grown on four different soils containing moderate to high amounts of heavy metals. The potential use of these species in the phytoremediation of metal polluted soils was assessed.

MATERIALS AND METHODS

Soil Sources Characterization and Preparation: Soil samples were collected from sites chosen for their industrial activities and/or historical backgrounds in Egypt. Sampling was conducted at four locations: an industrial and municipal waste site at Makhar El Saeel (Helwan), a site exposed to Cu and Pb smelter emissions (Tounsey), a tannery effluent polluted soil (Max), and a low metal agriculture farm soil. Soil were mixed in large containers and air-dried at room temperature, then crushed and sieved to remove rocks and undecomposed organic materials. Soil mechanical analysis was carried out by the pipette method according to[16]. The percentage water-holding capacity was determined according to[17]. Soil pH was determined after mixing 1 g of soil in 2.5 ml water for about 5 min, allowed ionic exchange to reach equilibrium prior to measuring pH[16]. Organic carbon content was measured by the rapid titration method[18]. Cation exchange capacity (CEC) was determined by the method of[19]. Total metals in soil were determined by digesting 500mg of soil in a mixture of concentrated HCl / HNO_3 (4:1, v / v)[20]. Water extractable metals were measured by shaking 10g (dry wt) moist field soil for 2-h in 20 ml deionized water[21]. Sample were filtered and acidified with HNO_3 before analysis. Metal concentrations in acid digest and extracts solutions were analysed by flame atomic absorption spectrophotometry (AAS).

Pot Experiment: To initiate the experiments, air-dried soils were mixed with the same volume of perlite (1:1, v/v) and about 2 kg placed into plastic pots (18 cm in diameter and 13 cm in length). Seeds of *H. annuus, Z. mays, S. bicolor, C. discoridies* and *C. dactylon* rhizomes were sown in plastic pots with four replicas for each treatment. The experiment was carried out in a greenhouse illuminated with natural light. The moisture content of each pot was maintained at 70% water holding capacity by weighing the pots two times per week. After germination, the seedlings were thinned to two plants per pot and grown for eight weeks. Tap water and nutrient solutions of KNO_3, $(NH_4)_2SO_4$ and K_2HPO_4 / KH_2PO_4 were added as needed.

Plant Harvest and Analysis: After eight weeks, plants were gently removed from the pots. Shoot and roots were separated and the lengths of both were measured. Plant shoots and roots were washed with deionized water, rinsed, and dried at 70^0C, and the dry matter (DM) measured. Plant materials were ground and two grams or less of milled plant matter was digested with a mixture of HCl/HNO_3 (4:1, v / v)[20], and the heavy metals in the digests were determined using AAS.

RESULTS AND DISCUSSIONS

Physico-chemical Properties of Soil: The four soils had different physicochemical properties and patterns of pollution (Table 1). Soil pH is one of the most influential parameters controlling the conversion of metals from immobile solid-phase forms to more mobile and/or bioavailable solution-phase forms. Soil pH in tannery effluent polluted soils and farm soils were slightly acidic (6.7 – 6.8, respectively), and pH of soils collected from Tounsey smelter site and Helwan industrial sites were in alkaline range (7.2 – 8.2, respectively). Sanders, 1983 reported that the solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils (approximately pH 5.0 to 7.0). The high pH values of soils could have accounted for a low transfer of metals from soil to plant. Low metal farm soils had a larger content of organic matter and a higher CEC than the other three soils. [23] reported that soils with high CECs could adsorb larger amounts of heavy metals than soils with low CEC. All soils were found to be sandy clay loam to sandy loam in texture.

Total metal content is important because it determines the size of the metal pool in the soil and thus the potential for metal uptake[24]. Therefore, soil samples were analyzed for total and water extractable Cr, Cu, Pb and Zn. Each soil sample exhibited a high concentration in one or more of the metals. Tounsey soil was the most contaminated, containing high metal concentrations (Table 1). Total concentrations of Zn, Cu, and Pb were 32.5, 22.8, and 1.9 g kg^{-1}, while water extractable concentrations were much lower with 0.16, 0.05, and 0.06 g kg^{-1}, respectively. Soils were collected from Helwan were highly contaminated with Zn (13.1 g kg^{-1}) compared with soils collected from Max and the agriculture farm soil (0.21 and 0.14 g Zn kg^{-1}, respectively). Contamination was a result of municipal waste and industrial effluent disposal. Chromium concentrations in soils were also elevated

Table 1: Physical and chemical characteristics of soils

Farm	Helwan	Max	Tounsey	
Soil texture	S*. C*. L*.	S.L.	S.L.	L. S.
CEC** (meq 100g^{-1})	43.5	26.0	34.8	17.4
pH (H_2O)	6.8	8.2	6.7	7.2
Organic matter (%)	4.4	0.13	2.6	2.2
Source of contamination	Fertilizer and pesticides	Iron industry electroplating and municipal waste	Tannery effluent	Cu and Zn smelters
Total Cr (mg kg^{-1})	176	180	16865	307
Mobile*** Cr (mg kg^{-1})	2.6	1.7	75.4	13.9
Total Cu (mg kg^{-1})	58.8	28.2	44.9	22800
Mobile Cu (mg kg^{-1})	0.5	0.48	1.1	53
Total Pb (mg kg^{-1})	49	122.5	124.5	1900
Mobile Pb (mg kg^{-1})	5.2	12.5	22.2	63.2
Total Zn (mg kg^{-1})	144	13100	208	32500
Mobile Zn (mg kg^{-1})	4.5	46.6	6.9	162.2

S= Sandy; C= Clay; L= Loam
CEC= Cation Exchange Capacity
Mobile= Water extractable metal

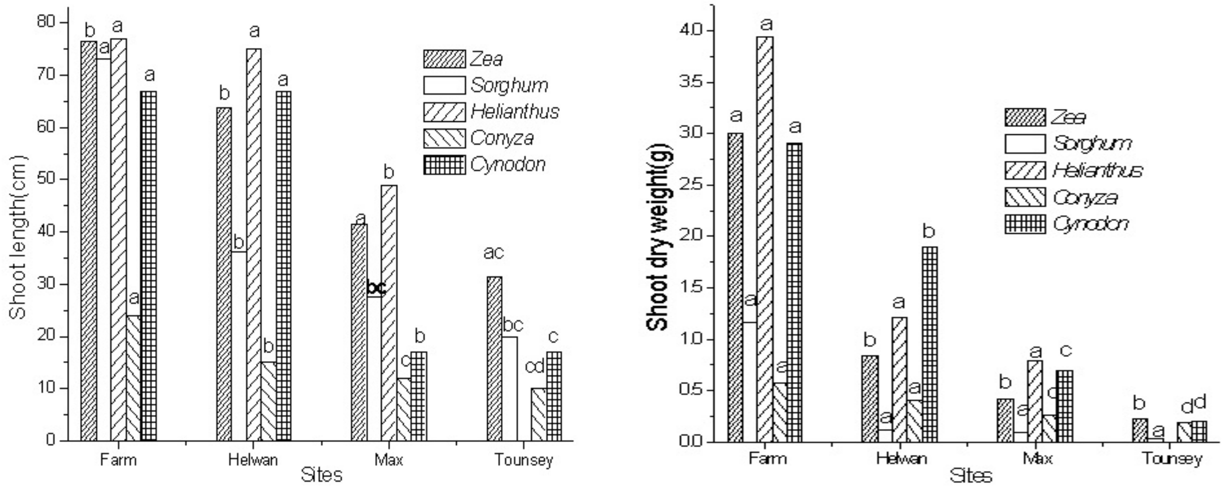

Fig. 1: Shoot length and dry weight of *Zea mays, Sorghum bicolor, Helianthus annuus, Conyza discoridies and Cynodon dactylon* grown in a greenhouse using four different metal contaminated soils. Mean values marked with the same letter are not significantly different at $P<0.05$

and varied from 0.17 to 16.86 g kg^{-1}. The highest chromium concentrations (16.86 g kg^{-1}) were recorded in the Max soils as a result of untreated tannery effluent wastes.

Metal variation was also recorded in the extractable metal content, this can be attributed to the behavior of trace metals in soil that depends not only on the level of contamination, as expressed by the total content, but also on the form and origin of the metal and the properties of the soils themselves[25,26]. Metals are present in soil as a natural component or as a result of human activities, such as smelting of metalliferous ores, electroplating, fuel production, fertilizer and pesticides application, and generation of municipal waste [27,28]. The extractable concentrations of Cr, Cu, Pb and Zn in the study sites were high compared to the values (70 – 400 mg Zn kg^{-1}, 100 – 400 mg Pb kg^{-1}, 60 – 125 mg Cu kg^{-1} and 75 – 100 mg Cr kg^{-1}), generally observed in agricultural soils and considered to be toxic according to[29,30,28].

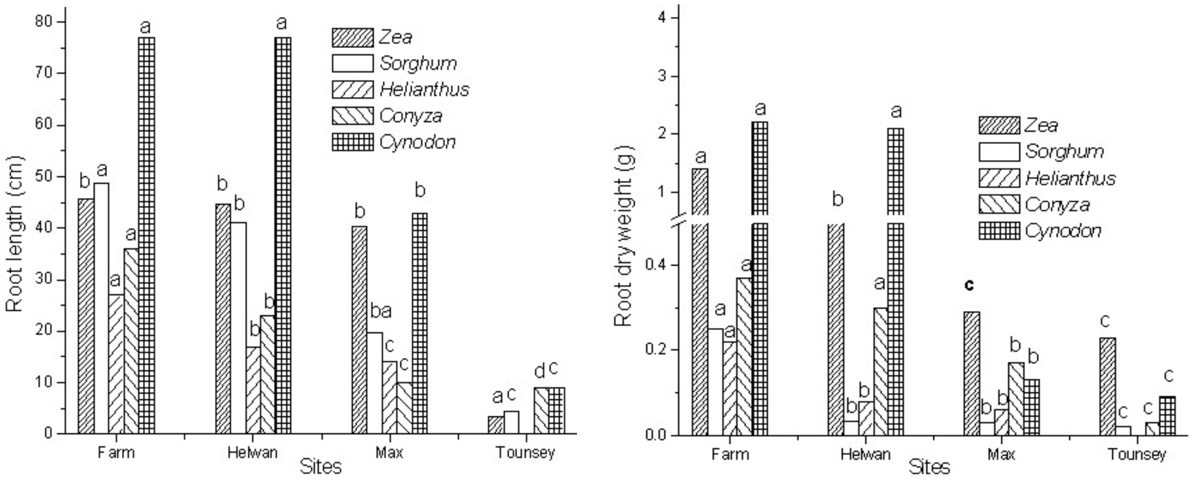

Fig. 2: Root length and dry weight of *Z. mays, S. bicolor, H. annuus, C. discoridies and C. dactylon* grown in a greenhouse using four different metal contaminated soils. Mean values marked with the same letter are not significantly different at P<0.05

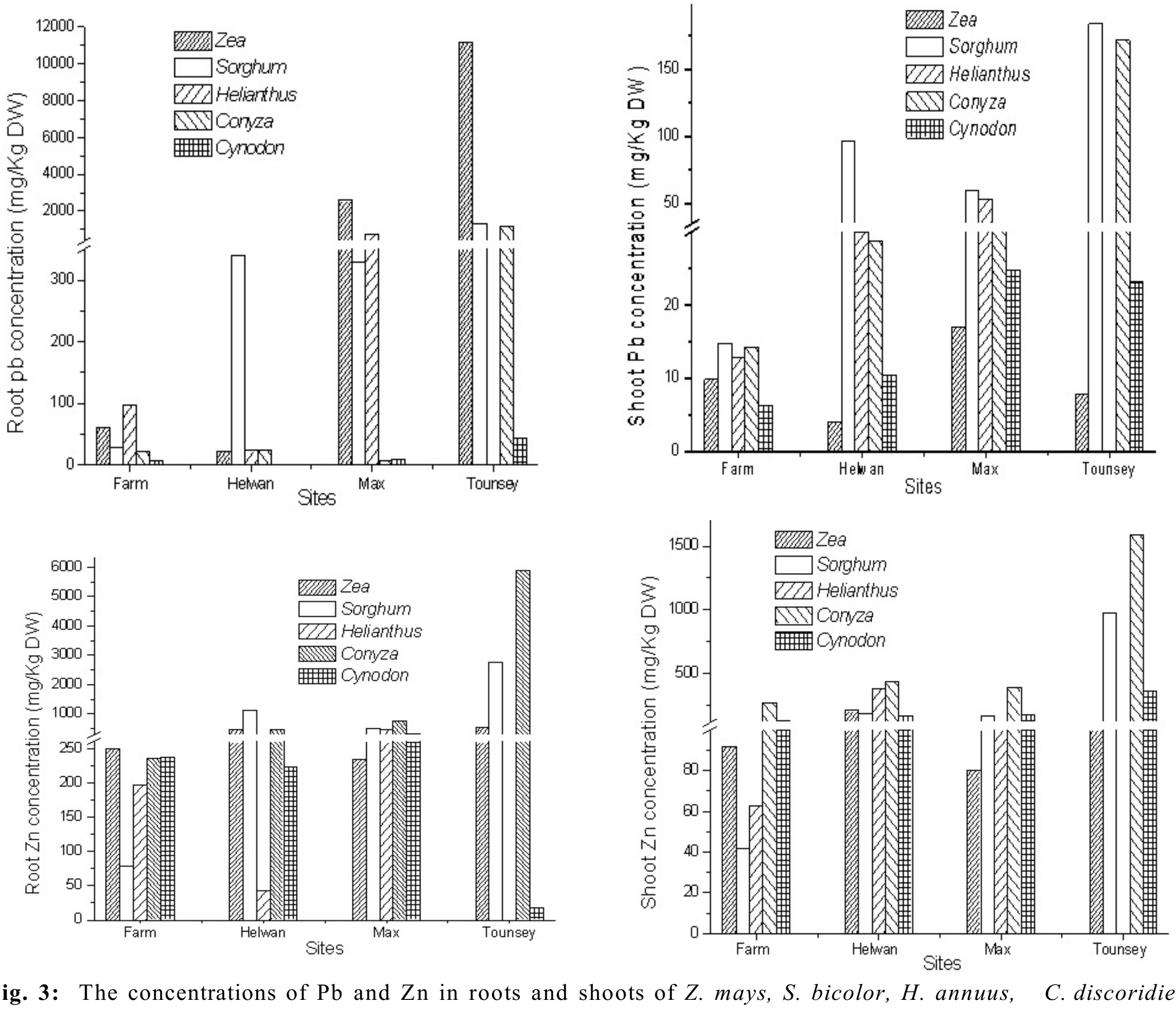

Fig. 3: The concentrations of Pb and Zn in roots and shoots of *Z. mays, S. bicolor, H. annuus, C. discoridies and C. dactylon* grown in different metal contaminated soils.

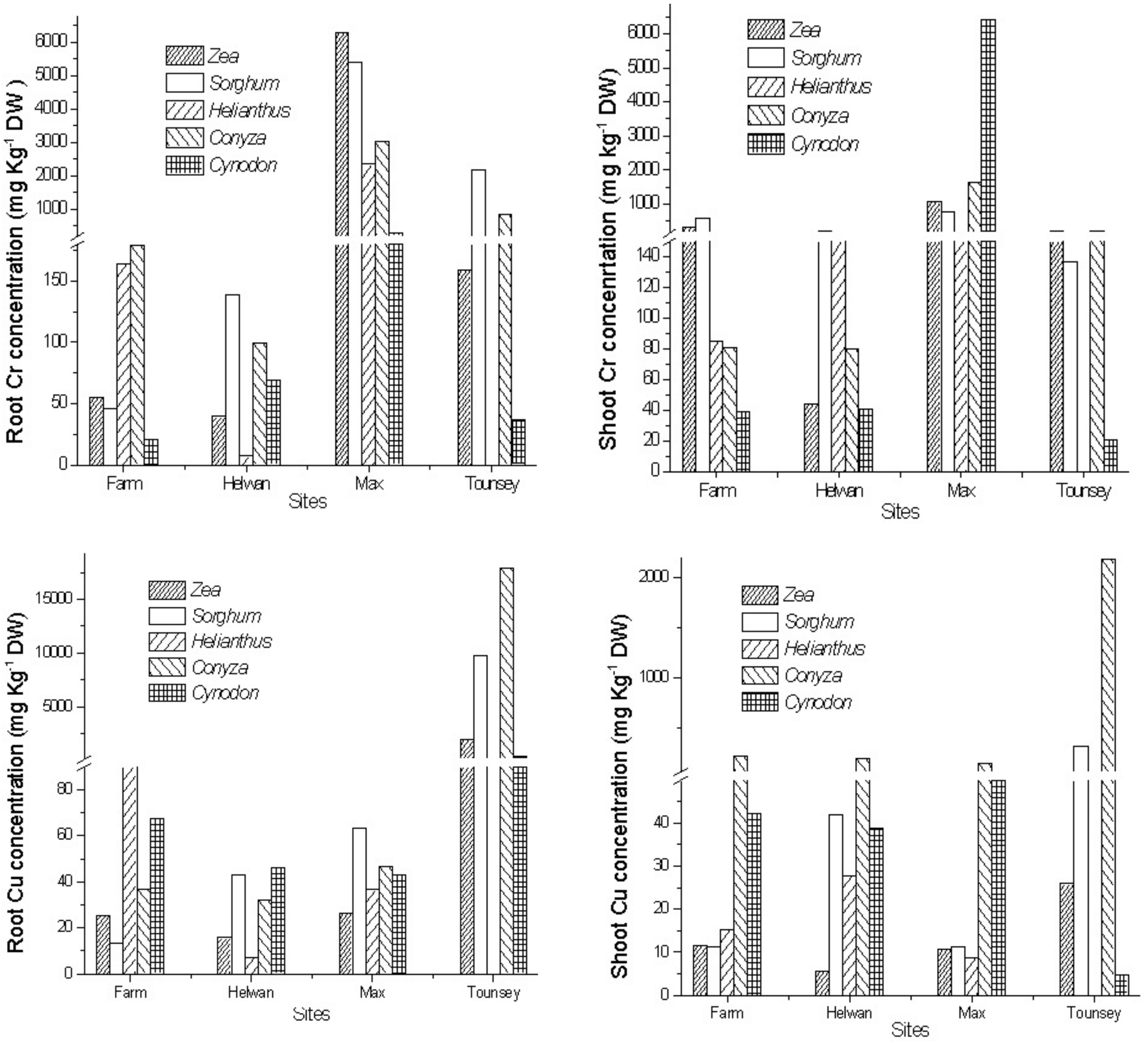

Fig. 4: The concentrations of Cr and Cu in roots and shoots of *Z. mays, S. bicolor, H. annuus, C. discoridies and C. dactylon* grown in different metal contaminated soils.

Plant Growth: Crop plants (*H. annuus*, *Z. mays*, and *S. bicolor*) and wild plants (*C. dactylon* and *C. discoridies*) were chosen for this study based on their high biomass, fast growth rates, and ability to remove heavy metals from contaminated sites[31, 32, 33, 34].

All five-plant species appeared healthy in the low and moderate metal contaminated soils (Farm and Helwan, respectively), whereas plant grown on the most Zn, Cu, and Pb contaminated Tounsey soils and Cr-Max contaminated soils showed yellowing to purpling of the leaves. In this experiment, there were significantly higher differences between the five plant species in shoot, root lengths and dry weights (Figure 1 and 2). The shoot and root length and dry weights were reduced in all plants grown on metal rich soils compared with the same plants grown on low and moderate metal contaminated soils. The root lengths in *Z. mays, S. bicolor, C. dactylon* and *C. discoridies* grown in Zn, Cu, and Pb rich Tounsey soils, were reduced by 13, 11, 9 and 4- fold lower than the root length of the same plants grown on the farm (control) soil, respectively. The shoot dry weights of *S. bicolor, Z. mays, C. discoridies* and *C. dactylon* grown on the Tounsey soils were also reduced by 39, 13, 4 and 3-fold, respectively, compared to the same plants grown in the control. Our results show that *H. annuus* is very sensitive to the highest Zn, Cu, and Pb contaminated soils. Shoot and root biomass of plant were depressed by growing on the Tounsey soil and died after 7 days. [33] reported that shoot and root biomass of sunflower seedlings was significantly smaller in the spill-affected soil than in the unaffected soil.[35] recorded that sunflower has a reasonable tolerance to heavy metals; it has been used for rhizofiltration because it has a high root uptake of metals but a low efficiency in their translocation from root to shoot.

Concentrations of Heavy Metals in the Shoots and Roots: Metal concentrations in plant tissues also differed among the five plant species grown on the same soils, indicating their different capacities for metal uptake. Lead concentrations in roots of plants was elevated and varied from 4 to 184 mg kg^{-1} DW. The highest concentrations of Pb (184 and 172 mg kg^{-1}) were found in the shoots of *S. bicolor* and *C. discoridies,* respectively, grown on the Tounsey soil. Lead concentrations were about 12.4 and 12.1 fold, respectively, higher than Pb concentration in the shoots of the same plants grown on the farm soil (Figure 3). However, the Pb concentration in the roots of *Z. mays*, *S. bicolor*, and *C. discorides* grown on the Tounsey soil was 185, 55, and 48 fold higher than Pb concentrations in the roots of the same plant species grown on low metal soils. Our results also indicated that *Z. mays* grown on the Max soils accumulated 43-fold higher Pb concentrations in roots than in plants grown on the farm soil. Plant species, *Z. mays*, *S. bicolor*, and *C. discoridies* had significantly higher concentrations of Pb in the roots than in the shoots. The ratio of Pb concentration in *Z. mays* roots to the total Pb concentration in the Tounsey soil was 5.9. [36] reported that the concentrations of Cd, Cu, Ni, Pb, and Zn were much higher in the roots than in the shoots.

C. discorides and *S. bicolor* accumulated significantly higher Zn concentrations in roots (5.9 and 2.8 g kg^{-1}) and shoots (1.5 and 0.9 g kg^{-1}), respectively, than other species grown on the Tounsey soil (Figure 3). Zn concentrations in the shoots of *H. annuus* were significantly higher than in roots by a factor of 8.7-fold. At the Max Cr-rich soils, *C. dactylon* attained a high concentration of Cr (6.4 g kg^{-1}) in the aboveground tissues, which was much higher than other plants, while *Z. mays*, *S. bicolor*, *C. discoridies* and *H. annuus* accumulated significantly higher Cr in its root tissues by a factor of 22, 19, 10, and 8- fold than *C. dactylon* grown on the same soils. However, Cu concentration (17.9 and 2.2 g kg^{-1}) in the roots and shoots of *C. discoridies*, respectively was significantly higher than the other four plant species grown on the Tounsey soils (Figure 4).[37] reported that *Solanum nigrum* and *Conyza Canadensis* can accumulate high concentrations of Cd.

Plants grown in metal-enriched substrata take up metal ions in varying degrees. Uptake is largely influenced by the availability of metals, which is in turn determined by both external (soil-associated) and internal (plant-associated) factors. Most previous studies have shown only poor correlations between metal uptake by plants and metal concentrations[38]. Our results shows that metal translocation into shoots appears to be restricted in cultivated plants so that harvesting such plants will not be an effective source of metal removal in soils. However, in the view of toxicology, this could be a desirable property, as metals would not pass into the food chain, and thus avoid potential risk to the environment.

REFERENCES

1. Cheng, S., 2003. Heavy metal pollution in China: origin, Pattern and control. Environ. Sci. Pollu. Res., 10, 192-198.
2. Turgut, C., 2003. The contamination with organochlorine pesticides and heavy metals in surface water in Kucuk Menderes River in Turkey, 2000-2002. Environ. Int. 29: 29-32.
3. Diels, L., van der Lelie, N., Bastiaens, L., 2002. New development in treatment of heavy metal contaminated soils. Rev. Environ. Sci. Biotech. 1, 75-82.
4. Lasat, M.M. 2002. Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. 31, 109-120.
5. Chaney, R.L., 1983. Plant uptake of inorganic waste constitutes. In: Parr et al (Eds) Land treatment of hazardous wastes. Noyes Data Corporation, Park ridge, N.J. pp: 50-76.
6. Baker, A.J.M., S.P. McGrath, C.M.D. Sidoli, R.D. Reeves, 1994. The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour., Conserv. Recyc. 11: 41- 49.
7. Suresh, B., Ravishankar, G.A. 2004. Phytoremediation-a novel and promising approach for environmental clean-up. Crit Rev Biotechnol. 24(2-3), 97-124
8. Baker, A.J.M., 1987. Metal tolerance. New Phyt. 106: 93-111
9. Baker, A.J.M., 1981. Accumulators and excluders-strategies in the response of plants to heavy metals. J. Plant Nutri. 3: 643-654
10. Baker, A.J.M., P.L. Walker, 1990. Ecophysiology of metal uptake by tolerant plants, CRC Press, Boca Raton, pp: 155-178.
11. De Vos, C.H.R., Schat, H., De Waal, M.A.M., Voojs, R., Ernst, W.H.O., 1991. Increased resistance to copper-induced damage of root cell plasmalemma in copper tolerant *Silene cucubalus*, Phys. Plant. 82, 523-528.

12. Baker, A.J.M., R.R. Brooks, 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecov. 1: 81-126.
13. Baker, A.J.M., S.P. McGrath, R.D. Reeves, J.A.C. Smith, 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In: N. Terry and G. Banuelos (Eds) Phytoremediation of contaminated Soil and Water. Lewis Publishers, Boca Raton, FL, pp: 85-107.
14. Ernst, W.H.O. 1996. Bioavailability of heavy metals and decontamination of soil by plants. Appl. Geochem. 11: 163-167
15. Ebbs, S.D., Kochian, L.V., 1997. Toxicity of zinc and copper to Brassica species: implications for phytoremediation. J. Environ. Qual. 5: 1424-1430
16. Black, B.C., D.D. Evans, J.I. White, L.E. Ensminger, F.E. Clark, 1982. Methods of Soil Analysis, Amer. Soc. Agron. Inc. Madison, Wisconsin USA
17. Alef, K. and P. Nannipieri, 1995. Methods in Applied Soil Microbiology and Biochemistry, Academic press, Harcourt Brace & Company, Publishers, London San Diego New York Boston Sydney Tokyo Toronto, pp. 105-121
18. Nelson, D.W., Sommers, L.E., 1986. Total carbon, organic carbon and organic matter. Methods for soil analysis, vol.2. ed. A. Klute, American Society of agronomy, Madison, wis. pp. 539-579
19. Thomes, G.W., 1982. Methods of soil analysis, Part 2 eds. A.L. Page Agronomy Monograph 9, Amer. Soc. Agron. Madison, WI, pp:159-165
20. McGrath S.P., Cunliffe, C.H., 1985. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Ni, Cr, Co, and Mn from soils and sewage sludge. J. Sci. Food and Agric. 36: 794-798
21. Angle, S.J., R. Chaney, D. Rhee, 1993. Bacterial resistance to heavy metals related to extractable and total metal concentrations in soil and media. Soil Biol. Biochem. 25: 1443-1446
22. Sanders, J.R., 1983. The effects of pH on the total and free ionic concentrations of manganese, zinc, and cobalt in soil solutions. J. Soil Sci. 34: 315-323
23. Chaney, R.L. P.M. Giordano, 1977. Microelements as related to plant deficiencies and toxicities, In: L.F. Elliott, F.J. Stevenson (Eds) Soils for management of organic wastes and wastewaters, American Society of Agronomy, Madison, WI, pp: 235-279
24. Ibekwe A.M., Angle J.S., Chaney R.L., van Berkum, P., 1995. Sewage sludge and heavy metal effects on nodulation and nitrogen fixation of legumes. J. Environ. Qual. 24: 1199-1204
25. Tessier, A. & Campbell, P.G.C. 1988. Partitioning of trace metals in sediments. In: Kramer, J.R. & H.E. Allen (Eds.), *Metal speciation: theory, analysis and application*. Lewis Publishers, Inc., Chelsea, MI. ISBN-10:0873711408
26. Chlopecka, A., Bacon, J.R., Wilson, M.J., Kay, J., 1996. Heavy metals in the environment. J. Environ. Qual. 25: 69-79
27. Wenzel, W.W., Jockwer, F. 1999. Accumulation of heavy metals in plants grown on mineralized soils of the Austrian Alps. Environ. Pollution 104: 145-155
28. Kabata-Pendias, A., Pendias, H. 2001. Trace elements in soils and plants, Boca Raton. FI.: CRC Press
29. Swaine, D.J. (1995). The trace-element content of soils, common bur. Soil Sci. Technolo. Common 48: 157-160
30. Brooks, R.R., 1987. Serpentine and its Vegetation. In: T.R. Dudley, Ecology, (Eds.), Phytogeography and Physiology Series, Portland, Or.
31. Murillo, J.M., Murillo, T., Maranon, F., Cabrera, R. L., 1999. Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. The Sci Total Environ. 242: 281-292
32. Chen, H., Cutright, T., 2001. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45: 21-28
33. Madejon, P., Murillo, J.M., Maranon, T., Cabrera, F., Soriano, M.A., 2003. Trace element and nutrient accumulation in sunflower plants two years after the Aznalcollar mine spill, Sci. Total Environ. 307, 239-257
34. Li, H., Wang, Q., Cui, Y., Dong, Y., Christie, P., 2005. Slow release chelate enhancement of lead phytoextraction by corn (*Zea mays* L.) from contaminated soil-a preliminary study. Sci. Total Environ. 339: 179-187
35. Kamnev, A.A. and van der Lelie, D. 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Bioscience Reports, 20: 239-258
36. Lombi, E., Zhao, F.J., Dunham, S.J., McGrath, S.P., 2001. Phytoremediation of heavy metal-contaminated soils: Natural hyperaccumulation versus chemically enhanced phytoextraction J. Environ Qual. 30: 1919-1926
37. Wei, S.H., Zhou Q.X., Wang, X., Cao, W., Ren L.P., Song, Y.F. 2004. Potential of weed species applied to remediation of soils contaminated with heavy metals. J. Environ. Sci. 16: 868-873
38. Greger, M., 1999. Metal availability and bioconcentration in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants-From Molecules to Ecosystems. Springer Press, Berlin, pp: 1-27
39. Suresh, B., Ravishankar, G.A. 2004. Phytoremediation-a novel and promising approach for environmental clean-up. Crit Rev Biotechnol. 24(2-3): 97-124