Caspian J. Env. Sci. 2005, Vol. 3, No.1, pp. 35~42

CJES
Caspian Journal of Environmental Sciences

[Research]

Heavy Metals Extraction Potential of Sunflower (*Helianthus annuus*) and Canola (*Brassica napus*)

***M. Solhi*[1*], *M.A. Hajabbasi*[2], *H. Shareatmadari*[2]**
1- Soil and Water Department, Isfahan Agricultural Research Center, Isfahan, Iran.
*2- Soil Science Department, College of Agriculture, Isfahan University of Technology, Isfahan 84154, Iran. *Corresponding author's e-mail: msolhi@ag.iut.ac.ir*

ABSTRACT

Phytoextraction is a remediation technology that uses plants to remove heavy metals from soil. The success of a phytoextraction process depends on adequate plant yield (aerial parts) and high metal concentrations in plant shoots. A pot experiment was conducted to investigate the combination effects of plants [sunflower (*Helianthus annuus*) and canola (*Brassica napus*)] with soil treatments (manure, sulfuric acid and DTPA). Treatments, included two plants and seven soil treatments, which were applied baeed on a completely randomized factorial design. Three replicates were used for each treatment.. The largest shoot dry weight biomass production occurred in manure treatments for both plants. The maximum shoot concentrations of Pb and Zn were 234.6 and 1364.4 mg kg^{-1} respectively in three mmoles DTPA kg^{-1} treatment of sunflower. Furthermore the results showed that sunflower had a higher extracting potential for removal of Pb and Zn from polluted soil.

Keywords: accumulation, canola, lead, phytoextraction, sunflower, zinc

Introduction

Heavy metal contamination is an increasing worldwide environmental concern (Body *et al*, 1991). The main sources of heavy metals in the environment are industrial, agricultural and urban activities. Contamination of soil-water-plant system with heavy metals is a form of chemical environmental load, which has health, economic and ecological importance (Alloway, 1995). Numerous studies have been conducted on remediation of heavy metal contaminated soil by employing thermal, chemical, physical and biological treatments, and significant progress has been made (Holden, 1989). These conventional methods are usually very expensive (Salt *et al*, 1995).

In recent years phytoextraction has been suggested by several authors as a green and low-cost technology to clean up metal polluted sites (Cunningham *et al.*, 1995; Kumar *et al*., 1995; Jorgensen, 1993; McGrath *et al*., 1993). This technique uses the ability of certain plants to accumulate heavy metals in a high concentration in their aboveground parts. The success of a phytoextraction process depends on biomass production and metal concentration in plant shoots (Raskin *et al*., 1994). Results of several studies under greenhouse or growth chamber conditions indicated that some crops and hyperaccumulating species have the potential to remove metals from polluted soils (Chaney, 1997; Shen *et al*., 1997).

Phytoextraction researches have been started using hyperaccumulators, like *Thlaspi caerulescens*, a member of the Brassicaceae family. Hyperaccumulators not only grow on high polluted soils, but also accumulate pollutants in a high concentration in their tissues (McGrath *et al*., 1993; Kumar *et al*., 1995). For instance, hydroponically grown *T. caerulescens* accumulated about 33600 mg Zn kg^{-1} in shoots (Salt *et al*., 1995). Some hyperaccumulators like *Ipomoea alpine* and *Haumaniastrum katangense* could accumulate about 12300 mg Cu kg^{-1} and 19800 mg Zn kg^{1} in their leaves, respectively (Baker and Walker, 1990). However, many of these species are slow growing and produce small amount of biomass and thus, cannot remove large quantities of heavy metals per unit of land area in a given period of time (Krueger *et al*., 1997). In contrast some plant species, producing a relatively large biomass, are capable of accumulating and tolerating moderate to high levels of heavy metals in their tissues. For instance, some varieties of corn (*Zea mays L.*), barley (*Hordeum vulgaris L.*) and ryegrass (*Lolium perenne L.*) have demonstrated significant heavy metal tolerance (Ebbs *et al*., 1997). On the other hand increasing and maintaining the bioavailability of heavy metals in soil solution, plays an important role in the phytoextraction process. Several chelating agents such as EDTA, DTPA, HEDTA, NTA and different organic acids have been used in pot and field experiments to enhance heavy metal uptake of plants (Kayser *et al*., 2000; Ebbs and Kochian, 1998; Blaylack *et al*., 1997; Huang and Cunningham, 1996). However in some cases in situ application of such chelates may pose the potential risk of water resources pollution. Sulfuric acid is one of the most abundant byproduct of petrochemical industry in Iran. It has relatively a low price (based on universal scale) due to low cost of transportation so it can be used as a decreasing pH agent in order to increase the bioavailability of metals. Although the metal concentrations in sunflower and canola are lower than hyperaccumulating plant species they produce a larger amount of aerial biomass. With regard to this our aims were: i) comparing the potential extraction of pb and Zn in sunflower and canola. ii) Study the effects of sulfuric acid, DTPA and composted manure treatments on enhancement of phytoextaction of sunflower and canola.

MATERIALS AND METHODS

Site description

Lead and zinc polluted soils [Fine, Loamy, Mixed, Typic, Torrifluent (Soil Survey Staff,1999)] were collected from surface around Bama mine at 20 km southwest of Isfahan city, central Iran (3598500N, 572000E, UTM, Zone 39) with the elevation of 1750 meters and mean annual precipitation of 145 mm.

Treatments and Statistical Design

The soil was incubated in the greenhouse at the temperature range of 18-25°C for 8 weeks. Soil moisture was raised to 80% of water holding capacity and maintained by periodical addition of water after weighing the pots. After incubation and bringing the soil to equilibrium, the soil was air-dried, grounded and passed through a 2 mm sieve. Then 5.0 kg of air-dried soil was placed in each plastic pot and fertilized with urea at a rate of 60.0 mg N kg^1 dry soil,, diammonium phosphate at a rate of 30.0 mg P kg^{-1} potassium sulphate at a rate of 40.0 mg K kg^{-1} . The experiment was conducted using a completely randomized factorial design containing two plants and seven soil treatments. Three replicates were used for each treatment. The treatments were: two levels of sulfuric acid [0.5 (S1) and 1.0 (S2) mmole acid kg^{-1} of pot dry soil], two levels of Diethylen Triamine Pentaacetic Acid [1.5 (D1) and 3 (D2) mmoles DTPA kg^{-1} of pot dry soil], two levels of composted manure [7.5 (M1) and 15 (M2) g kg^{-1} of pot dry soil], and control (C). About 15 seeds of sunflower [*Helianthus annuus* (*cv.hybride, hysun 25*)] and canola [*Brassica napus* (*cv.hybride, hyola* 401)] were sown in separate pots for respective treatments. After germination, the seedlings were trimmed to five plants per pot and grown for 60 days. The DTPA and acid solution were applied with irrigation water whereas composted manure was mixed thoroughly with soil before sowing. Soil moisture was maintained at 60% of water holding capacity based on soil moisture characteristic curve, which was plotted at 10, 30, 50, 100, 300, 500, 1000 and 1500 kPa (Klute, 1986).

Samples Collection and Analyses

Plants were harvested by cutting the shoots at the soil surface and removing the roots from the pots. The shoots and roots were washed with tap water, rinsed with deionized water and dried at 80° C for 24 h. The dry weight of shoots and roots were measured as dry biomass (DBM). Sub-samples of 2.0 g were digested in 6.0 ml of 65% HNO_3, 2.0 ml 2% H_2O_2 and 2.0 ml of distilled water, and heated at 100 °C for 25 minutes. The solution was then filteredrated through Whatman filter paper No 42. For Soil sampling the soil was thoroughly removed from the pot at moisture of about field capacity. The plant roots were then carefully removed from the soil by gently shaking the soil. The remaining soil was mixed and a 250 gram sample was collected for chemical analysis after harvesting. Soil pH was measured in 1:2.5 soil distilled water suspensions. Clay, silt and sand percentage were determined by hydrometer method (Day, 1965). Organic carbon (OC) was determined using wet oxidation method (Walkley and Black, 1934). Total Pb and Zn concentrations in soil samples were extracted using a mixture of $HClO_4$, HF and HNO_3

(Pratt, 1965). Ten ml of concentrated nitric acid was added to 2.0 gram of soil and kept overnight. Three drops of concentrated H_2SO_4 and 10 ml HF were added to the samples and the temperature raised slowly up to 200°C. Fifteen ml of concentrated HNO_3, 2 ml of concentrated H_2SO_4 and 5 ml of $HClO_4$ were added to solution and extracted through Whatman filter paper No 42. DTPA Extractable concentrations of Pb and Zn were determined using the method of Lindsay and Norvel (1978). The extracting solution contains 0.005 M DTPA, 0.01 M CaCl.H2O and 0.1 M triethanolamine (TEA). Ten g of air-dried soil were placed to polyethylene bottle, 20 ml of extract was added and shaken for 2.0 hours andfiltered through Whatman filter paper No.42. The Pb and Zn concentration of soils and plants were measured using Flame Atomic Absorption Spectrophotometry (FAAS) Perkin Elmer model 2380.

Statistical analyses

Statistical analyses were performed on log-transformed concentration (data were log normal). Regression analyses were performed with SAS software version 6.12. Analysis of variance (ANOVA) was performed using the GLM procedure (general linear model) of SAS 6.12, to compare treatment effects on heavy metal content in soil and plant tissues. If the F-value indicated significant difference ($p<0.05$). Mean comparisons were carried out using Duncan test.

RESULTS AND DISCUSSION

Some physicochemical properties of soil are summarized in Table 1. The selected soil was clay loam, nonsaline, low in organic carbon and rich in Pb and Zn inherited from mine parent material. The average Pb and Zn total concentrations of the soil were 1564 and 2739 mg kg^{-1}, respectively while DTPA extractable concentr-ations of Pb and Zn were 29 and 182 mg kg^{-1}, respectively. Variation of DTPA extractable Pb and Zn concentration during 8 weeks of incubation are shown in figure 1. Eight weeks incubation of soil resulted in a gentle increase of DTPA extractable concentrations of Pb and Zn and then reached an equilibrium in the soil with nearly constant levels of metal concentrations.

Table 1. Physicochemical properties of studied soil

Parameters	Amount
EC_e	1.8 dS m^{-1}
pH	7.3
OC	1.1%
Total Pb	1564 mg kg^{-1}
DTPA Ext.Pb	29 mg kg^{-1}
Total Zn	2739 mg kg^{-1}
DTPA ext. Zn.	182 mg kg^{-1}
Clay	390 g kg^{-1}
Silt	360 g kg^{-1}
Sand	250 g kg^{-1}

OC: Organic carbon
EC_e: Electrical conductivity of saturated paste

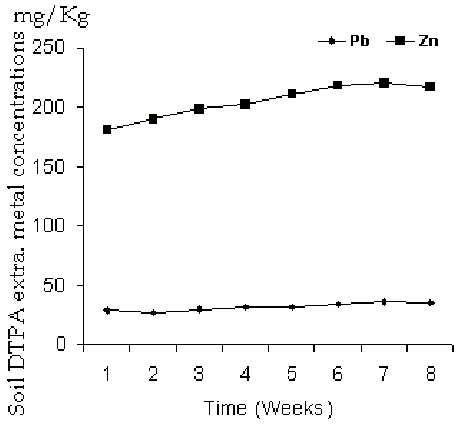

Fig 1. Variation of DTPA extractable metal concentrations during incubation time

Effects of treatments on biomass production

The ANOVA analysis showed a significant difference ($p<0.05$) for biomass production among the treatments (Table 2). Both composted manure treatments (M1 and M2) increased shoot biomass of sunflower and canola significantly ($p<0.05$). The largest shoot dry biomass production occurred in manure treatment both for sunflower (76.5 g per pot) and canola (46.7 g per pot). Composted manure improved physico-chemical and biological properties of soil and resulted in a significant increment of plant growth (Paul, 1984). The least values of shoot dry biomass of sunflower and canola were found in the D2 treatment (Table 2). Treating the soil with 3 mmoles DTPA kg^{-1} increased the metals availability thus phytotoxicity may inhibit plant growth and biomass reduction. Applying 1.5 mmoles DTPA kg^{-1} reduced dry biomass of canola by 24.0% but it had no significant effect on dry biomass of sunflower indicating sunflower was more tolerant than canola. Shen (1997) reported that after 7d of EDTA (3.0mmole kg^{-1}) application, dry matter yield of cabbage, hanelt, mung bean and wheat decreased significantly compared with those without EDTA treatment. Sulfuric acid treatments did not show any significant change in biomass production of sunflower and canola.

Table 2. Dry biomass (DBM) and dry biomass coefficient (DBMC) of sunflower and canola in different treatments.

Plant	Tre. [1]	DBM (g) [2]	DBMC
Sunflower	S1	67.0b	0.88
	S2	64.2b	0.84
	D1	65.1b	0.85
	D2	64.1b	0.84
	M1	73.8a	0.96
	M2	76.5a	1.00
	C	65.7b	0.86
Canola	S1	37.0d	0.48
	S2	35.6d	0.47
	D1	28.5de	0.37
	D2	21.5e	0.28
	M1	43.3c	0.57
	M2	46.7c	0..61
	C	36.2d	0.47

[1]S1 and S2 stand for 0.5 and 1.5 mmoles sulfuric acid kg^{-} of pot soil, D1 and D2 stand for 1.5 and 3.0 mmoles DTPA kg^{-1} of pot soil, M1 and M2 stand for 7.5 and 15.0 g kg^{-1} of pot soil, and (c) for control.
[2]Values followed by the same letter within columns are not significantly different at $p<0.05$
DBM: Dry weight of aerial parts (g)
DBMC: The ratio of dry weight biomass to the maximum observed value

Effects of treatments on Pb shoot and root concentrations of plants

The ANOVA analysis showed significant differences in shoot and root Pb concentrations of sunflower and canola ($p<0.05$). Maximum Pb concentrations were found in D2 treatment in shoot of sunflower, whereas for canola the maximum shoot Pb concentrations occurred in the D1 treatments (Table 3). Compared to control, Pb shoot concentrations of sunflower increased by 1.10, 1.60 and 2.00 fold in manure, acid and DTPA treatments, respectively. The values for canola were 1.50, 1.70 and 2.26 fold (Table 3). Although the increasing rate of Pb concentrations in shoot of canola was more than that of sunflower but the absolute shoot Pb concentrations of sunflower were higher than that of canola.

Root Pb concentrations of sunflower increased 1.20, 1.70 and 1.90 fold in manure, acid and DTPA treatments respectively compared to control. The same values for canola were 1.20, 1.40 and 2.00 fold (Table 3). The greatest increasing rate of shoot and root Pb concentrations occurred in DTPA treatments (D1 and D2) both for sunflower and canola (2.00, 2.26, 1.90 and 2.00 fold).

Effects of treatments on Zn shoot and root concentrations of plants

The ANOVA analysis showed significant differences in shoot and root Zn concentrations of sunflower and canola ($p<0.05$). The maximum Zn concentrations were found in D2 treatment in shoot of sunflower and canola (Table 3). Zinc concentrations in shoot of sunflower increased 1.87, 2.04 and 2.80 fold in manure, acid and DTPA treatments in comparison with control. The same values for canola were 1.30, 1.46 and 1.78 fold (Table 3) which were significantly lower than that of sunflower ($p<0.05$).

Root Zn concentrations of sunflower increased 1.30, 1.50 and 1.80 fold in manure, acid and DTPA treatments whereas the values for canola were 1.40, 1.70 and 2.25 fold (Table 3). In the case of Zn also the greatest increasing rate of Zn concentration in shoots and roots of sunflower and canola were observed in DTPA treatments (D1 and D2) (2.80,1.78,1.80 and 2.25 fold).

The results showed that DTPA treatments increased the supply of Pb and Zn for root uptake of sunflower and canola by increasing

Table 3. Pb and Zn concentrations of soil and plants (mg kg^{-1})

Plant	Tre.[1]	Soil DTPA Extraction Pb[2]	Shoot Pb	Root Pb	Soil DTPA Extraction Zn	Shoot Zn	Root Zn
Sunflower	S1	69b	138b	369b	69b	138b	369b
	S2	94a	231a	451a	94a	231a	451a
	D1	90a	230a	450a	90a	230a	450a
	D2	94a	235a	467a	94a	235a	467a
	M1	58bc	128b	291bc	58bc	128b	291bc
	M2	59bc	128b	301bc	59bc	128b	301bc
	C	33c	115b	238c	33c	115b	238c
Canola	S1	65b	79.bc	251c	65b	79.bc	251c
	S2	60bc	84bc	04bc	60bc	84bc	04bc
	D1	95a	109b	333bc	95a	109b	333bc
	D2	90a	106b	347bc	90a	106b	347bc
	M1	57bc	79bc	205bc	57bc	79bc	205bc
	M2	55bc	65bc	185d	55bc	65bc	185d
	C	39c	48c	165d	39c	48c	165d

[1]S1 and S2 stand for 0.5 and 1.5 mmoles acid kg^{-1} of pot soil, D1 and D2 stand for 1.5 and 3.0 mmoles DTPA kg^{-1} of pot soil, M1 and M2 stand for 7.5 and 15.0 g kg^{-1} of pot soil, and (c) for control.
[2]Values followed by the same letter within columns are not significantly different at $p<0.05$

metal availability in soil. Making the metals available, DTPA treatments were more efficient than that of the other treatments, so that shoot and root Pb and Zn concentrations were significantly higher than that of manure and acid treatments (Table 3). In addition Pb and Zn concentrations in shoot and root of sunflower were more than that of canola for all treatments, showing a higher potential for metals removal. Lombi *et al*. (2001) reported that using of EDTA in two soils (French and UK soils) increased the concentrations of Cd, Cu and Zn in the root of *Thlaspi caerulescens* by one to three fold as compared to control. Application of EDTA had no significant effect on the concentrations of metals in the shoot except for Zn which showed a 50% increase in the UK soil.

Soil and plant shoot metal relationships

The metal concentrations of plant shoots had a positive linear correlation with soil DTPA extractable metal concentrations (Fig 2). The relationship between Pb and Zn DTPA extractable concentrations in soil and Pb and Zn shoots of sunflower and canola were as follows (n=24):

For sunflower: (P<0.01)

$Shoot_{Pb}$=2.1896 $Soil_{DTPA\ Extra.Pb}$ +16.57, R^2=0.7697 (Fig. 2a)

$Shoot_{Zn}$=2.9279$Soil_{Zn\ DTPA\ Extra..Zn}$+13.589, R^2=.9191 (Fig. 2b)

For canola (P<0.01)

$Shoot_{Pb}$=0.9329$Soil_{DTPAExtra.Pb}$+20.038, R^2=0.7425 (Fig. 2c)

Shoot $_{Zn}$=1.3757$Soil_{Zn\ DTPAExtra..Zn}$+206.7 , R^2=0.8580 (Fig. 2d)

Stronger correlations existed between Zn concentrations in soil and shoots for both canola and sunflower.

Wenger *et al*. (2002) stated that in *Zea mays* shoot Zn, linearly increased to about 1400 mg kg^{-1} dry weight as $NaNO_3$-extractable Zn concentration in the soil increased to about 60 mg kg^{-1}. Lehoczky (1996) also found a positive linear correlation between DTPA extractable Cd and Zn of soil with Cd and Zn concentration of upper plant parts.

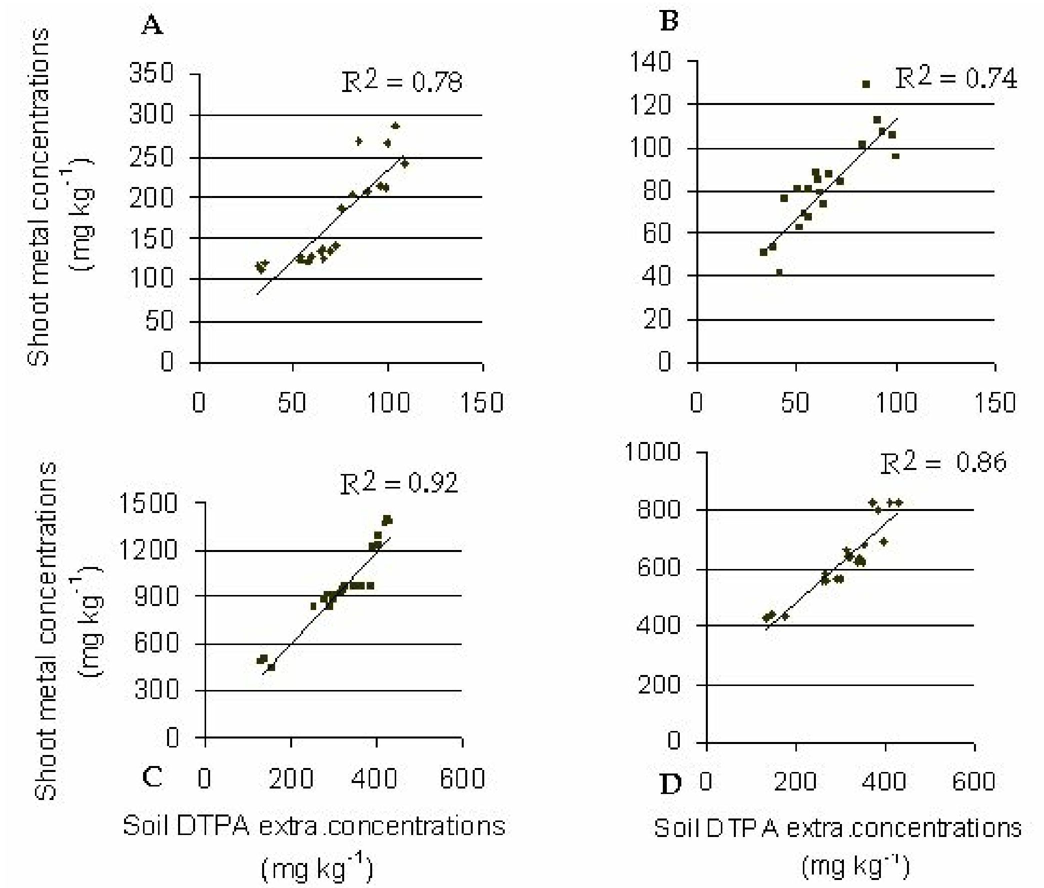

Fig 2. Correlation between soil DTPA extractable Pb and shoot Pb concentration of sunflower (a) and canola (b), Correlation between soil DTPA extractable Zn and shoot Zn concentration of sunflower (c) and canola (d).

Pb Uptake

Lead shoot:root ratios of sunflower were significantly greater than canola in all treatments (Table 4) that shows less resistance in translocation of Pb from root toward shoot in sunflower. Although Lombi *et al*., (2001) indicated that using EDTA could efficiently overcome the diffusion limitation of metals to root surface and resulting a lower Pb shoot/root ratio but our results showed that these ratios are small in all treatments including control for canola. Therefore the small shoot/root ratios in canola cannot merely be related to the chelating agents.

Lead soil plant transfer coefficient (SPTC) which is defined as the ratio of shoot metal concentrations to the background soil total concentration is shown in Table V. SPTC of sunflower were about two times as much as canola's, indicating sunflower can extract more Pb than canola per kg of dry mass. Metal removal potential of accumulators is greatly related to the biomass production and metal concentration of aboveground tissues; therefore in this study shoot dry biomass also was considered. In this regard, uptake index (UI), which is obtained by multiplying of shoot dry biomass coefficient by shoot metal concentration, was recommended. Dry biomass coefficient (DBMC) also is defined as ratio of shoot dry biomass of a specific treatment to the maximum value of dry biomass among all treatments (Huang *et al*., 1997). UI is a relative criteria having the capability of ranking the treatments based on their respective metal removal. The larger metal UI, the higher potential of metal removal.

The largest amount of Pb UI was obtained for sunflower (196.0) in D2 treatment while for canola (45.0) obtained in M1 treatment (Table 4). Therefore the most efficient treatment for Pb removal was 3 mmoles DTPA kg^{-1}(D2) with sunflower. The Pb UI of canola in D2 treatment was smaller than values observed for acid treatments (Table 4). The reason may be metal phytotoxicity in D2 treatments, which resulted a noticeable decline in dry biomass production of canola.

Zn Uptake

The maximum value of Zn shoot/root ratio for sunflower was 1.21 in M1 treatment and for canola was 1.61 in M2 and control. Zn shoot:root ratios for canola were greater than that for sunflower in all treatments (Table 4) indicating that Zn translocation from root toward shoot in canola was greater than that in sunflower. Although the Zn shoot:root ratios of canola were more than that in sunflower the absolute Zn concentrations in root and shoot of sunflower were more than those in canola. The maximum values of Zn SPTC in sunflower and canola were found in D2 treatment (Table 4). Zinc SPTC in sunflower were significantly higher than that in canola in all treatments ($p<0.05$) showing a higher accumulating potential of Zn in upper parts of sunflower. The high Zn concentrations in sunflower at D2 treatment are in good agreement with findings of Kayser *et al*. (2000) in a greenhouse study where *Nicotiana tabacum* and *Zea mays* were found to take up a great amount of Zn when metal solubility in soil was enhanced by addition of elementary sulfur.

Table 4. Pb and Zn shoot/root ratio, Soil Plant Transfer Coefficient (SPTC) and Uptake index (UI)

Plant	Tre.[1]	Pb Shoot/Root Ratio	Pb SPTC	Pb UI	Zn Shoot/Root Ratio	Zn SPTC	Zn UI
Sunflower	S1	0.37	0.09	121	1.16	0.34	820
	S2	0.51	0.15	193	1.13	0.35	804
	D1	0.51	0.15	195	1.14	0.45	1047
	D2	0.50	0.15	196	1.09	0.50	1142
	M1	0.44	0.08	123	1.21	0.32	848
	M2	0.43	0.08	128	1.15	0.31	851
	C	0.49	0.07	99	1.07	0.17	398
Canola	S1	0.31	0.05	38	1.52	0.24	313
	S2	0.41	0.05	39	1.29	0.23	293
	D1	0.33	0.07	41	1.33	0.27	274
	D2	0.31	0.07	30	1.22	0.30	230
	M1	0.39	0.05	45	1.44	0.21	321
	M2	0.35	0.04	40	1.61	0.21	334
	C	0.29	0.03	22	1.61	0.16	206

[1]S1 and S2 stand for 0.5 and 1.5 mmoles acid kg^{-1} of pot soil, D1 and D2 stand for 1.5 and 3.0 mmoles DTPA kg^{-1} of pot soil, M1 and M2 stand for 7.5 and 15.0 g kg^{-1} of pot soil, and (c) for control.

SPTC: The ratio of aerial metal concentration to background soil total concentration

UI:It is obtained by multiplying of aerial metal concentration to dry biomass coefficient

Calculated Zn UIs for sunflower were much greater than those for canola (Table 4). The maximum Zn UI for sunflower was found in D2 treatment (1142.0) while the maximum Zn UI of canola was found in M1 treatment (334.0). The most efficient treatment for Zn removal also was sunflower with 3 mmoles DTPA kg^{-1} treatment.

CONCLUSIONS

This study was launched to evaluate the effects of different levels of DTPA, sulfuric acid, manure and control on biomass production and Zn and Pb accumulation in plant tissues. The species showed different response due to treatment changes,so that the maximum aerial biomass was obtained in M2 treatment both for canola and sunflower. The maximum Pb and Zn concentrations were found in D2 treatment for sunflower and canola whereas the minimum concentrations of Zn and Pb were found in control. Metal concentrations in plant shoots had a positive linear correlation with soil DTPA extractable method of analyzing. When sunflower was treated with 3 mmoles of DTPA kg-1 soil, the most efficient combination of plant , treatment for Pb and Zn extraction was observed.

ACKNOWLEDGMENTS

This work was a part of PhD dissertation at the Department of Soil Science , Isfahan University of Technology and supported by Soil and Water Department of Isfahan Agricultural Research Center. We are grateful to Dr. A. A. Shahabi and Dr. S. Ismaeelkhanian for their valuable help. The contribution of the reviewers is appreciated

REFERENCES

Alloway, B. J. (1995) *Heavy Metals in Soils*, 2nd Ed; Blackie Academic and Professional, London, England, pp 287.

Baker, A. J. M. and Walker, P. I. (1990) Ecophysiology of Metal Uptake by Tolerant Plants. In J.A. Shaw (ed), *Heavy Metal Tolerance in Plants: Evolutionary Aspects*; CRC Press, Boca Raton, FL,USA, pp. 157-177.

Blaylock, M, J., Salt, D. E., Dushenkov, S, O., Gussman, C., Kapulnik, Y., Ensley, B.D. and Raskin, I. (1997) Enhanced Accumulation of Pb in Indian mustard by Soil Applied Chelating Agents. *Environ. Sci. Technol*. **31**, 860-865.

Body, P. E., Dolan, P. R. and Mulcahy, D. E. (1991) Environmental Lead, A review. Crit. Rev. Environ. Control **20**, 299-310.

Chaney, R. L., Malik, M., Li, Y. M., Brown, S. L., Brewer, E. P., Angle, J. S. and Baker, A. J. M. (1997) Phytoremediation of Soil Metals. *Curr. Opin. Biotechnol*. **8**, 279-284.

Cunningham, S. C., Berti, W. R. and Huang, J. W. (1995) Phytoremediation of Contaminated Soils. *TIBTECH* **13**, 393-397.

Day, P. R. (1965) Particle Fractionation and Particle Size Analysis. In C.A. Black (ed.), *Method of Soil Analysis* Part 1. American Society of Agronomy, Madison, Wisconsin, USA, pp. 545--565.

Ebbs, S. D., Kochian, L. V. (1998) Phytoextraction of Zinc by Oat (*Avena sativa*), Barely (*Hordeum vulgaris*) and Indian mustard (*Brassica junceae*). *Environ. Sci. Technol*. **32**, 802-806.

Ebbs, S. D., Lasat, M. M., Brady, D. J., Cornish, J., Gordon, R. and Kochian, L.V. (1997) Phytoextraction of Cadmium and Zinc from a Contaminated Soil. *J. Environ. Qual*. **26**, 1424-1430.

Holden, T. (1989) *How to Select Hazardous Waste Treatment Technologies for Soils and Sludges* : Alternative, Innovative, and Emerging Technologies. Noyes Data Corporation, Park Ridge, NJ.pp.341.

Huang, J. W., Chen J., Berti, W. B .and Cunningham, S. D. (1997) Phytoexraction of Lead-Contaminated Soils: Role of Synthetic in Lead Phytoextraction. *Environ. Sci. Technol*. **31**, 800-805.

Huang, J. W., Cunningham, S. D. (1996) Lead Phytoexraction: Species Variation in Lead Uptake and Translocation. *New Phytol*. **134**, 75-34.

Jorgensen, S. E. (1993) Removal of Heavy Metals from Compost and Soil by Ecotechnological Methods. *Ecological Engineering*. **2**, 89-100.

Kayser, A., Wenger, K., Attinger, W., Felix, H.R., Gupta, S.K. and Schullin, R.: 2000, Enhancement of Phytoextraction of Zn, Cd and Cu from Calcareous Soil: The Use of NTA and Sulphur Amendments. *Environ. Sci. Technol*. **24**, 217-225

Klute, A. (1986) Water Retention: Laboratory methods, In A. Klute (ed), *Method of Soil Analysis*, Soil Science Society of America, Madison, Wisconsin,USA, pp. 635-662.

Krueger, E. L., Anderson, T. A. and Coats, J. R. (1997) Phytoremediation of Soil and Water Concentrations, Symp. Series 664; ACS, Washington DC.

Kumar, P. B. A. N., Dushenkov, V., Motto, H. and Raskin, I. (1995) Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils. *Environ. Sci. Technol.* **29**, 1232-1238.

Lehoczky, E., Szabados, I. and Marth, P. (1996) Cd Content of Plants as Affected by Soil Cd Concentration. Commun. *Soil .Sci. Plant Analysis*. **27,** 1765--1777.

Lindsay, W. L., Norvell, W. A. (1978) Development of a DTPA Soil Test for Zinc, Iron, Manganase 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.

McGrath, S. P. (1988) Phytoextraction for Soil Remediation. In R. R.Brooks (ed.). *Plants that Hyperaccumulate Heavy Metals*. CAB international, Wallingford, Oxon, Uk, New York. pp. 261--287

McGrath, S. P., Sidoli, M. D., Baker, A. J. M. and Reeves, R. D. (1993) The Potential for the Use of Metal Accumulating Plants for the In Situ Decontamination of Metal Polluted Soils. In *Integrated Soil and Sediment Research*. H. J. P. Eijsackers and T.Hamers (Eds.). Kluwer Academic publishers, Dordrecht, the Netherlands. pp. 673--676.

Paul, E. A., (1984) Dynamics of Organic Matter Soils. *Plant Soil* **76**, 275--285.

Pratt, P. F. (1965) Digestion with Hydrofluoric and Perchloric Acid for Total Potassium and Sodium. In C. A. Black(ed.) *Methods of Soil Analysis*, Part 2 Soil Science Society of America, Madison, Wisconsin, USA, pp. 1019--1021.

Raskin, I., Kumar, P. B. N. A., Dushenkov, V. and Salt, D. E. (1994) Bioconcentration of Heavy Metals by Plants. *Curr. Opin. Biotechnol*. **5**, 285--290.

Robinson, B. H., Leblanc, M., Petit, D., Brooks, R.R., Kirkman, J.H. and Gregg, P. E. H. (1988) The Potential of *Thlaspi caerulescens* for Phytoremediation of Contaminated Soils. *Plant and Soil*. **203**, 47--56.

Salt, D. E., Blaylock, M., Kumar, N. P. B. A, Dushenkov, V., Ensley, B. D., Chet, I. and Raskin, I. (1995) Phytoremediation: A Novel Strategy for the Removal of Toxic Elements from the Environment Using Plants. *Biotechnol*. **13**: 468--475.

Shen, Z. G., Zhao, F. J. and McGrath, S. P. (1997) Uptake and Transport of Zinc in the Hyperaccumulator *Thlaspi caerulescens* and Nonhyperaccumulator *Thlaspi ochroleucum*. *Plant Cell Environ*. **20**, 898--906.

Soil survey staff. (1999) *Soil Taxonomy: A basic system of Soil Classification for making and interpreting. Soil Surveys*, 2nd edn. Agric. Handbook, Vol. 436. USA. Govt. print. Office, Washington, DC.pp. 869.

Walkley, A., Black, C. A. (1934) An Examination of the Degtareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method .*Soil Sci*. **37**, 29--38.

Wenger, K., Gupta, S. K., Furrer. G. and Schulin,R (2002) Zinc Extraction Potential of Two Common Crop Plants, Nicotiana tabacum *and* Zea mays. *Plant and Soil.* **242**, 217--225.

(Received: May. 2, Accepted June 3, 2005)