Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb, and Zn

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ABSTRACT

The use of plants to remove heavy metals from soil (phytoremediation) is expanding due to its cost-effectiveness as compared to conventional methods and it has revealed a great potential. Since contaminants such as Pb or Cd have a limited bioavailability in the soil, methods to facilitate their transport to the shoots and roots of plants are required for successful phytoremediation. The objective of this study was to investigate the effects of addition of different rates (0, 3, 6 and 12 mmol/kg) of ethylene diaminetetraacetate (EDTA) on heavy metal availability in soils contaminated with 50 mg/kg Cd ($CdCl_2$), 50 mg/kg Cu ($CuSO_4$), 50 mg/kg Pb [$Pb(NO_3)_2$] and 50 mg/kg Zn ($ZnSO_4$), and on the capacity of canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) plants to uptake Cu, Cd, Pb and Zn in a growth chamber. Results indicated that EDTA application increased heavy metal availability and uptake by plants. Significant differences were obtained in both species and plant parts. As for plant species tested, canola was more effective in the uptake of Cu, Cd, Pb and Zn. Root heavy metal uptake of both species was higher than shoot heavy metal uptake.

Keywords: canola; Indian mustard; heavy metal availability; EDTA; phytoextraction

It is almost impossible to imagine soil without at least trace levels of heavy metals. However, human, natural and anthropogenic activities concentrated some of these elements in certain areas up to dangerous levels for living organisms (Chatterjee and Chatterjee 2000, Kim et al. 2001). Use of sludge or urban composts, pesticides, fertilisers and emission from municipal waste incinerators, car exhausts, residues from metalliferous mining and metal smelting industry polluted extensive areas throughout the world (Zantopoulos et al. 1999, Herawati et al. 2000, Brun 2001). An excessive accumulation of heavy metals can have deleterious effects on soil fertility, affect ecosystem functions and constitute a health risk to animals and human beings.

The major problem hindering plant remediation efficiency is that some of the metals are immobile in soils and their availability and phytoextraction rate are limited by solubility and diffusion to the root surface. Chemical enhancements were used to overcome this problem. Several studies documented that chelating agents such as ethylenediamine-tetraacetic acid (EDTA), *N*-(2-hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA) and citric acid (CA) can be used to increase metal mobility, thereby enhancing phytoextraction (Elless and Blaylock 2000, Chen and Cutright 2001, Chen et al. 2003). Laboratory studies showed that EDTA is effective in removing Pb, Zn, Cu and Cd from contaminated soils, although extraction efficiency depends on many factors such as the lability of heavy metals in soil, the strength of EDTA, electrolytes, pH and soil matrix (Elliott and Brown 1989, Brown and Elliot 1992, Heil et al. 1999, Papassiopi et al. 1999).

Huang et al. (1997) and Blaylock et al. (1997) were able to achieve rapid accumulation of Pb in shoots higher than 1% of shoot dry biomass with EDTA, the most commonly used chelating agent.

Supported by the Foundation for Generous Financial Support of Atatürk University, Project No. 2004/261.

Much of the previous work on chelate-assisted soil washing and phytoextraction has focused on Pb. The aim of the present study was to investigate the ability of EDTA in enhancing the uptake and phytoextraction of Cd, Cu, Zn and Pb from heavy metal contaminated soils by the use of canola (*Brassica napus*) and Indian mustard (*Brassica juncea* L.) plants under laboratory conditions.

MATERIAL AND METHODS

The soil was sampled in a depth of 0–15 cm from agricultural fields in Erzurum province, Turkey (39°55'N, 41°61'E), dried indoors until it could be crumbled to pass through a 4 mm-sieve for pots experiment and a 2 mm-sieve for analyses of physicochemical properties. The soil is classified as Ustorthents according to the soil taxonomy (Soil Survey Staff 1999). Some physical and chemical properties of soil are given in Table 1.

Soil was transferred to polyethylene pots (20 cm diameter and 15 cm depth). Each soil sample was treated with 50 mg/kg Cd ($CdCl_2$), 50 mg/kg Cu ($CuSO_4$), 50 mg/kg Pb [$Pb(NO_3)_2$] and 50 mg/kg Zn ($ZnSO_4$) as pollutants. The soil contamination was performed by adding a specific amount of heavy metals; they were dissolved in deionised water into each pot (2000 g soil/pot) and then saturated, air dried at room temperature and thoroughly mixed. The wetting-drying mixing process was repeated to ensure soil equilibrium for one-month period under natural light at a minimum temperature of 10–11°C and maximum of 25–30°C and a relative humidity of about 30–40%. After the incubation period, soils contaminated with heavy metals were treated with ethylene diaminetetra acetate (EDTA) at the rates of 0, 3, 6, and 12 mmol/kg. Concentrations of EDTA were based on upper soil surface layer and were sprayed on the soil surface, following procedures described by Vogeler et al. (2001).

Plants were grown in a growth chamber (light 300 mmol/m^2/s/16 000 lux). They were maintained in a 20/18°C (day/night) growth chamber with 14 h daylight, and a relative humidity 38/32% (day/night). Preliminary tests on polluted soil indicated that both canola (*Brassica napus*) and Indian mustard (*Brassica juncea* L.) performed poorly when 2 seeds were sowed in pots containing this soil; 2 weeks after germination the seedlings of both plants appeared stunted as compared to the controls and their leaves had purple colour with necrotic areas. After a few days most of them died. Thus canola and Indian mustard seeds were germinated for five days at 25°C (approximately 640 000 plant/ha). To prevent emergence failures, 40 seeds were sown in each pot; after the first pair of true leaves appeared seedlings was thinned to 20 plants per pot. After plant sowing, each pot was fertilised with N, P, and K using urea (120 mg N/kg), calcium phosphate (100 mg P/kg), and potassium sulphate (50 mg K/kg) as a basal fertilising. The pots were weighed daily and irrigated with deionised water to replace water lost throughout evapotranspiration. Water content of the soil was adjusted to 70% of field capacity. The plants were harvested 96 days after germination. After the harvest, fresh subsoil samples were collected in each pot to determine 1M NH_4NO_3-extractable Cd, Cu, Pb and Zn. Samples of Cd, Cu, Pb and Zn extracts were analyzed by atomic absorption spectrophotometer (AOAC 1990).

Table 1. Some physical and chemical properties of soil

Parameter		
Particle size distribution (%)	Sand (%)	30.7
	Silt (%)	35.9
	Clay (%)	33.4
Organic C	g/kg	7.0
pH	(1:2.5 soil/water)	7.31
Total N	g/kg	1.15
Available P	P_2O_5 Olsen (mg/kg)	15.50
CEC	$cmol_{(+)}$/kg	15.20
EC	dS/m	1.15
Exchangeable cations		
K	$cmol_{(+)}$/kg	2.20
Ca	$cmol_{(+)}$/kg	11.50
Mg	$cmol_{(+)}$/kg	2.25
Na	$cmol_{(+)}$/kg	0.25
Available heavy metals (1M NH_4NO_3 extract solution)		
Zn	mg/kg	2.30
Cu	mg/kg	1.00
Cd	mg/kg	0.13
Pb	mg/kg	0.78
Total heavy metal		
Zn	mg/kg	32.2
Cu	mg/kg	18.6
Cd	mg/kg	10.51
Pb	mg/kg	8.65

Plants were harvested by cutting the shoots from the soil surface and washed with deionised water. Plant roots were separated from the soil and washed with water until free of soil and then washed three times with deionised water. Plant shoots and roots were dried for 48 hours at 70°C and were ground. Sub-samples of ground plant materials were analyzed. Cd, Cu, Pb and Zn were determined after wet digestion of dried and ground sub-samples in a HNO_3-$HClO_4$ acid mixture. Cd, Cu, Pb and Zn analysis was done by atomic absorption spectrometry (AOAC 1990).

The transportation index (T_i) gives the shoot/root heavy metal concentration and depicts the availability of the plant to translocate the metal species from roots to shoot at different chelating concentrations (Zayed et al. 1998). It is calculated as follows:

T_i = heavy metal in leaves (mg/kg)/heavy metal in roots (mg/kg) × 100

Plants tolerant to toxicity levels of trace elements respond by exclusion, indication or accumulation of metals (Baker 1981). Plants are selected according to the needs of the application, contaminants of concern and species producing high-biomass. The aim of phytoextraction is to recover the metals concentrated in the aboveground portion of the biomass by harvesting and examining the biomass (Schnoor 1997). Canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) plants were selected according to the information given by Baker (1995), Lasat (2000) and EPA (2000).

The experiment design was randomized block design. Each pot was considered as a replicate and all of the treatments were repeated three times. So, the total number of pots was 96. All data were subjected to a two-way analysis of variance (ANOVA) and separated by LSD using (SAS) statistical software (SAS 1982).

RESULTS AND DISCUSSION

Dry matter yield of plants. Application of EDTA to heavy metal contaminated soils significantly decreased root and shoot dry matter yields of both plants; plants also showed a significant decrease when EDTA addition levels were higher than 3 mmol/kg. According to the level of added EDTA, dry matter yield of canola and Indian mustard plants are given in Table 2. EDTA applications at the highest rate (12 mmol/kg) visibly affected plant growth. The total dry weight of biomass of canola and Indian mustard was affected by the contaminated soil; on average, the metals caused a reduction of about 75% in root and shoot dry matter of both plants. Similar results in other hyperaccumulator plants were reported by Chen and Cutright (2001), Peralta-Videa et al. (2002), Zhao et al. (2003), Gardea-Torresdey et al. (2004), Turan and Angın (2004), Turgut et al. (2004), Hajiboland (2005), Tlustoš et al. (2006).

Efficiency of EDTA complexing agents in enhancing soil Cu, Cd, Pb and Zn desorption. To evaluate the effect of heavy metal availability in soil on plant uptake, soil samples were collected and soluble Cu, Cd, Pb and Zn were measured. Effects of EDTA application significantly increased heavy metal availability in soils. Heavy metal concentration in the soil increased with increasing levels of EDTA added to both canola and Indian mustard planted soils (Table 3). When 12 mmol/kg EDTA was added, NH_4NO_3 extractable Cu, Cd, Pb and Zn availability in canola and Indian mustard planted soil increased up to 126, 233, 138, 200% and 102, 225, 140, 211%, respectively, as compared to the

Table 2. Effects of EDTA complexing agents on dry matter weight of canola and Indian mustard plants (g/pot dw)

EDTA application (mmol/kg)	Canola		Indian mustard	
	root	shoot	root	shoot
0 (control)	23.55 ± 1.05 a	192.5 ± 3.24 a	16.26 ± 0.90 a	171.22 ± 4.10 a
3	18.50 ± 0.84 b	136.65 ± 5.10 b	11.25 ± 0.20 b	121.45 ± 1.28 b
6	9.05 ± 0.74 c	107.30 ± 2.15 c	10.63 ± 0.05 c	94.93 ± 0.36 c
12	5.65 ± 0.12 c	47.85 ± 1.35 c	6.23 ± 0.05 c	41.72 ± 0.21 d
LSD(0.01)	2.13	18.14	257	14.12

Significance of values that were tested is shown in columns of each EDTA application rate; EDTA: Ethylene Diamine Tetra Acetate

Table 3. Effects of EDTA on Cu, Cd, Pb and Zn in canola and Indian mustard planted soil (mg/kg)

EDTA application (mmol/kg)	Cu	Cd	Pb	Zn
Canola				
0 (control)	3.05 ± 0.14 d	2.30 ± 0.08 d	2.15 ± 0.10 c	5.10 ± 0.2 8 d
3	4.15 ± 0.09 c	4.12 ± 0.10 c	3.13 ± 0.08 b	8.44 ± 0.26 c
6	6.50 ± 0.15 b	5.27 ± 0.14 b	4.58 ± 0.12 a	10.20 ± 0.24 b
12	10.15 ± 0.10 a	7.13 ± 0.11 a	5.13 ± 0.12 a	11.50 ± 0.22 a
LSD(0.01)	0.95	1.20	0.75	1.10
Indian mustard				
0 (control)	3.15 ± 0.15 d	2.31 ± 0.10 d	2.17 ± 0.11 c	5.72 ± 0.208 d
3	4.20 ± 0.10 c	4.15 ± 0.10 c	3.14 ± 0.10 b	8.75 ± 0.20 c
6	6.80 ± 0.20 b	5.20 ± 0.11 b	4.62 ± 0.13 a	10.35 ± 0.25 b
12	10.25 ± 0.25 a	7.20 ± 0.12 a	5.20 ± 0.15 a	11.60 ± 0.24 a
LSD(0.01)	0.82	1.29	0.79	1.15

control treatment. Recent studies have reported that complexation of Cu, Zn, B, Cd, Mo and Pb by different organic and inorganic chelates could play an important role in controlling heavy metal solubility and concentration in soil (Naidu and Harter 1998, Irenem and Yang 1999, Fischerová et al. 2005, Lesage et al. 2005, Meers et al. 2005, Finžgar et al. 2006).

Heavy metal concentration in shoots and roots. Application of EDTA at the rates of 3, 6 and 12 mmol per kg significantly increased Cu, Cd, Pb and Zn concentration in shoots and roots in both plants (Figures 1 and 2). As expected, the concentration of heavy metals measured in the tissues of plants grown on polluted soil was higher than in the controls. The increase rate was often 10-fold or more.

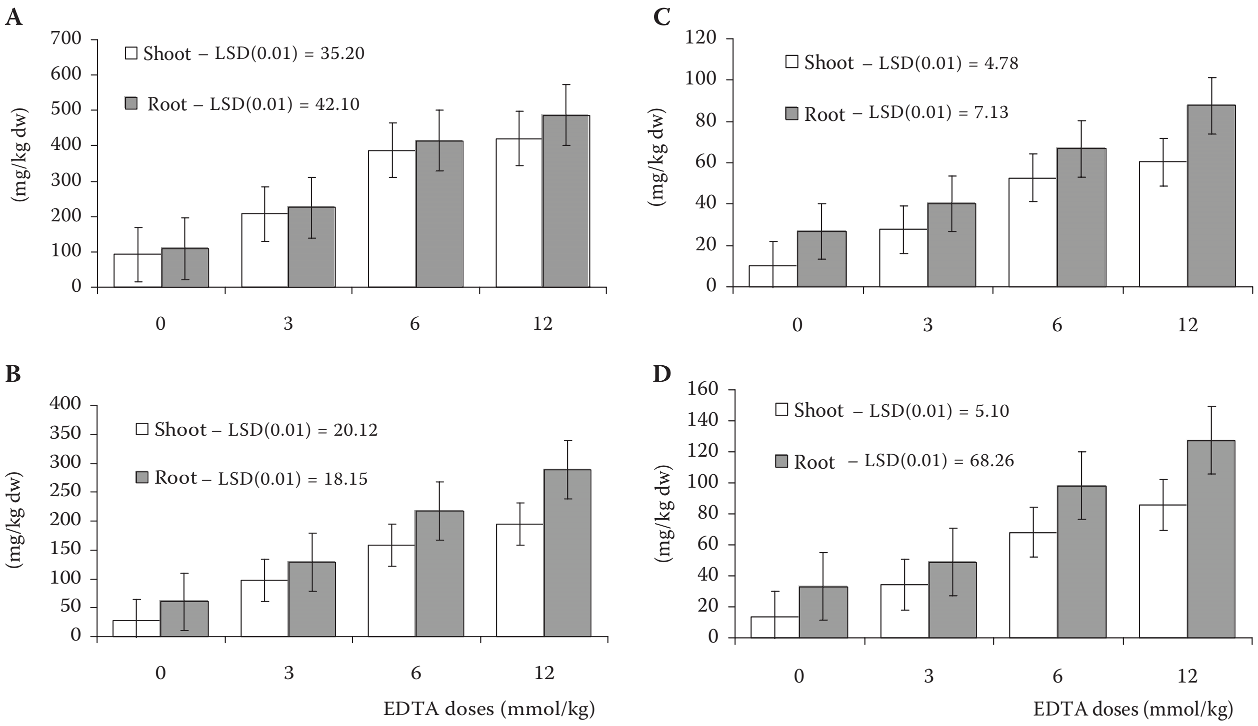

Figure 1. Effects of EDTA application on Zn (A), Cu (B), Pb (C) and Cd (D) content of canola root and shoot parts

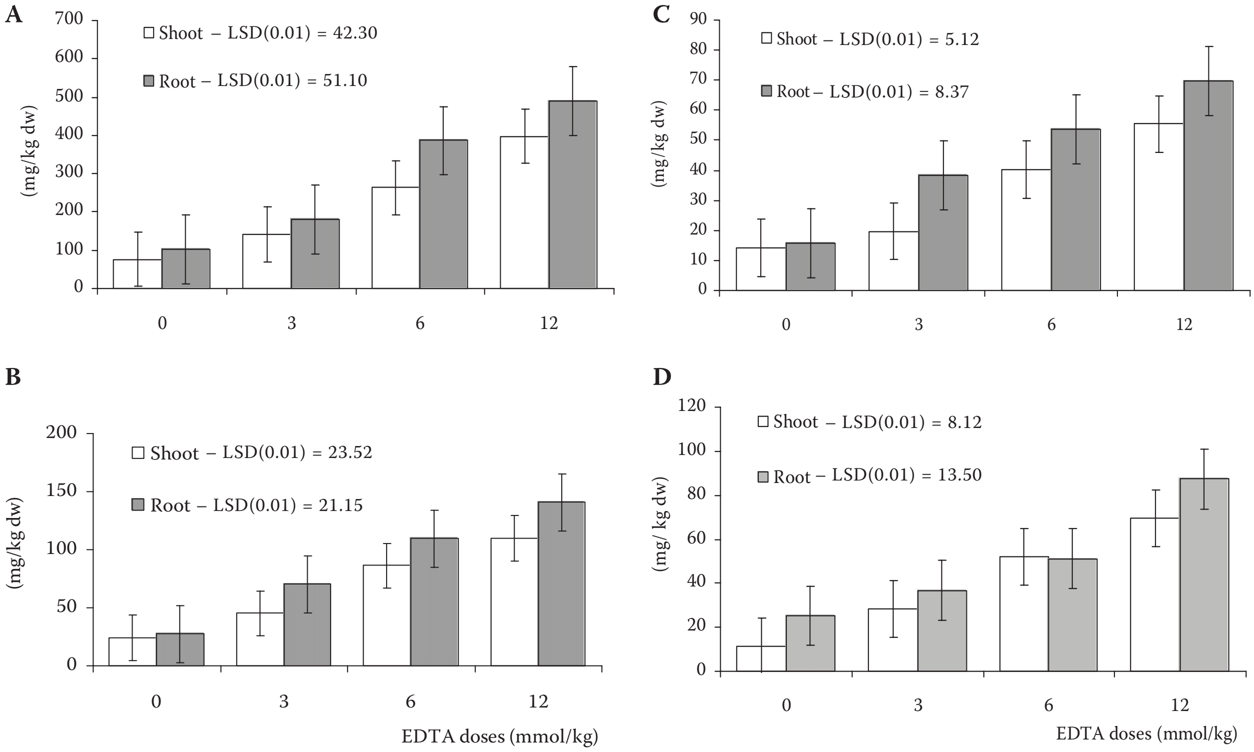

Figure 2. Effects of EDTA application on Zn (A), Cu (B), Pb (C) and Cd (D) content of Indian mustard root and shoot parts

Heavy metal concentration in shoots and roots of both plants significantly increased when EDTA addition levels were higher than 3 mmol/kg. Application of EDTA at rates up to 6.0 mmol/kg significantly increased heavy metal concentration in both plants; however rates over 6.0 mmol/kg decreased total heavy metal uptake significantly due to decreasing total dry matter weight. A statistically significant difference between species was evidenced in the phytoextraction of heavy metals: canola was the most effective in the uptake of Cu, Cd, Pb and Zn. Similar result were reported by Epstein et al. (1999), Kirkham (2000), Liphadzi et al. (2003), Thayalakumaran et al. (2003), Turan and Angın (2004).

Root heavy metal uptake was greater than shoot heavy metal uptake. In all EDTA application rates, heavy metal concentrations in roots were about 4–6 times higher than in shoots. Among the EDTA doses, 6 mmol/kg doses were the most effective for Cu, Cd, Zn and Pb uptake in both plants.

The shoot/root cadmium concentration index (T_i) is shown for each plant species in Table 4. Maximum transport of Cu, Cd, Pb and Zn was observed in Indian mustard at 6 mmol/kg EDTA application. At 6 mmol/kg EDTA application, the order of T_i was Zn > Cu > Cd > Pb in both plants. The big difference between root and shoot concentrations indicates an important restriction of the internal transport of Cu, Cd, Pb and Zn from roots to shoot, resulting in higher root concentrations rather than translocation to shoots.

Phytoextraction efficiency is related to both plant metal concentration and dry matter yield. Thus, the ideal plant species to remedy a contaminated site should be a high yielding crop that can both tolerate and accumulate the target contaminants. Indian mustard demonstrated to accumulate moderate levels of Pb, Zn, Cu (Kumar et al. 1995, Ebbs and Kockhian 1997). When synthetic chelate was used, concentrations of 1.5% in the shoot of *B. juncea* were obtained from soil containing 600 mg Pb/kg (Blaylock et al. 1997). Canola also showed a good metal accumulation performance. Figure 1 showed that shoot and root Cu, Cd, Pb and Zn uptake by plants growing in soil treated with EDTA were significantly higher than those of the controls. Shoot and root Cu, Cd, Pb and Zn uptake by canola and Indian mustard in 12 mmol/kg EDTA application were nearly six-fold and four-fold higher than the control in shoot and root, respectively.

The results of this study demonstrated that EDTA is an efficient soil amendment in enhancing Cu, Cd, Pb and Zn desorption from soil and in increasing Cu, Cd, Pb and Zn accumulation in plants. It is possible that Cu, Cd, Pb and Zn could

Table 4. The relative shoot/root concentrations [transportation index (T_i)] of Cu, Cd, Pb and Zn in canola and Indian mustard plants grown for 96 days (%)

EDTA application (mmol/kg)	Cu	Cd	Pb	Zn
Canola				
0 (control)	49.39	40.36	38.43	85.17
3	75.28	70.28	69.15	92.12
6	82.85	79.34	78.74	93.51
12	67.37	67.26	68.83	86.28
Indian mustard				
0 (control)	47.24	45.23	39.87	75.19
3	64.28	76.91	51.57	78.32
6	83.80	81.75	76.32	98.19
12	78.21	79.74	79.59	81.13

be accumulated in vacuoles of leaves as an EDTA complex. This has already been proven for Zn using the non-invasive technique for X-ray absorption spectroscopy (XAS). Salt et al. (1999) determined the ligand environment of Zn in different tissues of *Thlaspi caerulescens* and found that Zn coordination in shoots occurred mainly via organic acids with a smaller proportion present as the hydrated cation. There are many indications that organic and inorganic complexion agents are involved in heavy metal tolerance, transport and storage in plants, including Al, B, Cd, Fe, Mo, Ni, Pb and Zn (Yang et al. 1997, Ma et al. 2001, Nigam et al. 2001, Vysloužilová et al. 2003). The development of metal-organic acid complexes was also indicated using chromatography (Sagner et al. 1998) and X-ray absorption spectroscopy (Kramer et al. 2000). As plant vacuoles are a major repository for organic acids, an association between metals and organic acids suggests that metal detoxification occurs by vacuolar sequestration. However, other strategies for metal tolerance and accumulation, such as binding to the cell wall or localisation in the apoplast, may also be involved (Vazquez et al. 1992, Kramer et al. 2000). The distribution of metals within plant tissues is therefore an important property that can act as an indirect indicator of detoxification mechanism. Because the distribution of metals between the apoplasm and symplasm of tissues, and between the cytosol and vacuole in cells, requires transmembrane transport, the energisation and functioning of membrane processes may be of key significance in hyperaccumulation.

The data obtained in our experiment confirmed that both plants are tolerant to heavy metals and that canola is more tolerant than Indian mustard. Similar results were reported by Epstein et al. (1999), Marchiol et al. (2004), Lim et al. (2004), Yanqun et al. (2004), Wu et al. (2004).

Phytoremediation is widely considered as a low cost and ecologically responsible alternative to the expensive physical-chemical methods currently practiced, and an emerging bio-based and low cost alternative technology in the clean up of contaminated soils. The future of the technique is still in development and there are some questions that need to be solved; for instance the optimization of the process and a greater understanding of how plants absorb, translocate and metabolize heavy metals. EDTA application to soil not only increased heavy metal availability in soils but also enhanced heavy metal content of the plant organs. This situation can be explained partly by their chelating capacity (Zhang and Schmidt 2000, Evangelou and Marsi 2001, Zhang et al. 2003). When EDTA is added to soil containing heavy metals (Cu, Cd, Pb and Zn), EDTA complex the soluble form of heavy metal. Chelating agent not only facilities heavy metal removal from the soil via plant uptake; it theoretically means that any metal that can be chelated and solubilized can be removed in the same manner, providing that the soil chemistry favors the forming of a chelate metal complex. The results of this study demonstrated that EDTA is an efficient soil amendment in enhancing Cu, Cd, Pb, and Zn desorption from soil and in increasing their accumulation in plants.

Significant differences were obtained in both species and plant parts. As for plant species tested, canola was the most effective in the uptake of Cu, Cd, Pb, and Zn. Root heavy metal uptake of both species was greater than shoot heavy metal uptake. As a conclusion, it can be said that EDTA facilitated Cu, Cd, Pb, and Zn phytoextraction and may contribute to an effective soil decontamination strategy. Application of EDTA at the rates of 3, 6 and 12 mmol/kg significantly increased Cu, Cd, Pb and Zn desorption period from soil by canola and Indian mustard plants. When 12 mmol/kg EDTA was added, NH_4NO_3 extractable Cu, Cd, Pb and Zn availability in canola and Indian mustard planted soils were two and three-fold higher than the control, respectively. Concentrations of Cu, Cd, Pb and Zn in shoot and root of both plants were also increased by EDTA addition. The application of EDTA at rates up to 6.0 mmol/kg significantly increased heavy metal concentration in both plants; however rates over 6.0 mmol/kg decreased total heavy metal uptake significantly due to decreasing total dry matter weight. Thus, in the case of the application of EDTA at the rate of 6.0 mmol/kg, soil available Cu (6.50–6.80 mg/kg) and Zn (10.20–10.35 mg/kg) removal periods necessary to clean up the canola and Indian mustard planted soils were about one and two years or growing periods with approximately 640 000 seeds/ha of canola and Indian mustard plants, respectively; available Cd (5.27–5.20 mg/kg) and Pb (4.58–4.62 mg/kg) removal periods were three and four years with approximately 640 000 seeds/ha, respectively. As for the total amount of Zn (82.2 mg/kg), Cu (68.6 mg/kg), Pb (58.65 mg/kg) and Cd (60.51 mg/kg) in soil, 3.6, 7.2, 18.8 and 14.9 years or growing periods, respectively, would be required to uptake the heavy metals by canola with approximately 640 000 seeds/ha. Similarly, 5.7, 14.7, 27.9 and 22 years or growing periods, respectively, would be necessary to use up the heavy metals by Indian mustard with approximately 640 000 seeds/ha.

In conclusion, EDTA-enhanced phytoextraction by canola would remove adequate quantities of heavy metals from the contaminated soil and it would therefore be an appropriate remediation technique for the soil. This study provides a promising start for biomass-based phytoextraction; it includes high biomass production species, and growing these species is practically easier than the production of hyperaccumulators. Next we should focus on the effects of crop rotations and crop cycle duration on metal uptake; organic chelate amended aspect must be tested in the field under different site-specific conditions and on multi-heavy metal contaminated soil.

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Received on June 29, 2006

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