Effectiveness of Phytoremediation Technologies to Clean Up of Metalloids Using Three Plant Species in Iran

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Effectiveness of Phytoremediation Technologies to Clean Up of Metalloids Using Three Plant Species in Iran

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ABSTRACT: Phytoremediation is a potential, innovative, and cost-effective technology for non-destructive remediation of heavy-metal contaminated soils. A field trial was conducted to evaluate the phytoremediation efficiencies of three plants and the effects of ethylenediaminetetraacetic acid (EDTA) or ammonium addition [$(NH_4)_2SO_4$ and NH_4NO_3] for assisting removal of heavy metals (Pb, Hg, and Cd) from contaminated soil. The tested plants include *Amaranthus retroflexus, Sorghum bicolor*, and *Lolium perrene*. Results showed that maximum concentration of Pb, Hg, and Cd were detected in shoots of *A. retroflexus, S. bicolor*, and *L. perrene* at high concentrations in pH=6.2. The application of EDTA as a chelating agent to soil was the most efficient to enhance the phytoavailability of Pb, Hg and Cd. The concentrations of Pb, Hg, and Cd in the shoots of *A. retroflexus* treated with EDTA were 57 mg/kg, 14.1 mg/kg, and 30 mg/kg, respectively. Results indicated that among the three plants, *A. retroflexus* had great potential in phytoremediation of contaminated soils. *Water Environ. Res.*, **86,** 43 (2014).

KEYWORDS: phytoremediation, *Amaranthus retroflexus, Sorghum bicolor, Lolium perrene*, ammonium, EDTA.

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Introduction

The removal of heavy-metal constituents from industrial wastewater is of paramount importance given the environmental problems associated with heavy-metal polluted soil. Tyler et al. (1989) reported that the release of heavy metals in biologically available forms by human activity might damage or alter both natural and manmade ecosystems. One of the effective and affordable technological solutions is the application of a process known as *phytoremediation*. Phytoremediation is the application of plants for in-situ or ex-situ treatment/removal of contaminated soils, sediments, and water. The green plants degrade, assimilate, metabolize, or detoxify inorganic and organic pollutants from the environment or render them harmless. It is a cost-effective "green" technology based on the use of specially selected metal-accumulating plants to remove toxic metals from soils and water (Garbisu and Alkorta, 2001). However, despite these advantages, phytoremediation has not been widely used in developing countries such as Iran. This is because of the limited knowledge of this technology. Some elements such as Cu^{2+}, Zn^{2+}, and Fe^{2+} are essential micronutrients for plant metabolism but can become extremely toxic when present in excess. Cadmium (Cd) is one of the most toxic heavy metals and is considered non-essential for living organisms. Lead (Pb) on its own has been discovered to be non-toxic to plants. Mercury (Hg) is a non-essential metal and can become extremely toxic (Gupta, 1980). Many studies have been conducted using ethylenediaminetetraacetic acid (EDTA) for phytoremediation of Pb, Zn, and Cd in contaminated soils (Kayser et al., 2000; Pucshenreiter et al., 2001). It has been recognized that EDTA is one of the most efficient chemicals in enhancing Pb phytoavailability in soil and subsequent uptake and translocation in shoots after treating for a few days (Chen and Cutright, 2001). Up to now, however, no data have been reported which demonstrate that EDTA could enhance Cd uptake in plants while stimulating the translocation of Cd from root to shoot (Jiang et al., 2003). It has also been reported that the application of ammonium to soil significantly enhances ^{137}Cs accumulation in plants (Jiang et al., 2003) and promotes heavy-metal phytoavailability from the contaminated soil (Pb, Zn, Cu, Cd, Ni) of smelter (Pucshenreiter et al., 2001). However, not all studies are encouraging in terms of heavy-metal removal rates achieved by addition of chemical amendments, such as EDTA, N-(hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA), organic acid, and nitrilotriacetic acid (Kayser et al., 2000). Although chelate agents such as EDTA have been shown to enhance phytoremediation of Pb from contaminated soil, some concerns have been expressed regarding the leaching of metal-chelate complexes to groundwater posing potential risks during extended periods. Several reports have indicated the possible threat of heavy metals to groundwater contamination (Kos and Lestan, 2003). Therefore, phytoremediation processes that use chelates must be designed properly to protect environmental safety.

It has been recognized that selection of plant materials and chemical amendments is still very important even today for promoting phytoremediation efficiency (Kayser et al., 2000). Liu et al.'s (2002) field investigations and hydroponic tests proved *Amaranthus retroflexus,* a species belonging to *Amarantaceae*, to be a Cd hyper-accumulator. *Lolium perrene*, a perennial grass belonging to the *Graminaceae*, has been widely known for its rapid growth and high biomass, as well as its high tolerance to heavy metals (Shu et al., 2002), and could be potentially used for phytoremediation of soils contaminated by heavy metals (Chen and Cutright, 2001). *Sorghum bicolor* is also a perennial grazing

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Table 1—Different concentrations of Pb, Hg, and Cd used in experiments.

Metal concentration	Pb (mg/kg)	Hg(mg/kg)	Cd (mg/kg)
High	300	6	15
Moderate	200	3	7.5
Low	100	2	5

plant with high biomass. Although the metal tolerance in this plant had not been studied, several sorghum species have been proved to be metal tolerant or Pb hyper-accumulators (Liu et al., 2002).

For the purposes of this study, three plants, *S. bicolor*, *A. retroflexus*, and *L. perrene* were used for the removal of Pb, Hg, and Cd from contaminant soils. A rectangle paddy-plot trial was conducted to

(1) evaluate the metal-accumulation capacity and phytoreme diation efficiency of *A. retroflexus*, *S. bicolor*, and *L. perrene* grown on soil contaminated by Pb, Hg, and Cd;
(2) determine of the effect of pH and different concentrations of metals on their remediation from contaminated soils;
(3) assess the effects of different chemical amendments on the phytoremediation efficiency of these plants; and
(4) determine which of the plants' tissue (i.e., roots or shoots) absorbs more heavy metals.

Methodology

Site Description. The study site was a farmland located at approximately 4 km east of Urmia city in the central part of West Azerbaijan Province, northwestern Iran, at latitude 37° 32′ N and longitude 45° 05′ E. It has a semi-arid climate with an annual average temperature and rainfall of 9.8 °C and 870 mm, respectively. The minerals mainly consist of pyrite and magnetite, with calcite, quartz, and muscovite as minor minerals (Imamalipoura and Mirmohammadi, 2012).

Field Experiment. Farmland covering an area of 1000 m^2 and consisting of six consecutive rectangular paddy plots was selected for conducting the phytoremediation field experiment. A portion of the contaminated farmland was set up to conduct the field experiment on April 1, 2011. The split-plot design was used in the present field experiment, in an effort to avoid heterogeneous conditions. Four plots (8 m × 3 m) were set up within this area, and each plot was further equally divided into four subplots (2 m × 3 m). Each subplot was planted with *A. retroflexus*, *S. bicolor*, and *L. perrene*, respectively. Each plant species was planted with the space of 20 cm × 20 cm for *L. perrene*, and 40 cm × 40 cm for *A. retroflexus* and *S. bicolor*. The experiments included two pHs (6.2 and 8.1) and three concentration levels (high, moderate, and low concentration) of heavy metals, as shown in Table 1. Four treatments were examined to investigate the effects of chemical amendments on phytoremediation: Treatment A, EDTA; Treatment B, $[(NH_4)_2SO_4]$; Treatment C, NH_4NO_3; and Treatment D, control (Shu et al. 2002). Seeds of *A. retroflexus*, *S. bicolor*, and *L. perrene*, purchased in Tehran (Seed and Plant Improvement Institute, Tehran, Iran), were sown at the experiment field on January 1, 2011, and seedlings about 10 cm in height were synchronously transplanted at the experimental farmland on April 1, 2011.

During the experiment period, weeding, watering, fertilizing, and loosening of the soil were done manually as needed. The N-P-K fertilizer (1:1:1) was added at the amount of 10 g per pot. On July 20, 2011, $(NH_4)_2SO_4$ and NH_4NO_3 were applied at a concentration of 10 mmol/kg, and dissolved EDTA was applied at rate of 6 mmol/kg on August 20, 2011, respectively. After approximately 140 days of cultivation, all the plants were harvested 1 week after the EDTA application, and 4 weeks after the application of $(NH_4)_2SO_4$ and NH_4NO_3. Associated soil samples from the study sites were also collected from root and shoot regions of plants.

Soil Analysis. Soil samples were air dried and ground to pass through 2-mm mesh sieve. The soil samples were analyzed for the following parameters: pH (water: soil = 1:2), organic matter ($K_2Cr_2O_7 + H_2SO_4$), and total metal (Pb, Hg, and Cd) content (digested by 5:1:1 HNO_3, HCl, and $HClO_4$). Concentrations of Pb, Hg, and Cd were measured by flame atomic absorption spectrophotometry (American Public Health Association [APHA] et al., 2005).

Plant Analysis. Plant samples (root and shoot) were thoroughly washed with de-ionized water to remove surface dust and soil, divided into root and shoot, completely dried at 80°C, weighted, and then milled to pass through a 2- mm mesh sieve. Each sample (0.5 g) was digested with concentrated HNO_3 (16 mol/L) and $HClO_4$ (12 mol/L) at the ratio of 5:1 (v/v), the concentrations of Pb, Hg, and Cd were determined by Atomic Absorption Spectrophotometer (APHA, 2005).

Phytoremediation Rate. The potential for phytoremediation depends on four variables: plant biomass, plant metal concentration, soil metal concentration, and soil mass in the rooting zone. The phytoremediation rate of plant was calculated by the following equation (Zhao et al., 2003):

$$\%\,\text{removal} = \frac{(\text{plant metal concentration} \times \text{Biomass}) \times 100}{(\text{soil metal concentration} \times \text{soil mass in the each zoon of plant})} \quad (1)$$

It was assumed that metal pollution occurs only in the active rooting zone, top 20-cm soil layer, which gives a total soil mass of 2600 t/ha (assuming a soil bulk density of 1.3 t/m^3).

Control Group. Control groups (to which no metal was added in soil) were selected to evaluate difference of heavy-metal concentration values between control and experimental plants.

Statistical Analysis. Data were examined by one-way analysis of variance followed by a least significant difference test using SPSS 11.0 (2001).

Results and Discussion

Properties of the Soil. It was necessary to determine the physical and chemical properties of the soil, such as soil pH, clay content, organic matter content, and nutritional status. Table 2 shows these properties. Soil pH affects the solubility and mobility of heavy metals. The soil pHs used in this study were 6.2 and 8.1, to compare acidity and alkalinity conditions. The clay loam soil and organic matter contents of soil used in this study (41 to 53) resulted in high cation-exchange capacity of the soil (23 $cmol_c$/kg). Nitrogen is an essential nutrient for plant growth, as determined in the studied soil. Heavy-metal concentration in soil is essential to determine the amount of heavy metals needed to reach treatment concentration.

Table 2—The physical and chemical properties of soil studied (*M* ± *SE*, *n* = 3).

Parameter	Unit	Content	Analyzed method
pH	—	7.5	Potentiometric
Organic matter	mg/kg	41 to 53±5	Walkley-Black
Soil texture	—	Clay loam	Hydrometer
Moisture content	%	27.4	Gravimetric
Cation exchange capacity	$cmol_c$/kg	23±2	Ammonium acetate
Total nitrogen	%	0.18	Kjeldahl
Available phosphorus	ppm	432±5	Mehlich's No.1
Available potassium	ppm	202±2	Atomic absorption spectrophotometer
Total Pb	mg/kg	85.23±8	Atomic absorption spectrophotometer
Total Hg	mg/kg	1.05±0.05	Atomic absorption spectrophotometer
Total Cd	mg/kg	1.25±0.5	Atomic absorption spectrophotometer

Dashes indicate not applicable.

Phytoremediation Potential of Contaminated Soils Using Three Plants. Tables 3 to 5 present the concentration and pH levels of different heavy metals in roots and shoots of the three plants in control and treated soils.

The concentrations of heavy metals in plants were compared with the concentrations of Pb, Hg, and Cd in roots and shoots of *A. retroflexus*, *S. bicolor*, and *L. perrene* with different treatment conditions (control and treatment plants), as presented in Tables 3 to 5. Trace concentrations of Pb, Hg, and Cd in control plant tissues were compared to the treated plants. In general, concentrations of Pb, Hg, and Cd in shoots were higher than in roots of *A. retroflexus* in high concentration and pH = 6.2. A minimum concentration of Pb was detected in low concentration and pH = 8.1 in shoots of *S. bicolor*, and minimum concentrations of Hg and Cd were also detected in low concentrations and pH = 8.1 in shoots of *L. perrene*. The soils associated with *A. retroflexus* contained the highest concentrations of total Pb (29.01 mg/kg), Hg (7.27 mg/kg), and Cd (15.15 mg/kg). The soils associated with shoots of *L. perrene* contained higher concentrations of total Pb (25.22 mg/kg) than in shoots of *S. bicolor*, whereas concentrations of Hg (6.05 mg/kg) and Cd (12.14 mg/kg) in shoots of *S. bicolor* were higher than in shoots of *L. perrene*.

Table 4—Phytoremidiation rates of heavy metals in shoot and root *Sorghum bicolor* in different pHs.

Contaminate concentration	pH	Plant section	Control (mg/kg)			Treatment (mg/kg)		
			Pb	Cd	Hg	Pb	Cd	Hg
High	6.2	Root	2.02	1.25	0.5	15.18	8.08	4.168
		Shoot	2.5	1.8	1.9	24.01	12.14	6.05
	8.1	Root	1.815	0.955	0.199	10.27	5.14	2.25
		Shoot	0.963	0.341	0.0015	8.25	4.123	1.99
Moderate	6.2	Root	1.98	1.05	0.45	14.10	7.88	3.668
			2.32	1.45	1.56	23.31	11.54	5.85
	8.1	Shoot	1.75	0.90	0.161	9.17	4.74	1.82
			0.873	0.321	0.0011	7.95	3.64	1.78
Low	6.2	Root	1.88	1.04	0.44	13.90	7.68	3.38
			2.12	1.15	1.36	22.11	11.00	5.45
	8.1	Shoot	1.34	0.75	0.12	8.99	4.04	1.43
			0.842	0.311	0.001	7.45	3.24	1.29

Effect of Additives. It was also found that the ethylenediaminetetraacetic acid (EDTA) treatment significantly increased Pb concentrations in shoots of *A. retroflexus*, *S. bicolor*, and *L. perrene* from 29.01 to 56 mg/kg, 24.01 to 53 mg/kg, and 25.22 to 52 mg/kg, respectively. With the application of $(NH_4)_2SO_4$ and NH_4NO_3, the concentrations of Hg in shoot of *S. bicolor* significantly enhanced from 0.12 up to 6.05 and 12 mg/kg, respectively, and Hg concentrations in shoot of *L. perrene* increased from 0.031 to 5.11 mg/kg ($p < 0.01$) and 11.9 mg/kg, respectively. The application of both $(NH_4)_2SO_4$ and NH_4NO_3 did not have significant effect on uptake and accumulation of Pb and Cd by the three plant species tested.

Total Amounts of Heavy Metals Accumulated in Plants. After 140 days cultivation, the total dry weight yields of *A. retroflexus*, *S. bicolor*, and *L. perrene* were 28, 17, and 5.5 t/ha, respectively. The Pb, Hg, and Cd uptake amounts (mg /m^2) in shoots and roots of these three plants are shown (Figures 1 to 3). In general, *A. retroflexus* accumulated the highest amounts of Pb, Hg, and Cd, in all treatments. The Hg uptake amounts in

Table 3—Phytoremediation rates of heavy metals in shoot and root *Lolium perrene* in different pHs.

Contaminate concentration	pH	Plant section	Control (mg/kg)			Treatment (mg/kg)		
			Pb	Cd	Hg	Pb	Cd	Hg
High	6.2	Root	0.512	0.321	0.1	12.01	6.12	2.05
		Shoot	1.001	0.815	0.325	25.22	10.03	5.11
	8.1	Root	0.012	0.101	0.051	10.12	3.11	1.51
		Shoot	0.1	0.2	0.1	18.02	6.17	2.1
Moderate	6.2	Root	0.462	0.291	0.08	11.01	5.19	2.00
		Shoot	0.901	0.715	0.285	23.21	9.03	4.01
	8.1	Shoot	0.09	0.091	0.031	9.11	3.01	1.02
		Shoot	0.09	0.18	0.07	16.03	5.07	1.98
Low	6.2	Root	0.422	0.25	0.08	10.02	5.12	1.90
		Shoot	0.871	0.825	0.255	22.21	8.09	3.86
	8.1	Shoot	0.085	0.081	0.042	9.01	2.59	0.99
		Shoot	0.08	0.17	0.06	15.91	5.06	1.68

Table 5—Phytoremidiation rates of heavy metals in shoot and root *Amaranthus retroflexus* in different pHs.

Contaminate concentration	pH	Plant section	Control (mg/kg)			Treatment (mg/kg)		
			Pb	Cd	Hg	Pb	Cd	Hg
High	6.2	Root	2.11	1.45	0.56	18.38	9.012	5.25
		Shoot	2.67	1.85	1.38	29.01	15.15	7.27
	8.1	Root	1.85	1.03	0.35	12.13	8.25	4.15
		Shoot	1.02	0.665	0.125	10.015	6.24	3.13
Moderate	6.2	Root	2.01	1.25	0.50	17.45	8.86	5.01
		Shoot	2.01	1.45	1.08	28.11	14.45	7.01
	8.1	Shoot	1.46	1.02	0.33	11.83	8.20	3.95
		Shoot	0.98	0.62	0.105	9.5	5.94	3.01
Low	6.2	Root	1.81	1.01	0.42	16.35	8.01	4.52
		Shoot	1.86	1.40	1.03	27.31	13.41	6.61
	8.1	Shoot	1.23	0.01	0.23	10.93	7.80	2.65
		Shoot	0.90	0.43	0.100	9.05	5.02	2.71

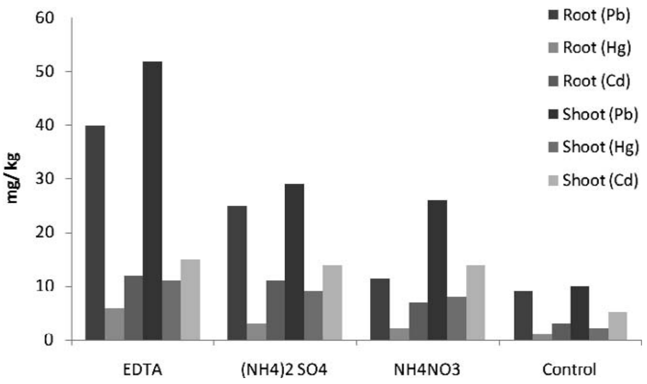

Figure 1—The uptake amounts of Pb, Hg, and Cd in the shoots and roots of *Lolium perrene* with different treatments for a period of 140 days at pH = 6.2.

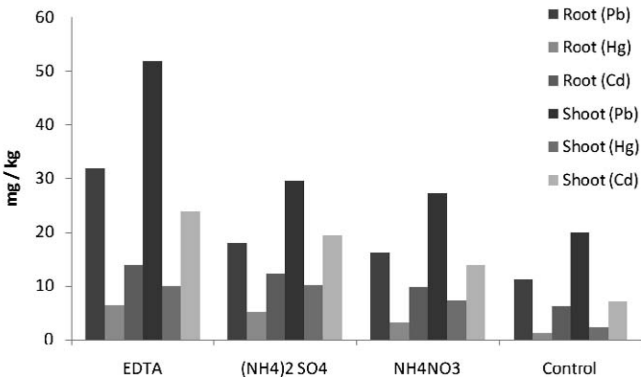

Figure 2—The uptake amounts of Pb, Hg, and Cd in the shoots and roots of *Sorghum bicolor* with different treatments for a period of 140 days at pH = 6.2.

shoots of all the three plants showed a similar level under the same treatment. The application of EDTA had significantly enhanced Pb accumulation, whereas $(NH_4)_2SO_4$ and NH_4NO_3 treatments slightly increased Hg and Cd uptake amounts in shoot of *A. retroflexus*. Figures 1 to 3 present phytoremediation of Pb, Hg, and Cd by *A. retroflexus*, *S. bicolor*, and *L. perrene* with different treatments. In general, *A. retroflexus* had the highest phytoremediation rate among the three plants. The application of EDTA remarkably enhanced Pb and Hg phytoremediation amount of *A. retroflexus* from 23 to 57, and 2.8 to 14.1 mg/kg, respectively. The addition of $(NH_4)_2SO_4$ and NH_4NO_3 increased the Cd phytoremediation amount of *A. retroflexus* from 8.2 mg/kg to 14 mg/kg and 12.1 mg/kg, respectively.

It has been recognized that high accumulation levels of heavy metal and high biomass production in plants are of paramount importance for successful phytoremediation (Roger et al., 2000). Up to now, more than 400 metal hyper-accumulator species have been reported in the world (Zhao et al., 2003), but only a few of these hyper-accumulators have been tested for phytoremediation (Ebbs et al., 1997). Field trials with selected metal hyper-accumulators, such as *Thlaspi caerulescens*, *Alyssum bertolonii*, and other species, demonstrate that these plant species accumulated high levels of Cd and Zn in shoot (Lombi et al., 2001). Field investigation and hydroponic experiments have proved that *A. retroflexus* is a hyper-accumulator of Cd (Liu et al., 2002). Their results showed that the concentration of Cd in shoot of *A. retroflexus* is 11.53 mg/kg under natural condition and the Cd concentration in shoot/root quotient was greater than 1 under both field and hydroponic conditions.

The results presented here further demonstrate that the plant had exceptional accumulation capacity for Cd, and the shoot of the species could accumulate 30 mg/kg Cd from the soil with low concentrations of Cd (total: 7.8 mg/kg), which was about 2- to 3-fold higher than the other two plants. Despite the concentration of Cd in shoots, the criterion for Cd hyper-accumulator (>100 mg/kg) could not be met, which may be attributable to the relatively low concentration of total Cd (7.8 mg/kg) in soil. Cd concentration in shoot of *A. retroflexus* was also significantly higher than that in the root and soil. The field experiment here indicated that the Cd phytoremediation amount of this species reached 12.1 mg/kg and 14 mg/kg with the applications of NH_4NO_3 and $(NH_4)_2SO_4$, respectively, and the EDTA treatment increased the amount of Pb and Hg phytoremediation by this species by 57 mg/kg and 14.1 mg/kg, respectively. Pb, Hg, and Cd accumulation amounts by *S. bicolor* and *L. perrene* were also significantly less than accumulation by *A. retroflexus*, as shown in Figures 1 and 2. The present results suggest that the relative low biomass hyper-accumulator, *A. retroflexus*, had significantly higher efficiency in phytoremediation of Pb, Hg, and Cd than a high-biomass plant.

Blaylock and Huang (2000) have compared five synthetic chelates (EDTA, diethylenetriaminepentaacetic acid, N-(hydroxyethyl)-ethylenediaminetriacetic acid, 1,2-cyclohexylene-aminotetraacetic acid, and ethylene glycol tetraacetic acid), and found that the application of EDTA significantly enhanced the concentration of Pb in plant shoots. Ebbs et al. (1997) reported that there was no significant difference in Hg concentration and uptake amount in the shoots of oat and wheat grass by addition of EDTA. A study by Dushenkov et al. (1999) suggested that the ammonium salts applied to assist phytoremediation in metal-contaminated soil increased the concentration of ^{137}Cs in plants.

In the present study, EDTA treatment increased the Pb phytoremediation amount of *A. retroflexus*, *S. bicolor*, and *L. perrene* by 4-, 3- and 4-folds compared with the control treatments (Figures 1 to 3). Those results are consistent with Blaylock and Huang's (2000) findings that EDTA was the most efficient chelating agent for increasing Pb accumulation in plant

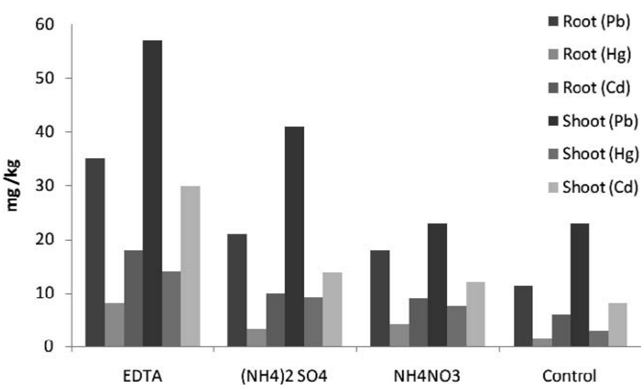

Figure 3—The uptake amounts of Pb, Hg, and Cd in the shoots and roots of *Amaranthus retroflexus* with different treatments for a period of 140 days at pH = 6.2.

shoots, whereas Hg and Cd accumulation and uptake amount had no remarkable response to the application of EDTA. The application of $(NH_4)_2SO_4$ and NH_4NO_3 had slightly enhanced the uptake amounts of Hg and Cd in shoots of *A. retroflexus*, *S. bicolor*, and *L. perrene*. In the present study, one cropping of *A. retroflexus* grown over 140 days removed approximately 29.01 mg/kg, 7.27 mg/kg, and 15.15 mg/kg for Pb, Hg, and Cd from the contaminated farmland without any treatment, respectively (see Table 5). In particular, that the uptake amounts by one crop with the application of EDTA of Pb, Hg, and Cd were 57 mg/kg, 14.1 mg/kg, and 30 mg/kg, respectively, suggest that *A. retroflexus* is an important candidate for phytoremediation of soil contaminated by heavy metals. Furthermore, *S. bicolor* was also advantageous in phytoremediation the contaminated soil with multiple metals because the plants could strongly accumulate three elements (Pb, Hg, and Cd) at the same time.

Conclusions

This study was conducted to evaluate the phytoremediation efficiencies of three plants at different conditions of pH and metal concentration at roots and shoots of plants and the effects of EDTA or ammonium addition [$(NH_4)_2SO_4$ and NH_4NO_3] for assisting heavy-metal (Pb, Hg, and Cd) removal from contaminated soil. Results indicate that, among the three plants, *A. retroflexus* has great potential in phytoremediation of contaminated soils. It appears that phytoremediation using *A. retroflexus* is more feasible when soil is contaminated by low levels of Cd and pH is 6.1 than when contaminated by Pb and Hg (the critical values of Pb, Hg, and Cd are 300 mg/kg, 6 mg/kg, and 15 mg/kg of soil, respectively). In particular, the uptake amounts of Pb, Hg, and Cd were 57 mg/kg, 14.1 mg/kg, and 30 mg/kg by one crop with the application of EDTA. EDTA is the most efficient chelating agent for increasing Pb, Hg, and Cd accumulation in plant shoots. Given these severe limitations of chelate-assisted phytoremediation and low phytoremediation rate of hyperaccumulator plants, further efforts to develop phytoremediation should focus on transporting gene technology or optimized agronomic methods. Therefore, further explorations are necessary for improving phytoremediation effects of *A. retroflexus*.

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