Plant and Soil (2005) 276:153–162
DOI 10.1007/s11104-005-3901-0

Chemically assisted phytoextraction of heavy metal contaminated soils using three plant species

P. Zhuang, Z.H. Ye, C.Y. Lan, Z.W. Xie & W.S. Shu[1]
*School of Life Sciences, and State Key Laboratory for Biocontrol, Sun Yat-sen (Zhongshan) University, Guangzhou, 510275 P.R. China. [1]Corresponding author**

Received 6 December 2004. Accepted in revised form 15 March 2005

Key words: ammonium, EDTA, heavy metals, phytoextraction, *Rumex K-1 (Rumex patientia × R. timschmicus), Vetiver zizanioides*, *Viola baoshanensis*

Abstract

Phytoextraction 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 phytoextraction efficiencies of three plants and the effects of EDTA or ammonium addition [$(NH_4)_2SO_4$ and NH_4NO_3] for assisting heavy metal (Pb, Zn, and Cd) removal from contaminated soil. The tested plants include *Viola baoshanensis*, *Vertiveria zizanioides*, and *Rumex K-1* (*Rumex patientia × R. timschmicus*). The application of EDTA soil was the most efficient to enhance the phytoavailability of Pb and Zn, but did not have significant effect on Cd. Lead phytoextraction rates of *V. baoshanensis, V. zizanioides* and *Rumex K-1* were improved by 19-, 2-, and 13-folds compared with the control treatment, respectively. The application of ammonium did not have obvious effects on phytoextraction of the three metals, except that the accumulations of Zn and Cd in shoot of *V. baoshanensis*. Among the three tested plants, *V. baoshanensis* always accumulated the highest concentrations of Pb, Zn, and Cd. The concentrations of Pb, Zn, and Cd in the shoots of *V. baoshanensis* treated with EDTA were 624, 795, and 25 mg kg^{-1}, respectively, and the phytoextraction efficiencies of this species for Pb, Zn, and Cd were also the highest among the three species. Results presented here indicated that *V. baoshanensis* had great potential in phytoremediation of soils contaminated by multiple heavy metals, although the dry weight yield was the lowest among the three plants.

Introduction

The paddy soils contaminated with heavy metal have posed a major environmental and human health problem around the world, due to mining activities, industrial processes, the use of synthetic product (e.g. paints) and other anthropic activities during the last century (Cambra et al., 1999; Dudka and Adriano, 1997). Therefore, remediation operations to remove heavy metals from contaminated soil are necessary (Baker et al., 1994; Geiger et al., 1993). Traditional remediation methods involve excavation and burial, or washing of polluted soils with strong acids and chelators (Michael and Huang, 2000; Steele and Pichhel, 1998), however, they have been losing public acceptance and economic favor, and phytoremediation as an alternative technology has emerged (Glass, 2000). Phytoextraction of heavy metal-contaminated soil, defined as the use of living green plants to transport and concentrate metals from the soil into the above-ground shoots, which are harvested with conventional agricultural methods (Baker et al., 1994; Kumar et al., 1995; Raskin et al., 1994), represents a novel, cost-effective 'green' remediation technology for clearing up polluted-soils, and has gained attention widely in last decade (Blaylock, 1999; Salt et al., 1995).

* FAX No: 00186-20-84113652. E-mail: ls53@zsu.edu.cn

Early phytoextraction research mainly focused on plants known as hyperaccumulators, whose shoot parts or leaves may contain >100 mg kg^{-1} Cd, >1000 mg kg^{-1} Ni and Cu, or >10,000 mg kg^{-1} Zn and Mn (dry weight) when grown in natural habitats (Baker and Brooks, 1998). However, phytoremediation potential may be limited by these plants due to the slow growth rate, low biomass production, and a reasonable time frame (e.g. 5–10 years) by remediation with little known agronomic characteristics (Cunninghan et al., 1995; Roger et al., 2000). In addition, the lower metal bioavailability in the soil and poor metal translocation from roots to shoots are major limiting factors for phytoextraction of metals from polluted-soils (Epstein et al., 1999; Huang et al., 1997). Therefore, several plants with higher biomass, such as maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.), have been also tested for their phytoextraction potential (Liphadzi et al., 2003; Lombi et al., 2001). Together with the application of chemical amendments, including chelators, soil acidifiers, organic acid, ammonium, these high biomass plants could partially eliminate these limiting steps as described above in metal phytoextraction (Chaney et al., 1997; Dushenkov et al., 1999; Salt et al., 1998).

Many studies have been conducted using EDTA for phytoextraction of Pb, Zn, and Cd in contaminated soils (Blaylock et al., 1997; Huang et al., 1997; 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; Huang et al., 1997). Up to now, however, no data has been reported that EDTA could enhance Cd uptake in the plant but appeared to stimulate 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 enhanced ^{137}Cs accumulation in plants (Lasat et al., 1998), and promote heavy metals 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 metals extraction rates achieved by addition of chemical amendments, such as EDTA, HEDTA, organic acid, and NTA (Cooper et al., 1999). Despite chelate agents, such as EDTA, have been shown to enhance phytoextraction of Pb from contaminated soil, some concerns have been expressed regarding the leaching of metal-chelate complexes to groundwater, posing potential risk during extended periods of time. Several reports have indicated the possible threat of heavy metals to groundwater contamination (Copper et al., 1999; Grčman et al., 2001; Huang and Cunningham, 1996; Kos and Leštan, 2003), therefore phytoextraction utilizing chelates must be designed properly to protect environmental safety.

It has been recognized that selection of appropriate plant materials and appreciate chemical amendments is still very important even today for promoting phytoremediation efficiency. The study on phytoextraction initiated in China since the 1990s, and several heavy metal hyperaccumulators have been identified, of which *V. baoshanensis* is a new species belonging to Violaeceae (Shu et al., 2003), and has been proved to be a Cd hyperaccumulator based on field investigation and hydroponic test (Liu et al., 2004). *Vetiver zizanioides*, a perennial grass belonging to gramineous plant, has been widely known for its rapid growth and high biomass, and high tolerance to heavy metals (Shu et al., 2002; Truong and Baker, 1998), and could be potentially used for phytoremediation of soils contaminated by heavy metals (Chen and Cutright, 2001). *Rumex K-1 (Rumex patientia* × *R. timschmicus)* is also a perennial grazing with high biomass and used for water erosion control in North China, although the metal tolerance in this plant had not been studied, but several *Rumex* plants have been proved to be metal tolerant species or Pb hyperaccumulators (Liu et al., 2002). Therefore, a field trial was conducted to: (1) evaluate the metal accumulation capacity and phytoextraction efficiency of *V. baoshanensis*, *V. zizanioides* and *Rumex K-1* grown on paddy soil contaminated by Pb, Zn, and Cd; and (2) assess the effects of different chemical amendments on the phytoextraction efficiency of these plants.

Materials and methods

Site description

The study site is a farmland nearby the Lechang lead/zinc (Pb/Zn) mine, which is located at about

4 km east of Lechang city in the northern part of Guangdong Province, South China. The Pb/Zn mine, located at latitude 24°40′ N and longitude 113°20′ E, has a humid subtropical climate with an annual average temperature and rainfall of 19.6 °C and 1522 mm, respectively. The minerals mainly consist of pyrite, sphaleritem galena, and magnetite, with calcite, quartz and muscovite as minor minerals (Shu et al., 2001). In the Pb/Zn mine area, approximately 25,000 tons of waste rocks and 30,000 tons tailings per year are produced with total dumping areas of 8,300 and 60,000 m^2, respectively (Shu et al., 2001). Local environment has been polluted seriously by discharge of Pb/Zn tailings effluent and dispersion of dust since the 1950s, as a result, the farmland around this mine are seriously contaminated by Pb, Zn, and Cd (Yang et al., 2004).

Field experiment

The experimental farmland near the Lechang Pb/Zn mine, covering an area of 0.8 ha and consisting of six consecutively rectangle paddy plots, was selected for conducting field experiment of phytoremediation. A portion of the contaminated farmland was set up to conduct the field experiment on April 1, 2003. The split-plot design was used in present field experiment, which might aid to overcome heterogeneous condition. 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), and each subplot was planted with *V. baoshanensis*, *V. zizanioides*, or *Rumex K-1*, respectively. Each plant species was planted with the space of 20 cm × 20 cm for *V. baoshanensis*, 40 cm × 40 cm for *V. zizanioides* and *Rumex K-1*. The experiment included four treatments with four replicates: treatment A (EDTA); treatment B [$(NH_4)_2SO_4$]; treatment C (NH_4NO_3); and treatment D (control). The three plants were all transplanted synchronously at the experimental farmland on April 1, 2003. The tillers of *V. zizanioides* (about 20 cm height), were collected from South China Institute of Botany, Guangzhou, seedlings of *V. baoshanensis* (about 3 cm height) was collected from Hunan Province. Seeds of *Rumex K-1*, purchased from Beijing, were sown at the experiment field on January 1, 2003 and about 10 cm height seedings were transplanted on April 1, 2003. During the experiment period, weeding, watering, fertilizing and loosening of the soil were done manually as needed. The NPK fertilizer (1:1:1) was added at the rate of 75 kg N ha^{-1}. On July 20, 2003, $(NH_4)_2SO_4$ and NH_4NO_3 were applied at concentration of 10 mmol kg^{-1}, and dissolved EDTA (Na_2–EDTA) was applied at rate of 6 mmol kg^{-1} on August 20, 2003, 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 zone of plants.

Sample 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), total metal (Pb, Zn, and Cd) contents (digested by 5:1:1 HNO_3, HCl, and $HClO_4$), and extractable metal (Pb, Zn, and Cd) contents [extracted by diethylenetriamine pentaacetic acid (DTPA)]. Concentrations of Pb, Zn, and Cd were measured by flame atomic absorption spectrometry (AAS) (Page et al., 1982). Plant samples were thoroughly washed with de-ionized water to remove surface dust and soil, divided into root and shoot, dried at 80 °C until completely dry, weighted and then milled to pass through 2-mm mesh sieve. Each sample (0.5 g) was digested with concentrated HNO_3 (16 mol L^{-1}) and $HClO_4$ (12 mol L^{-1}) at the ratio of 5:1 (v/v), the concentrations of Pb, Zn, and Cd were determined by AAS (Allen, 1989). Quality control for soils and plants was based on the use of certified samples (GBW 07406 and 07602), samples from inter-laboratory comparisons, internal control samples and duplicates of the analysis.

Phytoextraction rate

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

$$\% \text{ of soil metal removed by one crop} = \frac{(\text{Plant metal concentration} \times \text{Biomass}) \times 100}{(\text{Soil metal concentration} \times \text{Soil mass in the rooting zone})}$$

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^{-1} (assuming a soil bulk density of 1.3 t m^{-3}).

Statistical analysis

Data were examined by one-way ANOVA followed by LSD test as available in the SPSS 11.0 statistical package.

Results

Chemical properties of the soils associated with the three plants

The pH, organic matter, total and DTPA-extractable metal concentrations of the soils are presented in Table 1. The soils of *V. baoshanensis* (pH = 6.28) and *V. zizanioides* (pH = 6.09) were slightly acidic, while the soil of *Rumex K-1* was neutral (pH = 7.13). In general, the soils contained low concentration of total organic matter, but high concentrations of total and DTPA-extractable Zn, Pb, and Cd. The soils associated with *V. zizanioides* contained the highest concentrations of total Pb (2078 mg kg^{-1}) and Zn (2472 mg kg^{-1}), while the soils associated with *V. baoshanensis* had the highest concentration of Cd (9.8 mg kg^{-1}).

The concentrations of heavy metals in plants

Table 2 compares the concentrations of Pb, Zn, and Cd in shoots and roots of *V. baoshanensis*, *V. zizanioides*, and *Rumex K-1* with different treatments. In general, concentrations of Pb, Zn, and Cd in shoots were higher than in roots of *V. baoshanensis* with different treatments. It was also found that the EDTA treatment significantly increased Pb concentrations in shoots of *V. baoshanensis, V. zizanioides* and *Rumex K-1* from 35 to 624 mg kg^{-1}, 19 to 32 mg kg^{-1}, and 19 to 194 mg kg^{-1}, respectively. With the application of $(NH_4)_2SO_4$ and NH_4NO_3, the concentrations of Zn in shoot of *V. baoshanensis* significantly enhanced from 479 up to 799 and 868 mg kg^{-1}, respectively, and Zn concentrations in shoot of *Rumex K-1* increased from 114 to 180 mg kg^{-1} ($P < 0.01$) and 147 mg kg^{-1}, 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.

Table 1. Selected chemical properties of the soils associated with *Viola baoshanensis, Vetiver zizanioides*, and *Rumex K-1* (*Rumex patientia* × *R. timschmicus*) (mean ± se, $n = 4$)

Parameter	Unit	*V. baoshanensis soil*	*V. zizanioides soil*	*Rumex K-1 soil*
pH (1:2)		6.3 ± 0.61 a	6.1 ± 0.36 a	7.1 ± 0.27 a
Organic matter	%	0.64 ± 0.01 a	0.68 ± 0.02 a	0.62 ± 0.02 a
Total Pb	mg kg^{-1}	975 ± 84 c	2078 ± 194 a	1608 ± 309b
Extractable Pb	mg kg^{-1}	63 ± 5.9 b	177 ± 41 a	117 ± 28 a
Total Zn	mg kg^{-1}	924 ± 94 c	2472 ± 65 a	1453 ± 228 b
Extractable Zn	mg kg^{-1}	42 ± 3.8 b	161 ± 33a	76 ± 6.9 b
Total Cd	mg kg^{-1}	9.8 ± 0.9 a	7.0 ± 0.56 b	6.0 ± 0.73 b
Extractable Cd	mg kg^{-1}	0.37 ± 0.03 b	0.29 ± 0.02 b	0.91 ± 0.01 a

Data with different letters in the same row indicate a significant difference at $p < 0.05$ according to the least significant difference (LSD) test.

Table 2. Concentrations of Pb, Zn, and Cd (mean ± se, $n = 4$, mg kg^{-1}) in shoots and roots of *Viola baoshanensis, Vetiver zizanioides*, and *Rumex K-1* (*Rumex patientia* × *R. timschmicus*) grown on contaminated farmland with different treatments

Species	Treatments	Pb		Zn		Cd	
		Shoot	Root	Shoot	Root	Shoot	Root
V. baoshanensis	A	624 ± 238 a	387 ± 162 a	795 ± 207 a	445 ± 96 b	25 ± 8.2 a	30 ± 19 a
	B	68 ± 21 b	381 ± 60 a	799 ± 135 a	762 ± 184 a	30 ± 4.9 a	20 ± 5.1 b
	C	56 ± 14 b	297 ± 21 b	868 ± 329 a	690 ± 125 a	30 ± 5.9 a	17 ± 3.4 b
	D	35 ± 8.4 b	223 ± 40 b	479 ± 51 b	669 ± 225 a	26 ± 8.8 a	18 ± 3.4 b
V. zizanioides	A	32 ± 7.9 a	161 ± 32 a	191 ± 37 a	329 ± 36 b	3.9 ± 0.13 ab	8.1 ± 1.1 b
	B	16 ± 2.2 b	142 ± 24 b	134 ± 41 b	559 ± 247 a	4.3 ± 0.18 a	13 ± 1.8 a
	C	14 ± 5.6 b	114 ± 25 c	194 ± 78 a	367 ± 95 b	4.1 ± 0.29 a	10 ± 1.0 ab
	D	19 ± 2.5 b	136 ± 19 b	144 ± 40 b	339 ± 75 b	3.7 ± 1.8 b	10 ± 1.8 ab
Rumex K-1	A	194 ± 122 a	52 ± 13 a	180 ± 39 a	164 ± 14 a	4.5 ± 0.18 a	4.0 ± 0.17 a
	B	17 ± 2.9 b	25 ± 13 b	147 ± 13 b	151 ± 10 a	4.5 ± 0.19 a	3.9 ± 0.05 a
	C	29 ± 14 b	21 ± 4.5 b	124 ± 6.6 b	150 ± 24 a	4.1 ± 0.18 a	3.7 ± 0.21 a
	D	19 ± 5.1 b	24 ± 6.9 b	114 ± 26 c	125 ± 13 a	4.2 ± 0.37 a	3.7 ± 0.16 a

Note: A: EDTA treatment; B: $(NH_4)_2SO_4$ treatment; C: NH_4NO_3 treatment; D: Control treatment. Data with different letters in the same species, plant tissues (shoot or root) and metal indicate a significant difference at $P < 0.05$ according to LSD test.

Total amounts of heavy metals accumulated in plants and phytoextraction rate

After 140 days' cultivation, the total dry weight yields of *V. baoshanensis, V. zizanioides*, and *Rumex K-1* were 6.5, 30 and 18 t ha^{-1}, respectively. The Pb, Zn, and Cd uptake amounts (mg m^{-2}) in shoots of these three plants are shown in Figure 1. In general, *V. baoshanensis* accumulated the highest amounts of Pb and Cd, in all treatments except Pb in coutrol. The Zn and Cd uptake amounts in shoot of all the three plants showed a similar level under the same treatment, while the application of EDTA had significantly enhanced Pb accumulation, while $(NH_4)_2SO_4$ and NH_4NO_3 treatments slightly increased Zn and Cd uptake amounts in shoot of *V. baoshanensis*. Phytoextraction rates of Pb, Zn, and Cd by *V. baoshanensis, V. zizanioides*, and *Rumex K-1* with different treatments are presented in Figure 2. In general, *V. baoshanensis* had the highest phytoextraction rate among the three plants, while there was no significant difference in *V. zizanioides*, and *Rumex K-1*. The application of EDTA remarkably enhanced Pb and Zn phytoextraction rates of *V. baoshanensis* from 0.01 to 0.19%, and 0.17 to 0.26%, respectively. The addition of $(NH_4)_2SO_4$ and NH_4NO_3 increased the Cd phytoextraction rate of *V. baoshanensis* from 0.66 to 1.24% and 0.81%, respectively.

Discussion

It has been recognized that high accumulation level of heavy metal and high biomass production of plant were of paramount importance for successful phytoextraction (Roger et al., 2000). Up to now, more than 400 metal hyperaccumulator species have been reported in the world (McGrath and Zhao, 2003), but only a few of these hyperaccumulators have been tested for phytoextraction (Baker et al., 1994; Brown et al., 1994; Ebbs et al., 1997). Field trials with selected metal hyperaccumulators, such as *Thlaspi caerulescens*, *Alyssum bertolonii*, and other species, illuminated that these plant species accumulated high levels of Cd and Zn in shoot (Baker et al., 1994; Lombi et al., 2001; Robinson et al., 1997). However, extensive researches have been carried out to select metal hyperaccumulating plants from high biomass production species (Kumar et al., 1995; Liphadzi et al., 2003; Lombi et al., 2001). It has demonstrated that metal hyperaccumulation could be achieved with selected high biomass agronomic crops in conjunction with soil amendments application to the contaminated soils (Roger et al., 2000). It was commonly known that a significantly high amount of plant biomass resulted in accumulating high heavy metal amount, and then an important quantity of metal can be removed

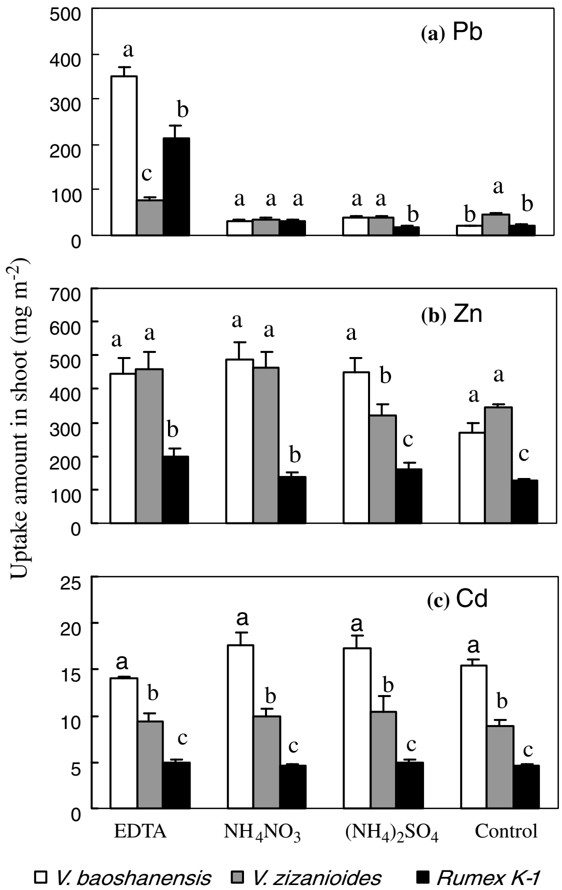

Figure 1. The uptake amounts of Pb (a), Zn (b), and Cd (c) in the shoots of *V. baoshanensis, V. zizanioides*, and *Rumex K-1* (*Rumex patientia* × *R. timschmicus*) with different treatments for a period of 140 days. Different letters in the same treatment indicate a significant difference at $P < 0.05$ according to the LSD test.

form the soil via plant accumulation (Blaylock and Huang, 2000). High-biomass trees such as willows or poplars have also been demonstrated have great potential to further efforts to develop phytoextraction (Liphadzi et al., 2003; Vervaeke et al., 2003).

Field investigation and hydroponic experiment have proved that *V. baoshanensis* is a hyperaccumulator of Cd (Liu et al., 2004). Their results have showed that the concentration of Cd in shoot of *V. baoshanensis* is 1168 mg kg^{-1} 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 demonstrated that the plant had exceptional accumulation capacity for Cd, and the shoot of the species could accumulated 30 mg kg^{-1} Cd from the soil with low concentrations of Cd (total: 9.8 mg kg^{-1}, DTPA: 0.3 mg kg^{-1}), was about 8–10 folds higher than the other two plants. Despite the concentration of Cd in shoot could not achieve the criterion for Cd hyperaccumulator (>100 mg kg^{-1}), which might due to the relatively low concentration of total Cd (9.8 mg kg^{-1}) and DTPA extractable Cd (0.3 mg kg^{-1}) in soil, Cd concentration in shoot of *V. baoshanensis* was also significantly higher than that in the root and soil. The field experiment here indicated that the Cd phytoextraction rate of this species reached 1.24 and 0.81% with the applications of NH_4NO_3 and $(NH_4)_2SO_4$, respectively, and the EDTA treatment increased Pb and Zn phytoextraction rates of this species by 0.19 and 0.26%, respectively. Pb, Zn and Cd phytoextraction rates and accumulation amounts by *V. zizanioides* and *Rumex K-1* were also

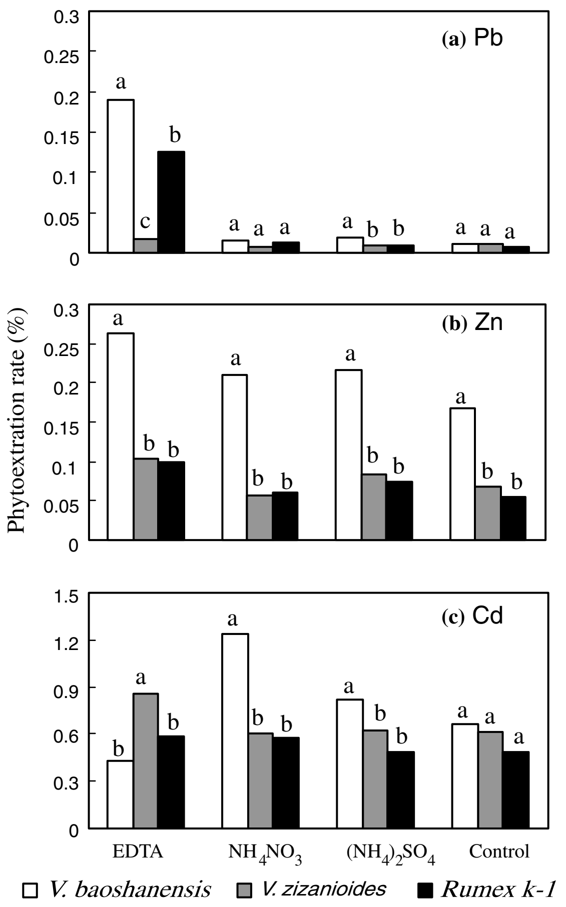

Figure 2. The phytoextraction rates of Pb (a), Zn (b), and Cd (c) by *V. baoshanensis, V. zizanioides*, and *Rumex K-1 (Rumex patientia* × *R. timschmicus)* with different treatments for a period of 140 days. Different letters in the same treatment indicate a significant difference at $P < 0.05$ according to the LSD test.

significantly less than those by *V. baoshanensis* (Figures 1 and 2). The present results suggested, that the relative low biomass hyperaccumulator, *V. baoshanensis*, had significantly higher efficiency in phytoextration of Pb, Zn, and Cd than high-biomass plant.

Blaylock et al. (1997) and Huang et al. (1997) have compared five synthetic chelates (EDTA, DTPA, HEDTA, CDTA, and EGTA), and indicated that the application of EDTA significantly enhanced the concentration of Pb in plant shoots. Ebbs and Kochian (1998) reported that there was no significant difference in Zn concentration and uptake amount in the shoots of oat and wheat grass by addition of EDTA. The ammonium salts applied to assist phytoextraction in the metal contaminated soil have been carried out by Lasat et al. (1998) and Dushenkov et al. (1999), and suggested that the ammonium salt treatments increased the concentration of ^{137}Cs in plants. In the present study, we detected that the EDTA treatment increased the Pb phytoextraction rate of *V. baoshanensis, V. zizanioides* and *Rumex K-1* by 19-, 2- and 13- folds compared with the control treatments (Figure 2). Those results were in line with the view that EDTA is the most efficient chelator in increasing Pb accumulation in plant shoots (Blaylock et al., 1997; Huang et al., 1997), while Zn and Cd accumulation and uptake amount had no remarkable response to the application of EDTA (Figures 1 and 2). The application of $(NH_4)_2SO_4$ and NH_4NO_3 had slightly enhanced the uptake amounts of Zn and Cd in shoots of *V. baoshanensis, V. zizanioides* and *Rumex K-1.*

It is generally noted that *Thlaspi caerulescens* is the suitable candidate plant for phytoremediation operation, which has a lower annual biomass but accumulate a very high Zn and Cd concentrations in the shoot (Brown et al., 1994, 1995; McGrath et al., 1993). It was also reported that three successive cropping of *T. caerulescens* remove approximately 200 and 8 mg kg^{-1} of Zn and Cd for a period of 391 days from the soil industrially contaminated with Zn (2920 mg kg^{-1}) and Cd (19 mg kg^{-1}) (Lombi et al., 2001). In present study, one cropping of *V. baoshanensis* grown over 140 days removed approximately 20, 268 and 15 mg kg^{-1} for Pb, Zn and Cd from the contaminated farmland without any treatment, respectively (Figure 1). Especially, the uptake amounts of Pb, Zn and Cd were 349, 445 and 14 mg by one crop with the application of EDTA, respectively, suggested that *V. baoshanensis* was an important candidate for phytoremediation of soil contaminated by heavy metals. Furthermore, *V. baoshanensis* also had an advantage to phytoremediate the contaminated soil with multi-metals, due to the plants could strongly accumulate three elements (Pb, Zn and Cd) at the same time. The more extensive use of *V. baoshanensis* stands in phytoextraction looks very promising through the successive cropping, due to this species is perennial plant with biomass production of 6.5 t ha^{-1}.

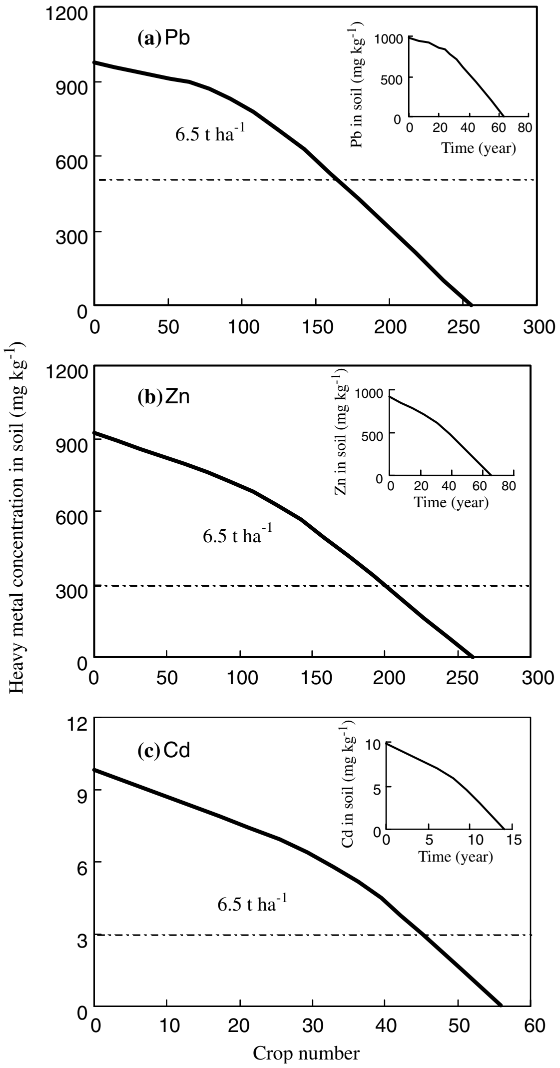

Figure 3. The concentrations of Pb (a), Zn (b), and Cd (c) in soil after successive croppings of *V. baoshanensis* with 6.5 t ha^{-1} biomass or during phytoremediating time frame. Horizontal lines represent the targets for phytoremediation.

Although *V. baoshanensis* had high potential for phytoremediation, it was a long way for using the plant for phytoremediation in practice, for example, based on the data of present trial (Figure 3). It would take 56 croppings of *V. baoshanensis* (14 years) to reduce soil Cd from the initial concentration of 9.8 mg kg^{-1} to 3 mg kg^{-1} with 6.5 t ha^{-1} biomass produce by the NH_4NO_3 treatment. And 256 and 260 croppings of *V. baoshanensis* (64 and 65 years, respectively) would be required to achieve the initial Pb and Zn concentration of 975 and 924 to 500 and 300 mg kg^{-1} soil Pb and Zn target with 6.5 t ha^{-1} biomass by the EDTA treatment, respectively. It appeared that phytoremediation using *V. baoshanensis* is more feasible when soil contaminated by low levels of Cd than that of Pb and Zn (the critical values of Pb, Zn, and Cd are 500, 300, and 3 mg $kg^{-1}$of soil respectively). The previous reports and present results show the limitations of phytoremediation only using hyperaccumulator, such as *V. baoshanensis* or *T. caerulescens*. Given these severe limitations of chelate-assisted phytoextraction and low phytoextraction rate of hyperaccumulator plants, further efforts to develop phytoextraction should focus on transporting genes technology or optimized agronomic methods. Therefore, further explorations are necessary for improving phytoextraction rate of *V. baoshanensis*.

Acknowledgements

This work was supported by the National "863" Project of China (No. 2001AA645010-3) and the National Natural Science Foundation of China (No. 30100024 and No. 40471117).

References

Allen S E 1989 Chemical Analysis of Ecological Materials. 2nd Edn. Blackwell Scientific Publications, Oxford.

Baker A J M and Brooks R R 1998 Terrestrial higher plants which hyperaccumulate metalic elements – a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81–126.

Baker A J M, McGrath S P, Sidoli C M D and Reeves R D 1994 The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Res. Conserv. Recy. 11, 41–49.

Blaylock M J, Salt D E, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley B D and Raskin I 1997 I. Enhanced accumulation of Pb in Indian mustard by soil-applied chelation agents. Environ. Sci. Technol. 31, 860–865.

Blaylock M J 1999 Field demonstrations of phytoremediation of lead contaminated soils. *In* Phytoremediation of Trace Elements. Ed. G S. Banuelos. & N E. Terry. Ann Arbor Press, Ann Arbor, MI .

Blaylock M J and Huang J W 2000 Phytoextraction of metals. *In* Phytoremediation of Toxic Metals Using Plants to Clean up the Environment. Eds. I Raskin. & B D Ensley. pp. 53–70. John Wiley & Sons, Inc., New York.

Brown S L, Chaney R L, Angle J S and Baker A J M 1994 Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soils. J. Environ. Qual. 23, 1151–1157.

Brown S L, Chaney R L, Angle J S and Baker A J M 1995 Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci. Soc. Am. J. 59, 125–133.

Cambra K, Martinez T, Urzelai A and Alonso E 1999 Risk analysis of area near a lead-and cadmium-contaminated industrial site. J. Environ. Health 8, 527–540.

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. Biotech. 8, 279–284.

Chen H and Cutright T 2001 EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45, 21–28.

Cooper E M, Sims J T, Cunningham S D, Huang J W and Berti W R 1999 Chelate-assisted phytoextraction of lead from contaminated soil. J. Environ. Qual. 28, 1709–1719.

Cunningham S C, Berti W R and Huang J W 1995 Phytoremediation of contaminated soils. TIBtech. 13, 393–379.

Dudka S and Adriano D C 1997 Environmental impacts of metal ore mining and processing: a review. J. Environ. Qual. 26, 590–602.

Dushenkov S, Vasudev D and Gleba D 1999 Phytoremediation of radiocesium-contaminated soil in the vicinity of Chernobyl, Ukraine. Environ. Sci. Technol. 33, 469–475.

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 site. J. Environ. Qual. 26, 1424–30.

Ebbs S D and Kochian L V 1998 Phytoextraction of Zn by oat (*Avena sativa*), barley (*Hordium vulgare*) and Indian mustard (*Brassica juncea*). Environ. Sci. Technol. 32, 802–806.

Epstein A L, Gussman C D, Blaylock M J, Yermiyahu U, Huang J W, Kapulnik Y and Orser C S 1999 EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. Plant Soil 208, 87–94.

Huang J W, Chen J, Berti W B and Cunningham S D 1997 Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol. 31, 800–805.

Huang J W and Cunningham S D 1996 Lead phytoextraction: species variation in lead uptake and translocation. New Phytol. 134, 75–84.

Geiger G, Federer P and Sticker H 1993 Reclamation of heavy metal contaminated soils: field soils germination experiments. J. Environ. Qual. 22, 201–207.

Glass D J 2000 Economic potential of phytoremediation. *In* Phytoremediation of Toxic Metals Using Plants to Clean up the Environment. Eds. Raskin, I and Ensley, B D. pp. 15–31. John Wiley & Sons, Inc., New York.

Grčman H, Velikonja-Bolta Š, Vodnik D, Kos B and Leštan D 2001 EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant Soil 235, 105–114.

Jiang X J, Luo Y M, Zhao Q G, Baker A J M, Christie P and Wong M H 2003 Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. Chemosphere 50, 813–818.

Kayser A, Wenger K, Keller A, Attinger W, Felix H R, Gupta S K and Schulin R 2000 Enhancement of phytoextration of Zn, Cd and Cu from calcareous soil: the use of NTA and sulfur amendments. Environ. Sci. Technol. 34, 1778–1783.

Knight B, Zhao F J, McGrath S P and Shen Z G 1997 Zinc and cadmium uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution. Plant Soil 197, 71–78.

Kos B and Leštan D 2003 Influence of a biodegradable ([S,S]-EDDS) and non-degradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. Plant Soil 253, 403–411.

Kumar N P B A, 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.

Lasat M M, Fuhrmann M, Ebbs S D, Cornish J E and Kochian L V 1998 Phytoremediation of a radiocesium-contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. J. Environ. Qual. 27, 165–169.

Liphadzi M S, Kirkham M B, Mankin K R and Paulsen G M 2003 EDTA-assisted heavy-metal uptake by poplar and sunflower grown at a long-term sewage-sludge farm. Plant Soil 257, 171–182.

Liu X M, Nie J H and Wang Q R 2002 Research on lead uptake and tolerance in six plants. Acta Phytoec. Sin. 26, 533–537 (in Chinese).

Liu 2004 *Viola baoshanensis*, a species that hyperaccumulates cadmium. Chinese Sci. Bull 49, 29–32 (in Chinese).

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.

Michael J B and Huang J W 2000 Phytoextraction of metals. *In* Phytoremediation of Toxic Metals Using Plants to Clean up the Environment. Eds. Raskin, I and Ensley, B D. pp. 53–70. John Wiley & Sons Inc., New York.

McGrath S, Sidoli C M D, Baker A J M and Reeves R D 1993 The potential for the use of metal-accumulation plants for the *in situ*, decontamination of metal-polluted soils. *In* Integrated Soil and Sediment Research: A Basis for Proper Protection. Eds. HJP Eijsackrs. & T Hamers. pp. 673–676. Kluwer Academic Publishers, Dordrecht, The Netherlands.

McGrath S P and Zhao F J 2003 Phytoextraction of metals and metalloids from contaminated soils. Curr. Opin. Biotech. 14, 277–282.

Page A L, Miller R H and Keener D R 1982 Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties (2nd edn.), Agronomy No. 9. American society of agronomy and soil science society of America, Madison, Wisconsin.

Pucshenreiter M, Stöger G, Lombi E, Horak O and Wenzel W W 2001 Phytoextraction of heavy metal contaminated soils with *Thlaspi goesingense* and *Amaranthus hybridus*: rhizosphere manipulation using EDTA and ammonium sulfate. J. Plant Nutr. Soil Sci. 164, 615–621.

Raskin I, Kumar N P B A, Dushenkov S and Salt D E 1994 Bioconcentration of heavy metals by plants. Curr. Opin. Biotech. 5, 285–290.

Robinson B H, Chiarucci A, Broods R R, Petit D, Kirkman J H, Gregg P E H and DeDominicis V 1997 The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. J. Geochem. Explor. 59, 75–86.

Roger D, Reeves R D and Baker A J M 2000 Metal-accumulating plants. *In* Phytoremediation of Toxic Metals Using Plants to Clean up the Environment. Eds. Raskin, I and Ensley, B D. pp. 193–220. John Wiley & Sons Inc., New York.

Salt D E, Blaylock M J, Kumar P B A N, Dushenkov V, Ensley B D, Chet I and Raskin I 1995 Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13, 468–474.

Salt D E, Smith R D and Raskin I 1998 Rhytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 643–668.

Shu W S, Ye Z H, Lan C Y, Zhang Z Q and Wong M H 2001 Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. Environ. Int. 26, 389–394.

Shu W S, Xia H P, Zhang Z Q, Lan C Y and Wong M H 2002 Use of vetiver and three other grasses for revegetation of Pb/Zn mine tailings: field experiment. Int. J. Phytor. 4, 47–57.

Shu W S, Liu W and Lan C Y 2003 Viola baoshanensis–a new species of violaceae from Human Province, China. Acta Sci. Nat. Univ. SunYat-sen 42, 118–119.

Steele M C and Pichtel J 1998 Ex-situ remediation of metal contaminated superfund soil using selective extractants. J. Environ. Eng. 124, 639–645.

Truong P N V, Baker D E. 1998 Vetiver grass for the stabilization and rehabilitation of acid sulfate soils. *In* Proceedings of Second National Conference on Acid Sulfate Soils, Coffs Harbour, Australia, pp. 196–198.

Vervaeke P, Luyssaert S, Mertens J, Meers E, Tack F M G and Lust N 2003 Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ. Pollut. 126, 275–282.

Yang Q W, Shu W S, Qiu J W, Wang H B and Lan C Y 2004 Lead in paddy soils and rice plants and its potential health risk around Lechang lead/zinc mine, Guangdong, China. Environ. Int. 30, 883–889.

Zhao F J, Lombi E and McGrath S P 2003 Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 249, 37–43.

Section editor: A.J.M. Baker