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Phytoextraction of Heavy Metals by Eight Plant Species in the Field

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Abstract Phytoremediation is an in situ, cost-effective potential strategy for cleanup of sites contaminated with trace metals. Selection of plant materials is an important factor for successful field phytoremediation. A field experiment was carried out to evaluate the phytoextraction abilities of six high biomass plants (*Vertiveria zizanioides, Dianthus chinensis*, *Rumex K-1* (*Rumex upatientia* × *R. timschmicus*), *Rumex crispus*, and two populations of *Rumex acetosa*) in comparison to metal hyperaccumulators (*Viola baoshanensis*, *Sedum alfredii*). The paddy fields used in the experiment were contaminated with Pb, Zn, and Cd. Our results indicated that *V. baoshanensis* accumulated 28 mg kg^{-1} Cd and *S. alfredii* accumulated 6,279 mg kg^{-1} Zn (dry weight) in shoots, with bioconcentration factors up to 4.8 and 6.3, respectively. The resulting total extractions of *V. baoshanensis* and *S. alfredii* were 0.17 kg ha^{-1} for Cd and 32.7 kg ha^{-1} for Zn, respectively, with one harvest without any treatment. The phytoextraction rates of *V. baoshanensis* and *S. alfredii* for Cd and Zn were 0.88 and 1.15%, respectively. Among the high biomass plants, *R. crispus* extracted Zn and Cd of 26.8 and 0.16 kg ha^{-1}, respectively, with one harvest without any treatment, so it could be a candidate species for phytoextraction of Cd and Zn from soil. No plants were proved to have the ability to phytoextract Pb with high efficiency.

Keywords Phytoextraction · Heavy metals · Bioconcentration factor · Field study · *Viola baoshanensis* · Phytoextraction efficiency

1 Introduction

Plant-based environmental remediation technology, or phytoremediation, has been widely pursued in recent years as an in situ, cost-effective potential strategy for the cleanup of trace metals from contaminated sites (Salt et al. 1995). The development of a commercially feasible technology (phytoextraction) depends on several factors including: identifying or creating an ideal phytoextraction plant, optimizing soil and crop management practices, and developing methods for biomass processing and metal extraction (Blaylock et al. 1997).

There are two main phytoextraction strategies proposed to clean up toxic metals from soil. The first phytoextraction approach is the use of metal hyperaccumulator species (Baker et al. 1994). Metal hyperaccumulator plants have been demonstrated to be potentially useful in soil cleanup, as they can take up significant amounts of metal from contaminated soils, but their low annual biomass production tends to limit their phytoextraction ability. As an example, *Thlaspi caerulescens* is generally referred to as a well-

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known Zn/Cd hyperaccumulator, which can accumulate and tolerate up to 10,000 mg kg^{-1} of Zn and 100 mg kg^{-1} of Cd in shoots (dry matter) without showing any symptoms of toxicity (Escarré et al. 2000). Although there are over 400 species of hyperaccumulator plants (Baker et al. 2000) and more are still being sought, very few studies have tested the feasibility of natural hyperaccumulators or other potential plants for phytoextraction performance under field conditions. From a practical aspect, the Zn/Cd hyperaccumulator *Thlaspi caerulescens* (Zhao et al. 2003) and the Cd hyperaccumulator *Viola baoshanensis* (Zhuang et al. 2005) may be suitable for phytoextraction of metal from moderately contaminated soil.

The other possible alternative is the use of non-accumulator plants, either high biomass plants or fast-growing trees that can be easily cultivated using established agronomic practices (Ghosh and Singh 2005; Meers et al. 2005; Solhi et al. 2005). Extensive work has been done on the heavy metal uptake capacity of high biomass crop plants, such as Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), and maize (*Zea mays*) (Szabó and Fodor 2006; Cui et al. 2004; Turgut et al. 2004). High biomass trees such as willows (*Salix spp.*) and poplars (*Populus spp.*) have also been demonstrated to have great potential in furthering efforts to develop phytoextraction (Liphadzi et al. 2003; Vervaekea et al. 2003). It is commonly known that a significantly high amount of plant biomass can compensate for a relatively low capacity for metal accumulation, resulting in the accumulation of a large amount of heavy metal which has been removed from the soil.

Phytoextraction efficiency is determined by two key factors: metal hyperaccumulating capacity and biomass production (McGrath and Zhao 2003). Clearly, if these factors affecting phytoextraction can be optimized, phytoremediation could be accelerated. One approach is to add chemical agents to the soil that increase metal uptake by plants. Several studies have reported that the application of chemical mobilizing agents, such as dissolved ethylene diamine triacetic acid (EDTA), *N*-(2-hydroxyethyl)-ethylene-diaminetriacetic acid (HEDTA), and diethylene-tetramine-pentaacetate acid (DTPA), can enhance the effectiveness of phytoextraction (Nowack et al. 2006; Turgut et al. 2004; Chen and Cutright 2001; Huang et al. 1997). In some cases, the in situ application of such chelators may pose the potential risk of groundwater pollution. Alternatively, processing of plant biomass production through crop management practices plays an important role in commercial phytoremediation. The majority of phytoextraction studies have focused on pot experiments and laboratory hydroponic studies (Solhi et al. 2005; Wenzel et al. 2003), whereas very few studies have attempted to evaluate the potential of natural hyperaccumulators or high biomass crops for phytoextraction under field conditions (McGrath et al. 2006; Hammer and Keller 2003; Vervaekea et al. 2003). This study was launched to compare the metal extraction potential of eight plant species, including natural hyperaccumulators and high biomass crops, growing in a metal-contaminated field, and to evaluate the phytoextraction efficiency of Pb, Zn, and Cd with the application of EDTA.

2 Materials and Methods

2.1 Site Description

The studied site is located on farmland near the Lechang lead/zinc (Pb/Zn) mine, which has a humid subtropical climate with annual average temperature and rainfall of 19.6°C and 1,522 mm, respectively. In the Pb/Zn mine area, approximately 25,000 tons of waste rock and 30,000 tons of tailings per year were produced with total dumping areas of 8,300 m^2 and 60,000 m^2, respectively (Shu et al. 2001). The local soil was seriously polluted by the discharge of Pb/Zn tailings effluent and dispersion of dust since the 1950s. The experimental farmland covered an area of 0.8 ha which was moderately contaminated with Pb, Zn, and Cd (Table 1).

2.2 Experimental Design

The field trial was conducted in a portion of the contaminated farmland. The experimental area was split into eight blocks and 32 plots (each plot 3 m× 2 m). One week before transplanting, the soil was fertilized with N: P: K (1:1:1) fertilizer which was added at a rate of 75 kg N ha^{-1}. The tested plants included two hyperaccumulators (*Viola baoshanensis* and *Sedum alfredii*) and six other metal tolerant plants with relatively high biomass, *Vertiveria zizanioides*,

Table 1 Selected chemical properties of soil in the field (mean ± SD, *n*=4)

Parameter	Unit	Concentration
pH (1:2)		6.5±0.41
OM	%	6.4±0.02
Total Pb	mg kg^{-1}	960±54
Extractable Pb	mg kg^{-1}	119±5.9
Total Zn	mg kg^{-1}	1,050±89
Extractable Zn	mg kg^{-1}	93±7.8
Total Cd	mg kg^{-1}	7.2±0.92
Extractable Cd	mg kg^{-1}	0.52±0.03

Dianthus chinensis, *Rumex K-1* (*Rumex patientia* × *R. timschmicus*), *Rumex crispus*, and two populations of *Rumex acetosa*. The seedlings of *V. baoshanensis*, *S. alfredii*, and *D. chinensis* (about 5 cm height) were cultivated in the laboratory for 1 month then transplanted to the field. The tillers of *V. zizanioides* (about 20 cm height) were collected from the South China Institute of Botany, Guangzhou. Seeds of *Rumex K-1*, *R. crispus*, and two populations of *Rumex acetosa* were purchased from Beijing, sown in the laboratory, then transplanted (about 10 cm height) to the field. Eight plant seedlings were transplanted to the designated field plots, with spacing of 20 cm×20 cm for all tested plants. A split-plot design was used in order to overcome heterogeneous soil conditions. Plants were watered and weeds were removed as necessary. After approximately 100 days of growth, the height of all plants ranged from 0.2 to 1.2 m, with *V. baoshanensis* and *S. alfredii* as the lowest and *V. zizanioides* as the highest, whereas the other four plants had medium heights of approximately 0.4–0.5 m. *V. baoshanensis*, *S. alfredii*, *V. zizanioides*, *D. chinensis*, *Rumex K-1*, and *R. crispus* were treated with EDTA (Na_2-EDTA solution) at a rate of 6 mmol kg^{-1}. One week after application of EDTA, eight plant specimens (four replicates) were harvested and weighed, and the associated soil samples were collected. The plant tissues were analyzed for concentrations of Pb, Zn, and Cd.

2.3 Plant and Soil Analysis

Plant samples were washed thoroughly with deionized water to remove surface dust and soil, divided into root and shoot, dried at 80°C until completely dry, weighed, and ground to <0.5 mm. Plant subsamples (0.5 g) of finely ground tissue were digested with concentrated HNO_3 (16 mol L^{-1}) and $HClO_4$ (12 mol L^{-1}) at a ratio of 5:1 (v/v). The soil samples were digested using a mixture of HNO_3–HCl–$HClO_4$ (5:1:1). The extractable heavy metals (Pb, Zn, and Cd) in soils were extracted with diethylene-tetramine-pentaacetate acid (DTPA). Quality control was addressed by routinely analyzing soil and plant standard reference materials (GBW 07406 and 07602) and including blanks in digestion batches. Metal concentrations in the plant and soil were determined by flame atomic absorption spectrometry (FAAS, Perkin–Elmer, 3030) (Allen 1989; Page et al. 1982).

Fig. 1 The total biomass production (t ha^{-1}, dw, mean ± SD, *n*=4) of *Viola baoshanensis, Rumex crispus, Rumex K-1, Vertiveria zizanioides, Dianthus chinensis, Rumex acetosa DSL, Rumex acetosa JQW, Sedum alfredii* grown in the contaminated field

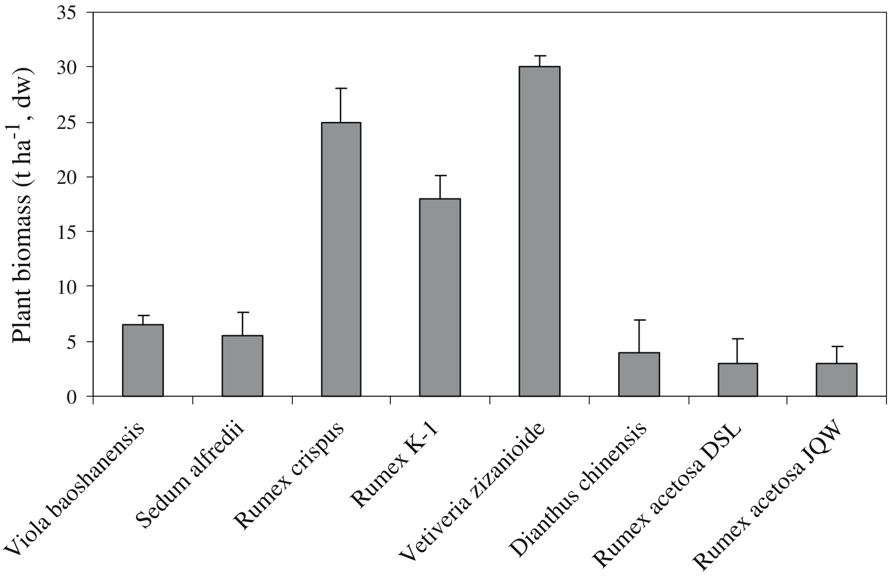

Table 2 Concentrations of Pb, Zn, and Cd in shoots and roots of *Viola baoshanensis*, *Rumex crispus*, *Rumex K-1*, *Vertiveria zizanioides*, *Dianthus chinensis*, *Rumex acetosa DSL*, *Rumex acetosa JQW*, *Sedum alfredii* grown in the contaminated field (mean ± SD, mg kg^{-1}, n=4)

Species		Pb	Zn	Cd
Viola baoshanensis	Shoot	37±4.8	514±24	28±5.2
	Root	223±23	669±130	18±3.4
Rumex crispus	Shoot	52±4.4	1340±105	8.1±1.0
	Root	71±20	1007±326	9.7±2.1
Rumex K-1	Shoot	19±2.9	114±15	4.2±0.21
	Root	24±4.0	125±7.2	3.7±0.09
Vertiveria zizanioides	Shoot	19±1.2	144±20	3.7±0.06
	Root	136±9.7	339±37	10±0.89
Dianthus chinensis	Shoot	146±27	282±43	6.0±0.22
	Root	163±9.6	228±35	7.2±0.43
Rumex acetosa DSL	Shoot	107±14	151±16	3.3±0.19
	Root	152±9.2	289±18	3.7±0.40
Rumex acetosa JQW	Shoot	41±9.2	708±24	4.0±0.01
	Root	86±5.9	866±89	4.2±0.36
Sedum alfredii	Shoot	104±6.5	6279±107	9.2±1.3
	Root	ND	ND	ND

ND no data due to the root of *Sedum alfredii* has rotted by drown in the field.

2.4 Phytoextraction Efficiency

Two indices were calculated to evaluate plants for phytoextraction purposes. The bioconcentration factor (BCF) was calculated by the following equation:

$$\mathrm{BCF} = C_{\text{harvested tissue}}/C_{\text{soil}}$$

where $C_{\text{harvested tissue}}$ is the metal concentration in harvested tissues and C_{soil} is the metal concentration in soil. To make a crude evaluation of the general phytoextraction efficiency of the plants, the depth of the rooting zone, the density of the soil, and the biomass production and mortality of the aboveground biomass components harvested were taken into account to calculate the phytoextraction capability. We assumed that metal pollution occurred only in the active rooting zone, the top 20 cm layer of soil, and that soil bulk density was 1.3 g cm^{-3}. We therefore propose the metal phytoextraction rate (PR), which is calculated as follows (Mertens et al. 2005; Zhao et al. 2003):

$$\mathrm{PR} = \left(C_{\text{plant}} \times M_{\text{plant}}/C_{\text{soil}} \times M_{\text{rooted zone}}\right) \times 100\%$$

where M_{plant} is the mass of the harvestable aboveground biomass produced in one harvest, C_{plant} is the metal concentration in the harvested component of the plant biomass, $M_{\text{rooted zone}}$ is the mass of the soil volume rooted by the species under study, and C_{soil} is the metal concentration in the soil volume.

2.5 Statistical Analysis

All analytical results were performed as the average of four replicates. Descriptive statistics were made using SPSS 13.0 and Excel (Microsoft Inc.) software packages.

3 Results

3.1 Biomass Production

The average biomass production of eight plants grown in the field is shown in Fig. 1. Great differences in biomass were observed between different plants. The total average biomass production (dry weight) was approximately 6.5 t ha^{-1} for *V. baoshanensis* and 5.5 t ha^{-1} for *S. alfredii*. The total average yields of the largest plants, *V. zizanioides*, *R. crispus*, and *Rumex K-1*, were 30 (25 t ha^{-1} for shoot), 25 (20 t ha^{-1} for shoot), and 18 (15 t ha^{-1} for shoot) t ha^{-1} (dry weight), respectively. The biomass production of *D. chinensis* and two populations of *Rumex acetosa* were low or similar to those of hyperaccumulators.

Table 3 Bio-concentration factor (BCF=$C_{\text{shoot}}/C_{\text{soil}}$) of Pb, Zn, and Cd of *Viola baoshanensis*, *Rumex crispus*, *Rumex K-1*, *Vertiveria zizanioides*, *Dianthus chinensis*, *Rumex acetosa DSL*, *Rumex acetosa JQW*, *Sedum alfredii* grown in the contaminated field

Species	Pb	Zn	Cd
Viola baoshanensis	0.05 b	0.77 c	4.80 a
Rumex crispus	0.06 b	1.61 b	1.57 b
Rumex K-1	0.01 c	0.08 d	0.71 c
Vertiveria zizanioides	0.01 c	0.06 d	0.53 c
Dianthus chinensis	0.18 a	0.34 cd	1.30 b
Rumex acetosa DSL	0.12 a	0.16 d	0.62 c
Rumex acetosa JQW	0.04 b	0.76 c	0.95 bc
Sedum alfredii	0.12 a	6.30 a	1.70 b

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 4 Phytoextraction rate of Pb, Zn, and Cd of *Viola baoshanensis*, *Sedum alfredii*, *Rumex crispus*, *Rumex K-1*, *Vertiveria zizanioides*, *Dianthus chinensis* grown in the contaminated field with or without EDTA treatment

Species	Pb		Zn		Cd	
	Control (%)	EDTA (%)	Control (%)	EDTA (%)	Control (%)	EDTA (%)
V. baoshanensis	0.01	0.12	0.11	0.19	0.88	0.90
S. alfredii	0.02	0.04	1.15	1.77	0.25	0.37
R. crispus	0.04	0.18	0.98	1.60	0.86	0.81
Rumex K-1	0.01	0.12	0.06	0.10	0.34	0.36
V. zizanioides	0.02	0.03	0.13	0.17	0.50	0.52
D. chinensis	0.02	0.04	0.03	0.04	0.10	0.11

3.2 Metal Accumulation in Plant Tissues

The concentrations of Pb, Zn, and Cd in shoots and roots are presented in Table 2. Lead accumulation in shoots varied between 19 and 146 mg kg^{-1}, with *D. chinensis* having the highest concentration and *Rumex K-1* the lowest. Concentrations of Zn in the shoots ranged from 114 mg kg^{-1} in *V. zizanioides* to 6279 mg kg^{-1} in the Zn hyperaccumulator *S. alfredii*. Shoot concentrations of Cd varied between 3.3 and 28 mg kg^{-1}, with the lowest accumulation observed in *Rumex acetosa* DSL and the highest observed for the hyperaccumulator *V. baoshanensis*. The accumulations of Cd in *V. baoshanensis* and Zn in *S. alfredii* were significantly higher than those of non-hyperaccumulating plants ($p<0.01$).

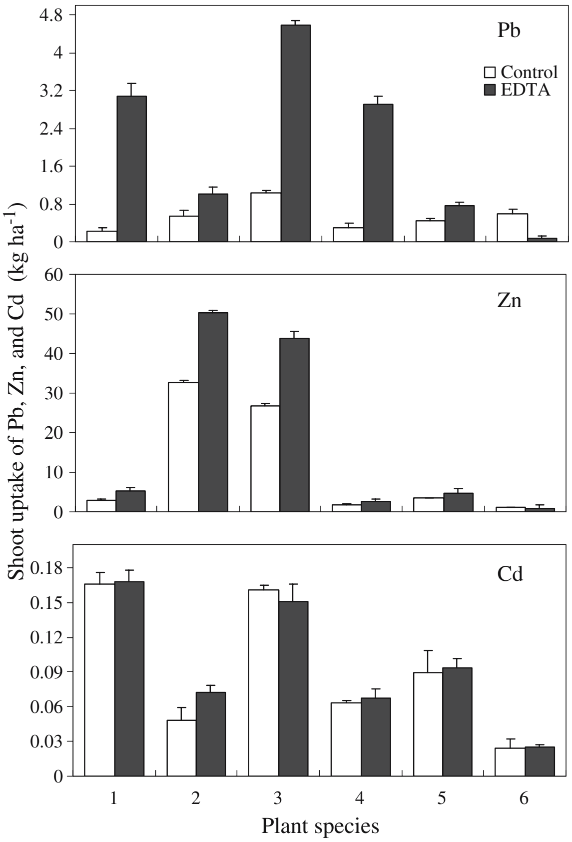

Fig. 2 The uptake of Pb, Zn, and Cd (kg ha^{-1}, □:Control, ■: EDTA) in shoots of 1, *Viola baoshanensis*; 2, *Sedum alfredii*; 3, *Rumex crispus*; 4, *Rumex K-1*; 5, *Vetiveria zizanioides*; 6, *Dianthus chinensis* (with one harvest) grown in the contaminated field with or without EDTA treatment

3.3 Bioconcentration Factor (BCF)

Table 3 shows the bioconcentration factors (BCF) of all tested plant species. The BCF values for Pb were lower than 0.2 for all the eight tested plants. The Zn BCFs of all plants varied between 0.06 and 6.30, with the lowest BCF in *V. zizanioides* and the highest BCF in *S. alfredii*. The BCFs for Cd varied between 0.53 and 4.80, with the lowest BCF observed in *Rumex acetosa DSL*, while *V. baoshanensis* had the highest.

3.4 Phytoextraction Rate (PR)

The phytoextraction rates (PRs) of Pb, Zn, and Cd for *V. baoshanensis*, *S. alfredii*, *R. crispus*, *Rumex K-1*, *V. zizanioides*, and *D. chinensis* grown in the contaminated field with or without EDTA treatment are reported in Table 4. The Cd hyperaccumulator *V. baoshanensis* had the highest PR for Cd (0.88%) among the tested plants. *S. alfredii* had the highest Zn PR (1.15%) among the tested plants without EDTA treatment. The PRs of *R. crispus* for Cd and Zn were 0.86 and 0.98%, respectively, due to its high biomass production. In this field experiment, EDTA treatment increased the Pb PRs of *V. baoshanensis*, *R. crispus*, and *Rumex K-1* by 13.9-fold (from 0.009 to 0.12%), 4.4-fold (from 0.04 to 0.19%), and 10.2-fold (from 0.01 to 0.11%), respectively, compared with the control treatment.

3.5 Effect of EDTA on Metal Uptake

Figure 2 exhibits the removals of Pb, Zn, and Cd (kg ha^{-1}) in shoots of *V. baoshanensis*, *S. alfredii, R. crispus*, *Rumex K-1*, *V. zizanioides*, and *D. chinensis* grown in the contaminated field with or without EDTA treatment. *V. baoshanensis* and *S. alfredii* could extract up to 0.17 kg ha^{-1} Cd and 32.7 kg ha^{-1} Zn with one harvest without any treatment, respectively. Lead uptakes of *Viola baoshanensis*, *Rumex crispus*, and *Rumex K-1* were 3.08, 4.59, 2.91 kg ha^{-1}, respectively, with the application of EDTA. EDTA treatment significantly increased the Pb uptake amounts of most of the tested plants, but had no remarkable effect on Cd and Zn uptake in the six tested plants, with the exception of *S. alfredii* and *R. crispus*.

4 Discussion

Plant biomass, bioconcentration factor, and soil mass are the three key variables that define the phytoremediation potential of a given plant species (Zhao et al. 2003). The field experiment here further suggested that the biomass production of hyperaccumulators (*V. baoshanensis* and *S. alfredii* with 6.5 and 5.5 t ha^{-1}, respectively) was clearly smaller than that of generic non-accumulating plants, such as *V. zizanioides* and *R. crispus*. The bioconcentration factor refers to the most important plant feature in phytoremediation: the uptake of metals, their mobilization into plant tissues, and storage in the aerial plant biomass (McGrath and Zhao 2003). The results from the present study showed that all tested plants had low Pb bioconcentration factor values, indicating that plants had difficulties in mobilizing Pb in the root zone. The bioconcentration factor values of *V. baoshanensis* for Cd (4.80) and *S. alfredii* for Zn (6.30) were significantly higher than those of the high biomass, non-accumulating plants ($p < 0.01$), due to the hyperaccumulators' strong abilities to accumulate the element in shoots. Metal uptake and translocation of an element from roots to shoots is basically linked to the element speciation, soil pH, and other factors. In this study, we determined that multi-contamination of farmland could affect plant performance. The field experiment here indicated that the Cd and Zn phytoextraction rates of *V. baoshanensis* and *S. alfredii* reached 0.88 and 1.15%, respectively, under natural conditions, and the phytoextraction rate of *R. crispus* was 0.86% for Cd and 0.98% for Zn, due to its adequate plant biomass. The results furthermore showed that *R. crispus* had a higher extracting potential for removal of Zn and Cd from multi-contaminated soil.

The goal of the phytoextraction process is to reduce heavy metal concentrations in contaminated soil to acceptable levels within a reasonable time frame. *V. baoshanensis* and *S. alfredii* could extract up to 0.17 kg Cd and 32.7 kg Zn per hectare, respectively, with one harvest without any treatment (Fig. 2). Hulina and Dumija (1995) reported that *R. crispus* had great potential to remove Zn and Cu, which was in line with our results. Interestingly, *R. crispus* could extract 0.16 kg Cd and 26.8 kg Zn per hectare with one harvest without any treatment, which was slightly lower than the phytoextraction values of *S. alfredii* and *V. baoshanensis*, respectively. This result was in line with the view that a greater shoot biomass of crops can more than compensate for lower shoot metal concentration in phytoextraction techniques (Ebbs et al. 1997). These phytoextraction values compared well to the extracted amounts of *T. caerulescens* based on a field trial by Hammer and Keller (2003), where the total amounts of Cd and Zn removed by *T. caerulescens* were 0.53 kg Cd and 20 kg Zn per hectare in acidic, Zn/Cd contaminated soil. Furthermore, the above values were in accord with previous field results from Robinson et al. (1998) and McGrath et al. (2000), where the maximum potential removal of Zn by *T. caerulescens* was found to be 25–50 kg ha^{-1}. Therefore, it is clear that *V. baoshanensis* and *S. alfredii* have similarly high capacities to extract and accumulate both Cd and Zn under field conditions as *T. caerulescens*.

The efficiency of phytoextraction is determined by both the metal accumulation ability of plants and the development of optimal agronomic management practices, including soil management practices to improve metal mobilization and crop management practices to develop a commercial cropping system (Chaney et al. 2000). The application of metal mobilizing agents to soils has been proposed as a way of chemically enhancing root uptake and translocation of metal contaminants from soil to plants, thereby improving phytoextraction (Liphadzi et al. 2003). The results in the field study here indicated that the application of EDTA to soils significantly enhanced Pb removal by most tested plants (Fig. 2), which was consistent with the findings of Blaylock et al. (1997) and Huang et al. (1997). EDTA treatment slightly enhanced the phytoextraction rates and uptake amounts for Zn in shoots of the tested plants. In the present study, the phytoextraction rate and uptake amount of Cd did not respond to the application of EDTA. It is generally noted in the literature that EDTA has taken a predominant place in increasing metal removal efficiency (Copper et al. 1999). However, some concerns have been expressed regarding the leaching of metal-chelate complexes to groundwater, posing potential risks over extended periods of time. Several reports have indicated the possible threat of heavy metal contamination in groundwater (Nowack et al. 2006; Copper et al. 1999), which has made some scientists and legislators frown on this technology to enhance phytoextraction. Therefore, phytoextraction utilizing chelators must be designed properly to ensure environmental safety.

5 Conclusions

A field study demonstrated that *V. baoshanensis*, *S. alfredii*, and *R. crispus* could extract Cd and Zn from metal contaminated soil with high efficiency. Higher bioaccumulation factors were found for Cd in *V. baoshanensis* and for Zn in *S. alfredii* and these resulted in greater extractions of Cd and Zn, respectively. The extraction ability of *R. crispus* to remove Cd and Zn was considerable, due to its higher biomass. No plants were proved to have the ability to phytoextract Pb with high efficiency. Addition of EDTA enhanced the accumulation of Pb in shoots of *V. baoshanensis*, *S. alfredii*, *Rumex K-1*, and *R. crispus*, however, the environmental risk of EDTA application should be considered.

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