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Chelator-enhanced phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with the hyperaccumulator plant (*Sedum alfredii* Hance)

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ABSTRACT

Enhanced phytoextraction of heavy metals using chelating agents and metal-hyperaccumulators has been proposed as an effective approach to removing heavy metals from contaminated soils. The effects of application of EDTA and citric acid (CA) on the growth of *Sedum alfredii* and its heavy metal uptake and accumulation were investigated using the pot-culture experiments. The application of EDTA (5 and 8 mmol kg^{-1}) and CA (5 and 8 mmol kg^{-1}) had inhibitory effects on the growth of the plants, resulted in 60.0%, 50.0%, 55.6% and 85.0% reduction in shoot dry biomass, respectively, compared with that in the control. However, the addition of chelators effectively increased the mobility of target heavy metals (Cd, Cu, Pb and Zn) in soils, and significantly enhanced the accumulation of these heavy metals in aerial parts of the plants. The concentrations of Cd, Cu, Pb and Zn increased by 2.37–4.86, 0.09–3.73, 0.33–5.06 and 3.71–6.06 times, respectively, compared with the control. The application of EDTA (5 and 8 mmol kg^{-1}) and CA (5 and 8 mmol kg^{-1}) could markedly enhance Cd and Zn accumulation, up to 970.6–1463.9 and 3288.3–5376.6 μg pot^{-1} DW, respectively. The most effective method for Cd and Zn accumulation was the treatment of 8 mmol kg^{-1} CA. As for Pb, the addition of 5 mmol kg^{-1} CA was obtained the maximum value of phytoextraction.

1. Introduction

The use of urban wastewater for crop production has been a worldwide agricultural practice in arid and semiarid regions due to the increasing scarcity and high cost of freshwater resources and food (Zhou and Song, 2004; Song et al., 2006; Mutengu et al., 2007; Chary et al., 2008). In China, urban wastewater has been considered as one of the most important freshwater resources and has been used for agricultural irrigation since 1950s (Wang et al., 2003; Song et al., 2006). However, the common practice to deal with the large volume of wastewater was discharged either untreated or after some preliminary treatments. Unfortunately, as one of unexpected side effects, large areas of soils were contaminated by heavy metals, especially cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn), which were the main harmful and toxic elements in China (Gu et al., 2003; Song et al., 2006). Excessive accumulation of heavy metals in agricultural soils has led to elevate heavy metal uptake by crops, and thus affect food quality and safety (Wang et al., 2001), which may cause a potential hazard to human health by way of food chain.

Therefore, the cleanup of heavy metal contaminated soils is emergent and imperative. Phytoremediation was considered as a alternative cleanup method by using plants such as trees, ornamentals and grasses to remove, destroy, or sequester hazardous contaminants from environmental media, such as soil, water and air (Chen and Cutright, 2001; Prasad, 2003; Zhou and Song, 2004). It is an emerging technique as a cost-effective, environmentally friendly, and technically applicable *in situ*, making it preferable to other chemical or mechanical techniques (Lombi et al., 2001; Prasad, 2003). At present, there are two strategies of phytoextraction: (1) continuous phytoextraction which depends on the natural ability of some plants to accumulate, translocate and resist high amounts of metals over the complete growth cycle (e.g., hyperaccumulations), and (2) chelate-enhanced phytoextraction based on the application of chelating agents to the soil to enhance metal uptake by plants (Garbisu and Alkorta, 2001; Zhou and Song, 2004; Alkorta et al., 2004). Hyperaccumulators or hyperaccumulating plants are capable of accumulating large amount of trace elements including nickel (Ni), arsenic (As), Zn, Cd, and Pb in their aboveground tissues without any toxic symptoms (Baker and Brooks, 1989; Sun et al., 2007). However, the use of those species for phytoremediation on a commercial scale is limited due to its low biomass production and slow growth rate. In order to compensate for the low metal accumulation, many researches have been conducted using synthetic chelators such as EDTA and

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natural low molecular weight organic acids (NLMWOA) to enhance the availability of heavy metals in soils and increase phytoextraction efficiency (Cooper et al., 1999; Garbisu and Alkorta, 2001; Chen et al., 2003; Evangelou et al., 2006; Turgut et al., 2004).

Sedum alfredii Hance was found as a new Zn/Cd hyperaccumulator and Pb accumulator (Yang et al., 2002, 2004; Xiong et al., 2004a,b). According to Yang et al. (2002), the wild populations of *S. alfredii* from Pb/Zn contaminated soils were found to contain from 4134 to 5000 mg kg^{-1} Zn in dry shoots. Under hydroponic cultures, Zn concentration and accumulation in shoots reached 19.67 g kg^{-1} and 19.83 mg $plant^{-1}$, respectively, when Zn supplying level reached 80 mg L^{-1}. Besides, *S. alfredii* has strong ability to uptake and accumulate Cd, the concentrations of Cd in leaves and stems reached a maximum of approximately 9000 and 6500 mg kg^{-1}, respectively. And the amount of Cd accumulated in shoots reached 2.9 and 3.2 mg $plant^{-1}$ at Cd level of 200 and 400 μmol L^{-1} (Yang et al., 2004). Zhou and Qiu (2005) also found that the Cd content in leaves reached 11 000 mg kg^{-1} when the plants were exposed to 600 μmol L^{-1} Cd. Xiong et al. (2004a,b) found that the Pb contents in shoot of *S. alfredii* were 0.17 and 1.17 g kg^{-1} at 200 and 1500 μmol L^{-1}.

The main objective of the current work was to assess induced phytoextraction of *S. alfredii* in heavy metal contaminated soil irrigated with industrial wastewater. To this point, chelator-induced phytoextraction study was carried out with EDTA and citric acid (CA) to determine the capacity of *S. alfredii* to phytoextract Cd, Cu, Pb and Zn from soil.

2. Materials and methods

2.1. Site characterization

The Zhangshi Irrigation Area located in the western suburb of Shenyang (Long. 123°11′18″–123°20′37″ E and Lat. 41°43′14″–42°48′41″ N) has been irrigated with industrial wastewater from 1960s to 1980s, and about 2800 ha areas were contaminated by Cd and other heavy metals. Wu et al. (1989) found that the Sluice Gate I was heavily polluted by heavy metals, the contaminated areas were up to 330 ha, and Cd concentrations in soil and rice were from 5 to 7, from 1 to 2 mg kg^{-1}, respectively. In the Sluice Gate II and the Sluice Gate III, the concentration of Cd in soil ranged from 3 to 5 mg kg^{-1}, and rice contained 0.2–0.4 mg kg^{-1} Cd. High Cd accumulation in rice seeds has posed a potential hazard to human health by way of food chain. In this area, the Cd concentration in human blood increased from 1.76 μg L^{-1} in 1978 to 2.23 μg L^{-1} in 1979 and up to 13.26 μg L^{-1} in 1982 (Wu et al., 1989).

2.2. Experimental procedure

2.2.1. Pot-culture experiment

Surface (0–20 cm) samples of air-dried, homogenized and sieved (10 mm) soil (2.5 kg) were fertilized with 250 mg kg^{-1} N (NH_4NO_3), 109 mg kg^{-1} P (K_2HPO_4) and transferred to plastic pots (15 cm height and 20 cm diameter). Three uniform seedlings of *S. alfredii* were transplanted into each pot. After growing for 30 days, different doses of 2 chelators (EDTA and CA) were applied to the soil. Treatments included one control (soil with no chelator) and 2 chelators amended to soil surface as solutions at doses of 5 and 8 mmol kg^{-1} dry weight (DW), which were referred as CK, EDTA-5, EDTA-8, CA-5, and CA-8. The tested soil samples were watered to reach 75% of the field water-holding capacity and maintained this humidity by daily watering throughout the cultivation, and a petri dish was placed under each pot to collect potential leachate during the experiment.

2.2.2. Analysis of heavy metal speciation

Cd, Cu, Pb and Zn speciation in the soil was performed using sequential extraction by Tessier et al. (1979). The exchangeable fraction was determined through extraction with 8 mL of 1.0 M $MgCl_2$ at pH 7.0 for 1 h. The carbonated-associated fraction was determined after extraction with 8 mL of 1.0 M NaAc adjusted to pH 5.0 with acetic acid for 5.0 h. The Fe–Mn oxide fraction was determined after extraction with 20 mL of 0.04 M NH_2·HCl in 25% (v) acetic acid (pH 2.0) for 6.0 h at 96 °C. The organic fraction was determined after extraction with 3 mL of 30% H_2O_2 and 0.02 HNO_3 (pH 2.0) for 2.0 h at 85 °C, followed by 3 mL 30% (v) H_2O_2 (pH 2.0) for 3.0 h at 85 °C and then 5 mL of 3.2 M NH_4Ac in 20% HNO_3 diluted to 20 mL at room temperature for 0.5 h. The residual fraction, the above four fractions subtracted from the total metal content.

2.3. Plant and soil analysis

The plants were immersed in a 0.01 M HCl solution to remove any external Cd and rinsed with deionized (DI) water (Aldrich et al., 2003). Subsequently, the plants were separated four parts: roots, stems, leaves and seeds. After that, they were dried at 100 °C for 10 min, then at 70 °C in an oven until completely dry. The plant and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). The concentration of Cd was determined using the atomic absorption spectrophotometry (WFX-120) method.

Bioaccumulation factor (BF) is defined as the ratio of metal concentration in the plants to metal concentration in the soil, and the transfer factor (TF) is defined as the ratio of the concentration in the stems to that in the roots.

2.4. Statistical analysis

All treatments were replicated three times in the experiments. The means and standard deviations (SD) were calculated by the Microsoft Office Excel 2003. One-way analysis of variance was carried out with SPSS10.0. When a significant ($P<0.05$ or $P<0.01$) difference was observed between treatments, multiple comparisons were made by the LSD test.

3. Results and discussion

3.1. Soil properties

Basic physico-chemical soil characteristics and heavy metal concentrations are listed in Table 1. The studied soil texture was silty clay with a moderate cation exchange capacity of 16.38 cmol kg^{-1} soil. The original total metal concentrations in the contaminated soil were 3.03±0.41 mg Cd kg^{-1}, 45.51±1.80 mg Cu kg^{-1}, 57.93±1.44 mg Pb kg^{-1} and, 168.81±16.39 mg Zn kg^{-1}, respectively. Based on the mean concentrations, the components in soils arranged in the following decreasing order: Zn>Pb>Cu>Cd, according to the environmental quality standards for heavy metals in soils (GB 15618-1995), which are considerably higher than the natural background values of heavy metals in soils of China, showing about 15.2-, 1.3-, 1.7-, and 1.7-fold, respectively. Especially, the content of Cd was 3 times more than the environmental quality standard (Grade III) for Cd in soils of China (GB 15618-1995), indicating that the soils were heavily contaminated by Cd, and which was consistent with Li et al. (2009), Xiong et al. (2004a,b), and Sun et al. (2008a,b), who reported that the Cd concentrations in topsoils of the Zhangshi Irrigated Region ranged from 0.42 to 3.93 mg kg^{-1}, from 2.10 to 4.30 mg kg^{-1} and

Table 1
Basic physical and chemical properties of the tested soil

Soil property	Soil value
pH	5.78±1.27
OM (%)	1.96±0.38
CEC (cmol kg^{-1})	16.38±3.48
Total-N (%)	0.96±0.39
Available-P (%)	16.32±5.31
Available-K (%)	96.83±31.56
Background Cd^{2+} level (mg kg^{-1} soil DW)	3.03±0.41
Background Cu^{2+} level (mg kg^{-1} soil DW)	45.51±1.82
Background Zn^{2+} level (mg kg^{-1} soil DW)	168.81±16.39
Background Pb^{2+} level (mg kg^{-1} soil DW)	57.93±1.44

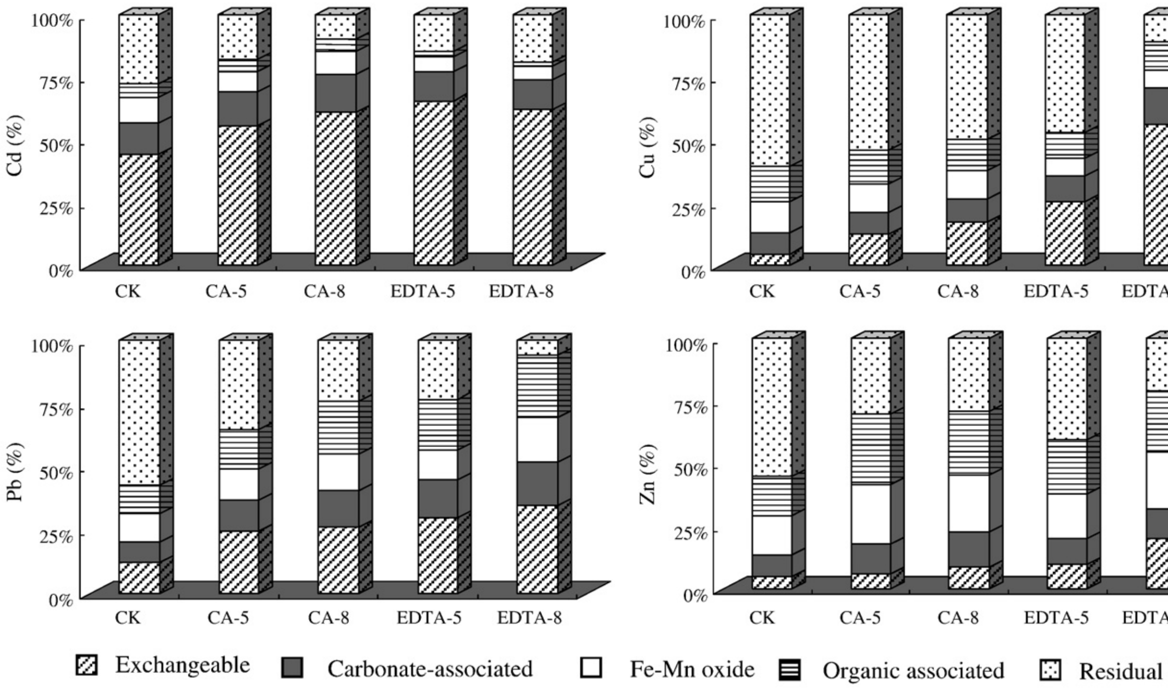

Fig. 1. Chemical speciation of Pb, Cd, Cu and Zn in studied soils.

from 0 to 7.57 mg kg^{-1}, respectively. Meanwhile, the mean Pb, Cu and Zn concentrations in the 0–20 cm soil layer were 40.46, 32.15 and 150.10 mg kg^{-1}, respectively (Li et al., 2009).

3.2. Effect of EDTA and CA on heavy metal speciation

The results of preliminary sequential extraction tests performed to evaluate heavy metal distribution in soil are depicted in Fig. 1. When no chelator was applied, the high proportion (44.4%) of Cd was associated with exchangeable fraction, which is in agreement with other studies on the high mobility of metals in the acid environment (Prechthai et al., 2008), while the most portions of Cu, Pb and Zn were observed in the residual form, up to 57.0%, 60.6% and 54.9%, respectively. The application of EDTA and CA could significantly ($P<0.05$) enhance exchangeable fraction of heavy metals, the portions of Cd, Cu, Pb and Zn with exchangeable fraction increased by from 0.25 to 0.47, from 1.00 to 1.81, from 1.67 to 11.03 and from 0.22 to 1.97 times, respectively, under the treatments of CA-5, CA-8, EDTA-5 and EDTA-8 compared with the control. It has been reported that the accumulation of metals in plants only occurs with high concentrations of metals in soils (Sun et al., 2007; Prechthai et al., 2008). EDTA and LMWOA can chelate and mobilize the heavy metals in soils, and have been used in decontamination or phytoremediation enhancement of metal polluted soils (Wu et al., 2003; Meers et al., 2005). However, the high mobilization of Cd, Cu, Pb and Zn in soils may result in substantial groundwater pollution (Wu et al., 2003; Evangelou et al., 2006; Neugschwandtner et al., 2008). In response to the enhancement of exchangeable fraction, the residual form of heavy metals was markedly reduced by the addition of EDTA and CA compared with the control.

3.3. Response of EDTA and CA to plant growth

It revealed that the application of EDTA and CA had inhibitory effects on plant growth, there were numerous brown dots on the leaves, and especially in the treatment of 8 mmol kg^{-1} EDTA, some plants even died after 2–5 days, indicating phytotoxicity of EDTA and CA to *S. alfredii*.

As shown in Fig. 2, the addition of EDTA and CA inhibited the growth of plants and the dry biomass yields of roots, stems, leaves and shoots were reduced with different degrees when compared with the plants in the control. The statistical analysis showed that there was no significantly difference in the dry biomass of the roots between the treatments of CA and CK. However, under the treatments of EDTA-5 and EDTA-8, the root biomass decreased significantly ($P<0.05$) relative to the control, resulting in 55.9% and 71.4% reduction, respectively. For aerial parts, the inhibitory effects of chelators on *S. alfredii* were in the decreasing order of CA-8<EDTA-5<CA-5<EDTA-8. The application of CA (5 and 8 mmol kg^{-1}) and EDTA (5 and 8 mmol kg^{-1}) had a significant ($P<0.05$) decrease in the dry biomass, up to 70.0%, 57.0%, 64.9% and 84.0% reduction for the stems, 53.3%, 45.4%, 49.3% and 85.7% reduction for the leaves, and 60.0%, 50.0%, 55.6% and 85.0% reduction for the shoots, respectively, compared with that in the control group. When the concentration of EDTA was up to 8 mmol kg^{-1}, leaf and shoot biomass reduced markedly compared with that in the treatments of CA-5, CA-8 and EDTA-5.

Numerous reports suggested that the addition of some synthetic chelators had a significantly adverse effect on plant growth (Grčman et al., 2001; Lai and Chen, 2005; Quartacci et al., 2006). The application

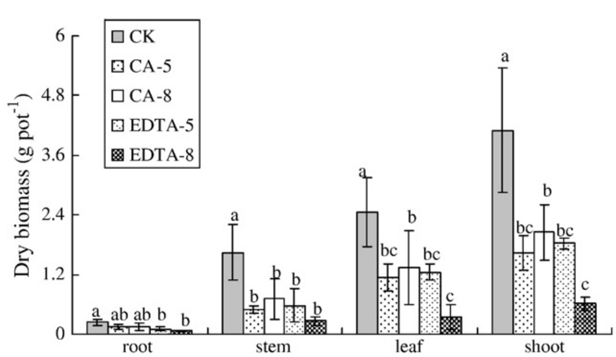

Fig. 2. The dry biomass of *S. alfredii* treated with EDTA and CA. The same letters are not significantly different at $P=0.05$ ($n=3$) between the same tissue of different treatments according to the LSD test. The statistical analysis was a one-way ANOVA.

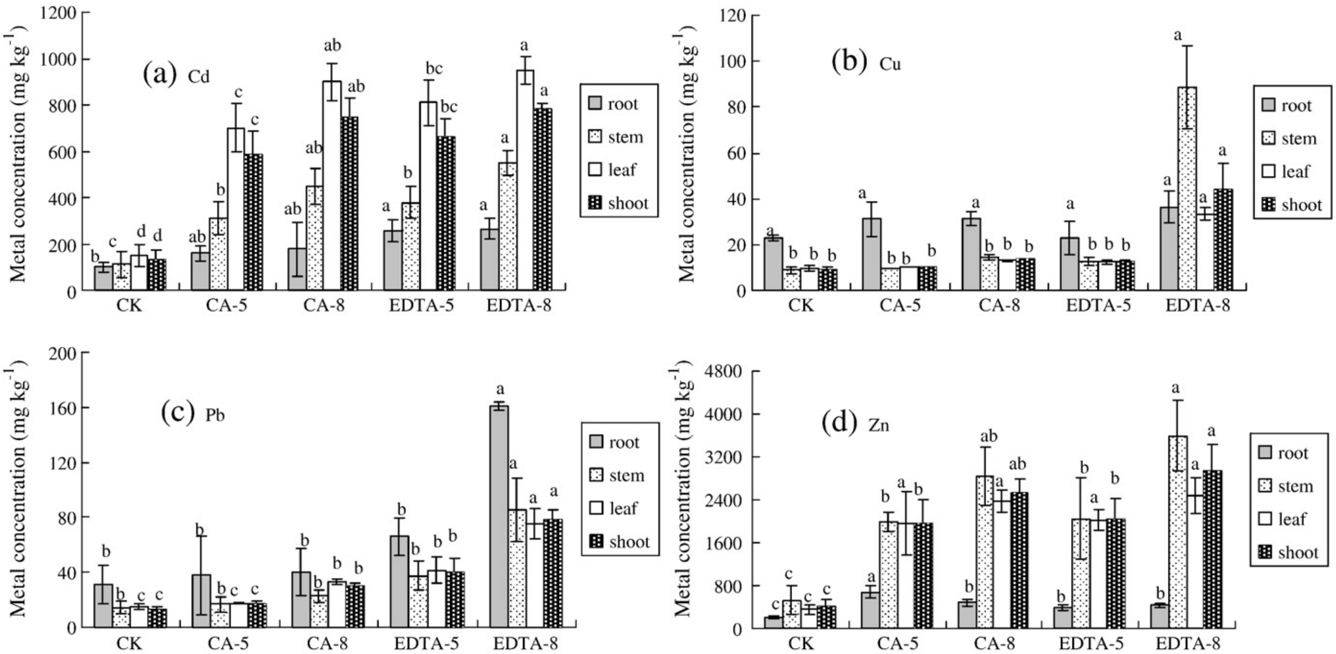

Fig. 3. Effects of application of chelators on the uptake of Cd (a), Cu (b), Pb (c) and Zn (d) in *S. alfredii*. The same letters are not significantly different at P=0.05 (n=3) between the same tissue of different treatments according to the LSD test. The statistical analysis was a one-way ANOVA.

of higher concentrations of NLMWOA showed toxic symptoms such as lower dry weight and chlorosis, and CA had significantly ($P<0.05$) toxic effects at the concentration of 125 mmol kg^{-1} (Evangelou et al., 2006). Quartacci et al. (2006) demonstrated that the dry weights of *B. juncea* shoots showed a significant reduction only following NTA amendment at 5 mmol kg^{-1}. In comparison with the growth of the plants in the control, those treated with NTA showed a 33% reduction in the dry biomass. Luo et al. (2005) found that the dry biomass of shoots decreased up to 60% and 52% that in the control for corn, and 76% and 61% for beans, respectively, on the 14th day after the application of EDTA and EDDS. According to Chen and Cutright (2001), adding HEDTA and EDTA led to a severe yield reduction in the biomass across the treatments, with more than 75% reduction. The severe reduction in the growth was attributed to the combination of heavy metal concentration and the addition of chelators that exceed the capacity of plants to activated defense systems, for instance, NLWOA may damage the plasma membranes which are normally stabilized by Ca^{2+} and Zn^{2+} ions (Kaszuba and Hunt, 1990), and synthetic chelating agents at high concentrations can also be toxic to plants (Cooper et al., 1999; Navari-Izzo and Quartacci, 2001; Luo et al., 2005).

3.4. Effects of EDTA and CA on metal concentrations of S. alfredii

As anticipated, the addition of EDTA and CA to the soils could increase heavy metal uptake by *S. alfredii* when compared with the control group (Fig. 3). As shown in Fig. 3a, adding EDTA and CA significantly enhanced the concentration of Cd in stems, leaves and shoots. When compared with the plants in the control, the application of CA-5, CA-8, EDTA-5 and EDTA-8 resulted in an increase in Cd accumulation at the rate of 0.61, 0.79, 1.61, and 1.68 times for the roots, 1.77, 2.98, 2.36, and 3.88 times for the stems, 3.70, 5.03, 4.43, and 5.37 times for the leaves, and 3.37, 4.56, 3.94, and 4.86 times for the shoots, respectively. In the present study, the ability of EDTA to absorb Cd was higher in comparison with CA and EDTA-8 was the more effective at increasing the concentration of Cd in the plants than other treatments.

There was a little difference in the concentration of Cu in the stems, leaves and shoots among the treatments of CK, CA-5, CA-8 and EDTA-5, and even in the roots, the concentration of Cu was not significantly changed under those treatments (Fig. 3b). However, the concentrations of Cu in stems, leaves and shoots treated with EDTA-8 markedly increased as compared with that in CK, CA-5, CA-8 and EDTA-5, and the amount of Cu in stems obtained a maximum level of 88.6 mg kg^{-1} DW.

Fig. 3c demonstrated that the concentration of Pb in roots was higher than that in aerial parts of the plants, which was consistent with the report by Xiong et al. (2004a,b). The effectiveness of chelator-enhanced Pb uptake in the plants was in sequence EDTA-8>EDTA-5>CA-8>CA-5>CK. The concentrations of Pb in roots, stems, leaves and shoots of the plants treated with EDTA-8 significantly increased in comparison with that in the treatments of EDTA-5, CA-8 and CA-5, respectively. And compared with that in the control, when 8 mg EDTA $kg^{-1}$was applied, the concentration of Pb in roots, stems, leaves and shoots increased at 5.21, 5.93 4.97 and 6.06-fold, respectively, reached 161.0, 84.5, 75.5 and 78.2 mg kg^{-1} DW.

The distribution of Zn in all parts of *S. alfredii* was also significantly affected by the application of chelators (Fig. 3d). The concentration of

Table 2
BFs, TFs and RFs of heavy metals in *S. alfredii*

Treatment mg kg^{-1}	Cd			Cu			Pb			Zn		
	BF	TF	RF	BF	TF	RF	BF	TF	RF	BF	TF	RF
CK	56.30	1.35	8.97%	0.20	0.41	0.03%	0.22	0.42	0.04%	2.28	2.04	0.38%
CA-5	183.38	3.66	12.18%	0.21	0.33	0.02%	0.29	0.45	0.02%	12.59	2.88	0.84%
CA-8	222.24	4.21	17.48%	0.30	0.43	0.02%	0.50	0.74	0.04%	15.61	5.28	1.32%
EDTA-5	228.24	2.56	14.01%	0.30	0.57	0.01%	0.70	0.61	0.05%	10.76	5.34	0.69%
EDTA-8	233.59	2.95	5.84%	0.99	1.21	0.03%	1.40	0.49	0.03%	19.17	6.78	0.49%

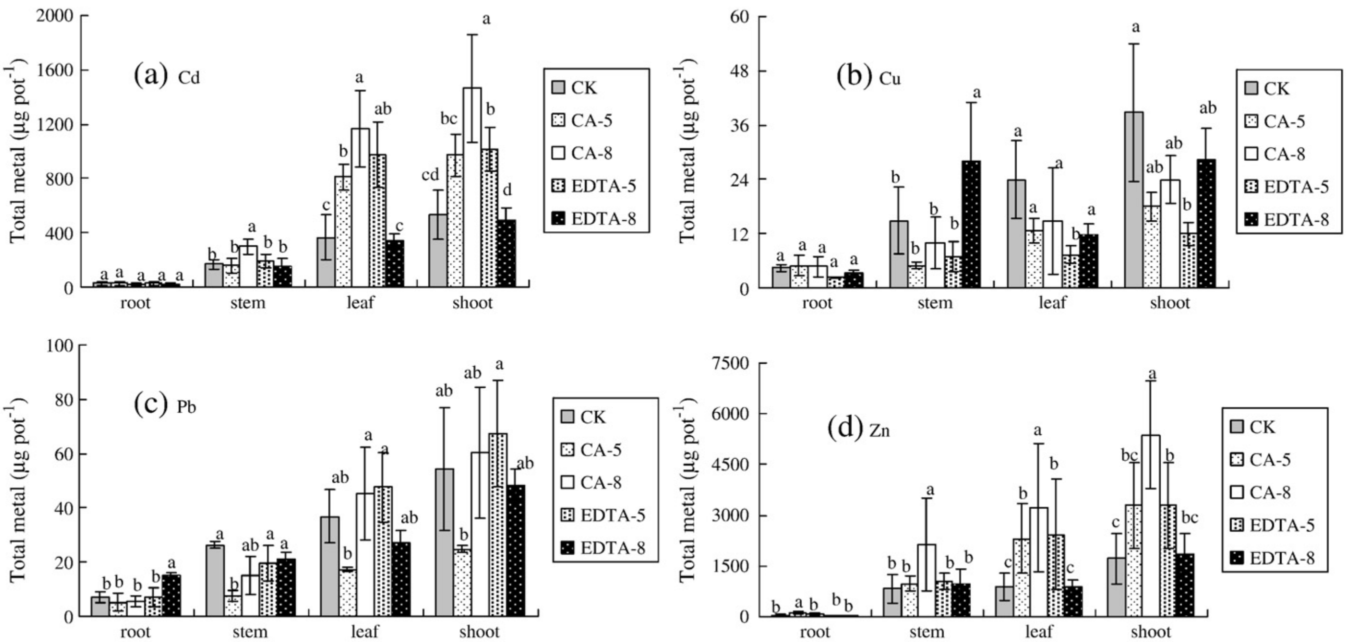

Fig. 4. Effects of chelators on the total metal accumulation in *S. alfredii*, (a) Cd, (b) Cu, (c) Pb and (d) Zn. The same letters are not significantly different at P=0.05 (n=3) between the same tissue of different treatments according to the LSD test. The statistical analysis was a one-way ANOVA.

Zn in stems, leaves and shoots was in sequence EDTA-8>CA-8>EDTA-5>CA-5, whereas the concentration of Zn in roots was in sequence CA-5>CA-8>EDTA-8>EDTA-5. The addition of CA-5, CA-8, EDTA-5 and EDTA-8 resulted in 0.87–2.34 times increase for roots, 2.90–5.85 times increase for stems, 4.59–6.04 times increase for leaves, 3.71–6.06 times increase for shoots compared with Zn in the control.

Yang et al. (2002, 2004) found *S. alfredii* as a Cd/Zn hyperaccumulator. In this study, the concentration of Cd in the plants was in the order of leaf>stem>root, and shoot>root, and the amount of Zn was in the sequence of stem>leaf>root, and shoot>root. The BFs and TFs of Cd and Zn were more than 1.0, which were 56.30–233.59 and 1.35–4.21, and 2.28–19.17 and 2.04–6.78, respectively (Table 2). Furthermore, the concentration of Cd in stems, leaves and shoots ranged from 112.3 to 548.2 mg kg^{-1}, from 149.1 to 949.4 mg kg^{-1}, and from 133.8 to 784.2 mg kg^{-1}, respectively. And the content of Zn in stems, leaves and shoots varied from 1979.2 to 3594.3 mg kg^{-1}, from 1957.2 to 2465.8 mg kg^{-1}, from 1962.6 to 2941.0 mg kg^{-1}, respectively. All the above-mentioned indexes could further validate that *S. alfredii* is a Cd/Zn-hyperaccumulator (Brooks, 1998; Zhou and Song, 2004).

3.5. Impact of EDTA and CA amendments on heavy metal accumulation

Total metal phytoextraction by the plants is shown in Fig. 4. Taking dry biomass reduction and heavy metal absorption into consideration, only Cd, Pb and Zn showed an increased uptake by leaves and shoots of *S. alfredii* after amendments of CA-8 and EDTA-5. The application of CA-8 obtained the maximum of Cd and Zn accumulation in leaves and shoots, and markedly enhanced metal absorption than other treatments, whereas, the maximum Pb accumulation in leaves and shoots was at the addition of EDTA-5. When compared with that in the control, the leaf and shoot metal accumulation increased by 2.19 and 1.75 times for Cd, 0.29 and 0.23 times for Pb, and 2.64 and 2.12 times for Zn, respectively, reached 1166.6 and 1464.0 μg pot^{-1} for Cd, 47.6 and 67.4 μg pot^{-1} for Pb, and 3236.6 and 5376.7 μg pot^{-1} for Zn, respectively. However, the application of EDTA-8 inhibited Cd and Pb accumulation in shoots due to the significant reduction in aboveground biomass. In the case of Cu, when the plants treated with chelators, there was a reduction in Cu absorption by stems, leaves and shoots (except for Cu accumulation in stems in the treatments of EDTA-8). The Cu accumulation in shoots resulted in 53.7%, 38.2%, 69.0% and 27.1% reduction, respectively, in the treatments of CA-5, CA-8, EDTA-5 and EDTA-8, relative to that in the control, because the increase of Cu translocation in the shoots after the application of chelators was not high enough to compensate for a decrease in the plant dry biomass.

3.6. Phytoextraction efficiency

The bioaccumulation factor (BF) and transfer factor (TF) were used to evaluate the effectiveness of a plant in metal accumulation and translocation (Sun et al., 2008a,b). As listed in Table 2, the BF and TF values of Cd, Cu, Pb and Zn increased with the application of EDTA and CA. The BFs and TFs of Cd and Zn were all greater than 1.0, and the BFs of Cu and Pb were also higher than 1.0 in the treatment of EDTA-8. Especially the BFs of Cd and Zn ranged from 183.38 to 233.59 and from 12.59 to 19.17, respectively, indicating high capability of Cd and Zn uptake and transport by *S. alfredii*.

The phytoextraction efficiency of plants depends not only on metal concentration in aboveground biomass, but to a great extent, on the biomass yield of the plants (Komárek et al., 2008; Neugschwandtner et al., 2008). The remediation factor (RF) is defined as the ratio of metal accumulation in shoots to that in soil (Mertens et al., 2005; Sun et al., 2008a,b), which is calculated as follows:

$$\mathrm{RF}(\%) = \frac{M_{\mathrm{shoot}} \times W_{\mathrm{shoot}}}{M_{\mathrm{soil}} \times W_{\mathrm{soil}}} \times 100\% \quad (1)$$

where M_{shoot} is the metal concentration in shoots of a plant (mg kg^{-1}); W_{shoot} is the plant dry aboveground biomass (g); M_{soil} is the metal content in soil (mg kg^{-1}) and W_{soil} is the amount of soil in a pot (g). As shown in Table 2, among the chelator induced treatments, the RF values of heavy metals arranged in the following decreasing order: Cd>Zn>Pb>Cu, and the highest RF value for Cd (17.48%) and Zn (1.32%) was obtained in the treatment of CA-8, and for Pb (0.05%) was in the treatment of EDTA-5, whereas the control variant gave the highest RF value of Cu (0.03%). Therefore, the application of these mobilizing agents is not needed to enhance the uptake of heavy metals by *S. alfredii*, and the chelator-induced manner is not a suitable method for Cu remediation due to its low efficiency (low remediation factors). The RFs of Cd, Cu, Pb and Zn were in the order of CA-8 > EDTA-5 > CA-5 > EDTA-8 > CK, CK > EDTA-8 > CA-8 > CA-5 > EDTA-5, EDTA-5 > CA-8 > CK > EDTA-8 > CA-5 and CA-8 > CA-5 > EDTA-5 > EDTA-8 > CK, respectively. The extraction efficiency of a plant is relevant to different chelators (Luo et al., 2005; Meers et al., 2005; Santos et al., 2006; Quartacci et al., 2006). The application of 5 mmol kg^{-1} soil EDDS to soil significantly increased concentrations of Cu in shoots, with maximum levels of 2060 and 5130 mg kg^{-1} DW in corns and beans, respectively, which were 45- and 135-fold higher than those in the corresponding control plants. Concentrations of Zn in shoots were also higher in the plants treated with EDDS than in those treated with EDTA. For Pb and Cd, EDDS was less effective than EDTA. The maximum Cu phytoextraction was found with the EDDS treatment. The application of EDTA and EDDS also significantly increased the shoot-to-root ratios of the concentrations of Cu, Pb, Zn and Cd in both plant species (Luo et al., 2005). Hence, despite the substantial enhancement of Cd, Cu, Pb and Zn concentrations in the plants, heavy metal absorption was not high enough to achieve extraction rates which would be necessary for practical use (Neugschwandtner et al., 2008). In our study, adding EDTA (5 mmol kg^{-1}) and CA (5 and 8 mmol kg^{-1}) to soil would be promising phytoextraction for soils contaminated by Cd, Pb and Zn.

3.7. The potential of using EDTA and CA for phytoextraction of contaminated soils

The strategy of phytoextraction is based on the fact that the application of chelators to soil significantly enhances metal accumulation by plants (Garbisu and Alkorta, 2001; Ruley et al., 2006), and the application of certain chelators to soil increases the translocation of heavy metals from soil into the shoots. A wide new range of possibilities for this field of metal phytorextraction has been opened (Blaylock et al., 1997), and EDTA has been proven to be very effective in facilitating the uptake of Pb, Cd, Cu, and Zn. Grčman et al. (2001) tested EDTA on the uptake of Pb, Zn and Cd by *Brassica rapa* and found that the concentrations of Pb, Zn and Cd in shoots were detected, up to 104.6, 3.2 and 2.3-times as much as that in the control. Blaylock et al. (1997) added EDTA at the onset of flowering stage, the results demonstrated that the concentration of 1.5% Pb in the shoots of *B. juncea* was obtained from soils amended with synthetic chelators such as EDTA. After the addition of EDTA, the concentrations of Cr, Ni and Zn were increased 26.23, 20.03 and 10.49-fold, respectively, compared with the control (Duo et al., 2005a). Similarly, it caused a 1.77, 1.11 and 1.87-fold increase in Cd, Zn and Pb metal concentration in shoots respectively, compared with that in the control (Santos et al., 2006). Duo et al. (2005b) reported that there was the highest BFs in *Lolium perenne* L and *Festuca arundinacea* L treated with EDTA at the level of 10 mmol kg^{-1}, which increased 9.68 and 1.33 times, respectively, when compared with that in the control.

Another possible alternative is NLMWOA, which is of particular importance due to their complexing properties, which play a significant role in heavy metal solubility and effect on microbial activity and rhizosphere physical properties (Nigam et al., 2000; Wu et al., 2003). And biodegradable chelants such as CA are more attractive than persistent chelators such as EDTA. After application of 5 and 10 mmol kg^{-1} CA, the TFs of Cr increased 2- and 3.5-fold relative to the control (Quartacci et al., 2005). The most significance of Ni uptake in the leaves was at the treatment of 3.0 g kg^{-1} CA, which was 4.3-fold higher than that at addition of 1.0 g kg^{-1} CA (Turgut et al., 2004). According to Jean et al. (2008), CA was more effective in increasing the uptake of Cr by *Datura innoxia* than EDTA. After application of 5 and 10 mmol kg^{-1} CA, the TF of Cr increased 2- and 3.5-fold relative to the control.

Although chelators may increase the effectiveness of phytoextraction by means of increasing the removable metal concentration, not all studies agree. It is important to take into account the biomass losses caused by the negative effects of chelants on a plant. Usually, the metal concentration in the plant increases after the amendment of chelators, while the biomass can result in a significant decrease. As a result, the metal accumulation capacity may decrease (Jean et al., 2008). The increase of Cd in soil solution induced by EDTA did not increase total Cd uptake in a plant that appeared to stimulate the translocation of the metal from roots to shoots when the plants appeared to be under Cd toxic stress (Jiang et al., 2003). Walker et al. (2003) studied the effects of two contrasting organic amendments and EDTA on metal uptake by *B. juncea*, *Raphanus sativus*, and *Beta maritime*, finding out that 2 mmol kg^{-1} EDTA soil had a limited effect on metal uptake. Similarly, other literatures have reported that the use of chelators such as EDTA and DTPA did not enhance (Athalye et al., 1995) and in some cases reduced heavy metal uptake by plants (Robinson, 1997). Furthermore, Chen et al. (2003) found that the amount of Pb or Cd adsorbed by a plant decreased with an increase in the concentrations of citric acid, and the decreasing rates of Pb adsorption were 1.87%, 4.35% and that of Cd 25.25%, 43.89%, respectively, compared with that in the control. And also, chelator enhancement is plant- and metal-specific and is subjective to inhibition when multiple heavy metals are present (Chen and Cutright, 2001; Turgut et al., 2004). Under multiply metal-contaminated soil, the high concentrations of heavy metals may cause antagonistic or synergistic interaction among the different metals that could have enhanced or reduced the capacity of plants to uptake the individual metals (Quartacci et al., 2006).

Moreover, the application of chelating agents to soil can cause prolonged negative effects upon the growth of plants and soil microfauna and they can be also persistent in the environment due to their poor photo-, chemo-, and biodegradability in soil environments (Nowack, 2002; Grčman et al., 2003). Most importantly, the use of EDTA may result in potential risks of surface and ground water pollution through the uncontrolled solubilization and migration of metals (Quartacci et al., 2006). Therefore, potential environmental risk should be considered when chelate enhancement is used to improve photoremediation efficiency (Jiang et al., 2003; Wu et al., 2004; Kos and Leštan, 2004). And the restrictions concerning chelator-assisted phytoextraction may be overcome only when easily biodegradable and low phytotoxic chelating agents are applied.

4. Conclusion

EDTA and CA had positive effects on metal bioavailability in soil and markedly enhanced metal uptake and accumulation in *S. alfredii*. The concentrations of Cd, Cu, Pb and Zn in aerial parts of the plants were significantly increased after the treatments of EDTA and CA compared with those in the control. However, the application of EDTA and CA caused negative effects on the growth of plants, such as the severe decrease in stem, leaf and shoot biomass. Because the enhancement of heavy metal uptake in the plants could offset the reduction of dry biomass, the total heavy metal accumulation in the plants increased with the addition of chelators, especially for Cd and Zn absorption in shoots. The treatment of CA-8 was obtained the highest phytoextraction efficiency of Cd and Zn, and for Pb was at the treatment of EDTA-5, whereas chelator-enhanced phytoextraction of Cu from such contaminated soils with several metals was not a suitable remediation approach. It is necessary to further investigate other methods for phytoremediation of metal-contaminated soils irrigated by industrial wastewater.

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