Influence of EDTA on Lead Transportation and Accumulation by *Sedum alfredii* Hance

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Z. Naturforsch. **62c**, 717–724 (2007); received January 5/March 12, 2007

Hydroponics and pot experiments were conducted to study the effects of ethylenediaminetetraacetic acid (EDTA) on Pb transportation and accumulation by two contrasting ecotypes of *Sedum alfredii* Hance. In hydroponics experiments, the accumulating ecotype (AE) showed more ability to tolerate Pb toxicity compared with the non-accumulating ecotype (NAE). When treated with equimolar mixtures of EDTA and Pb, maximum Pb accumulation occurred without any phytotoxicity symptoms. Pot experiments with Pb contents of 400 mg kg^{-1} showed that 5 mM EDTA is the optimum dose for the phytoextraction of soils contaminated with relatively low Pb levels; in contrast, increasing EDTA addition resulted in increased Pb accumulation in the shoots of AE in soils with high Pb content (1200 mg kg^{-1}). The post-harvest effects of EDTA on available Pb were strong compared with those without addition of EDTA (CK). Within the initial 7 days almost no differences of water-soluble Pb were noted in soils contaminated with both levels of Pb but after 2 weeks, water-soluble Pb started to decrease significantly compared with before. Considering the toxicity and biodegradability of synthetic chelators, it can be concluded that the chelate-assisted technique is more suitable for soils contaminated with low Pb levels and to avoid environment risks; a suitable dose of chelators must be considered before application.

Key words: EDTA, Lead, Phytoremediation

Introduction

Soil pollution caused by lead (Pb) is a widespread global problem (Tandy *et al.*, 2006), and poses critical concerns to human health and environmental issues (Diels *et al.*, 2002). Phytoremediation of heavy metal-contaminated soil is an emerging technology that aims to extract or inactivate metals in soils (Salt *et al.*, 1998). Being cost-effective it has received attention recently and has been implemented for environmental benefits (Chaney *et al.*, 2005).

In spite of elevated Pb levels in contaminated soils, only a fraction of Pb is readily available for plant uptake. The bulk of soil metal is commonly found as insoluble compounds not available for the transport into roots, and consequently affects the metal uptake by hyper-accumulating plants. Recently many synthetic chelators, such as EDTA (ethylenediaminetetraacetic acid), DTPA, HEDTA, EDDS, CDTA and EGTA, have been applied in Pb-contaminated soils to increase the mobility and bioavailability of Pb, thereby increasing the amount of accumulated Pb in the aerial parts of phytoextracting plants (Chen *et al.*, 2004; do Nascimento *et al.*, 2006; Lai and Chen, 2005; Luo *et al.*, 2006; Tandy *et al.*, 2005; Turgut *et al.*, 2005). Among these chelators, EDTA has been found to be the most efficient in increasing the concentration of water-soluble Pb (Blaylock *et al.*, 1997; Wu *et al.*, 1999).

For *in situ* phytoremediation of Pb-contaminated soils, it appears that the chelate-assisted phytoextraction strategy may be proved more effective than one based on the natural ability of certain wild plant species for metal hyper-accumulation (Baker *et al.*, 1991; Chaney *et al.*, 1972). For more than 40 years, synthetic chelators have been applied to supply plants with micronutrients in both soil and hydroponics cultures. Nevertheless the mechanisms by which chelators enhance metal accumulation are still not well characterized (Wallace and Wallace, 1992).

0939–5075/2007/0900–0717 $ 06.00

Sedum alfredii Hance is a newly discovered Zn/Cd hyper-accumulator growing in old Pb/Zn-mined areas of southeast China, and later proved to be a Pb-accumulating species (He *et al.*, 2002; Yang *et al.*, 2002, 2004). He *et al.* (2002) reported that *S. alfredii* was able to accumulate 1182 mg/kg Pb in shoots with a fast growth rate and relatively large amount of biomass. However, compared to Zn and Cd, the phytoextraction of Pb by *S. alfredii* was not high enough, and the application of the chelate-assisted technique on Pb-contaminated soil by *S. alfredii* is still not studied. In the present study, for the stimulatory role of chelators in Pb uptake, the role of EDTA in Pb accumulation and transportation was investigated in a Pb-accumulating plant, *Sedum alfredii* Hance, grown in pot and hydroponics cultures.

Materials and Methods

Plant materials and soil characterization

Two different ecotypes of *Sedum alfredii* Hance, *i.e.* the accumulating ecotype (AE) and non-accumulating ecotype (NAE), were collected from an old Pb/Zn mine area and a tea garden of Hangzhou suburb, Zhejiang Province of China.

Agricultural farm soil was taken from a ranch on the Hua Jia Chi campus of Zhejiang University, Hangzhou, Zhejiang Province China. The samples were sieved through a 2 mm sieve and air-dried for 3 d. The soils were contaminated artificially with Pb, as $Pb(NO_3)_2$, at the content of 400 and 1200 mg kg^{-1} soil, respectively. NH_4NO_3 and KH_2PO_4 were applied as basal fertilizers at the rates of 0.43 and 0.33 g kg^{-1}, respectively (Wu *et al.*, 2004). After adding heavy metals and fertilizers, the soils were equilibrated for 15 d, undergoing five cycles of saturation with distilled water and air-drying. After equilibrating the soil for 15 d, the following parameters were determined: pH value (solid/distilled water 1:2.5, w/v); total organic matter (450 and 600 °C, after heating for 6 h in a muffle furnace); total nitrogen content; total phosphorus and water-soluble P; total potassium and water-soluble N; water-soluble K; total Pb, Zn, Cu and Cd contents (mixed acid digestion with concentrated HNO_3/HCl/HF 3:1:1, v/v); and water-soluble metal contents (solid/distilled water 1:2.5, w/v) (Bao, 2000).

Hydroponics experiment

Healthy and equal-sized shoots of both ecotypes were chosen and grown in Hoagland solution. After pre-culturing for 14 d, the plants were transferred to 2.5 l pots. Before the treatments, composition of nutrient solution was modified by adjusting the KH_2PO_4 concentration to 0.025 mM in order to prevent precipitation of Pb (He *et al.*, 2002). Six different treatments were used, *i.e.* (1) Pb alone (control); (2) Pb/0.02 mM EDTA; (3) Pb/0.05 mM EDTA; (4) Pb/0.1 mM EDTA; (5) Pb/0.2 mM EDTA, and (6) Pb/0.4 mM EDTA. Pb was used as $Pb(NO_3)_2$ at 0.1 mM. The experiment was randomly arranged with three replicates for each treatment. Plants were grown under glasshouse conditions with natural light, day/night temperature of 25 ~ 30 °C and humidity of 70 ~ 90%. Nutrient solution pH value was adjusted daily to 5.5 with 0.1 M NaOH or 0.1 M HCl; the solution was continuously aerated and the treatments renewed every 3 d. The experiment was terminated after 12 d of treatment. At harvest, root morphological parameters such as root length, surface area, diameter, and volume were determined with a root automatism scan apparatus (Min Mac, STD1600$^+$), equipped with WinRHIZO software (Regent Instruments Co). Root and shoot parts were separated, washed thoroughly with distilled water and fresh weights were noted. These parts were then oven-dried at 70 °C after which dry weights were recorded.

Pot experiment

To study the application of EDTA on phytoextraction from soils by AE plants, two levels of Pb-contaminated soils, *i.e.* treated with Pb levels of 400 and 1200 mg kg^{-1} soils, were used. After pre-culturing for three weeks in hydroponics, the seedlings of AE plants were transferred to pots containing 2.5 kg soil. The soil moisture content was maintained at 60% of the water-holding capacity (by weight) adding distilled water every 2 d. Plants were grown in a greenhouse at 30 °C and 24 °C during the day and night, respectively. After two months, EDTA was added to the contaminated soil at the concentrations 1, 5, 10 mM, respectively. Control pots (CK), *i.e.* without amendments of chelating agents, were also maintained in triplicates. All the chelators were purchased in reagent grade and stored at appropriate temperature as recommended by the manufacturers. Plants were harvested on the 10th day after EDTA application. After harvest, soil samples were collected from the soil at day 0, 3, 7, 14, 21 and 30 to study the effects of EDTA on water-soluble Pb in the soils.

Heavy metal analyses of plants and soils

Plant samples were ground with a stainless steel mill, and then passed through 0.1 mm nylon sieve used for Pb analysis. 0.1 g of each plant sample was digested using the $HNO_3/HClO_4$ digestion method. The digested solutions were washed into 50 ml flasks using distilled water. The plant Pb concentrations were determined using ICP-MS (Agilent 7500a, Tokyo, Japan).

To determine the water-soluble Pb content in the soil, distilled water was added to the soil (soil-to-water ratio 1:5, w/v) and the suspensions were shaken for 30 min. The suspensions were then centrifuged and the supernatant was filtered through a 0.45 μm filter paper, acidified with HNO_3 and analyzed for the Pb concentrations by ICP-MS (Agilent 7500a).

Data analysis

Statistical analysis was performed using the SPSS statistical package (version 11.0). All values reported in this work are means of at least three independent replications. Data were tested at significant levels of $P < 0.05$ by two-way ANOVA.

Results and Discussion

Effects of EDTA on growth responses of S. alfredii grown in hydroponics culture

During the application of the chelate-assisted technique, chelator addition may influence the biomass of the plants, so the addition dosage must be considered (Blaylock *et al.*, 1997; Chaney *et al.*, 1997). In hydroponics experiments, the addition of EDTA did not affect the shoot dry weight of AE plants except for the treatment level of 0.4 mM EDTA, at which shoot weight was decreased by 10.6% as compared with CK ($P < 0.05$); in contrast, no impact of EDTA treatment was noted on the shoot dry weight of NAE (Fig. 1A). The results might be attributed to two reasons: first the available Pb in the solution was considerably higher before EDTA addition compared with soil environments, and second the treatment time might be not long enough for the present hydroponics experiment. However, it could be noted that EDTA levels of 0.2 and 0.4 mM caused a significant decrease in root dry weights of both ecotypes of *S. alfredii* compared with CK ($P < 0.05$) (Fig. 1B).

The root growth responses for morphological parameters are listed in Table I. From the results

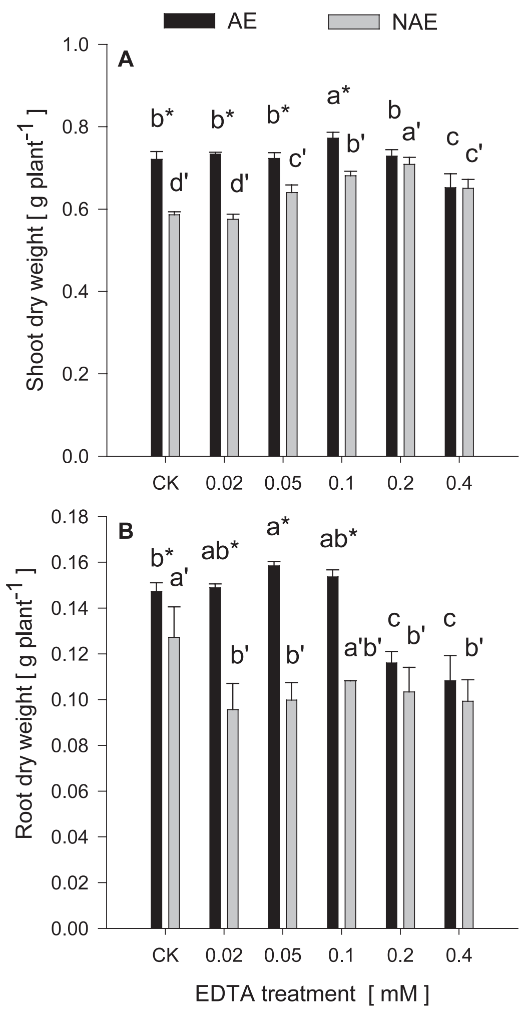

Fig. 1. Dry weight of shoots (A) and roots (B) of both ecotypes of *Sedum alfredii* Hance exposed to various EDTA concentrations for 12 days in hydroponics experiments. Values are means ± SD ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among all the treatments. An asterisk (*) significantly differs AE from NAE plants ($P < 0.05$).

it is obvious that root length of AE plants increased at 0.1 mM EDTA, which was 1.41-fold compared with CK ($P > 0.05$). The same trend could be traced in the root surface area and volume, *i.e.* the addition of 0.1 mM EDTA produced highest growth responses ($P > 0.05$). All the parameters involving root length, surface area and volume of AE plants were always higher than that of NAE plants; however, there were no significant differences in the root diameter of the two ecotypes. From the results of plant biomass and

Table I. Changes in selected root morphological parameters with different Pb and EDTA treatments for the two ecotypes of *Sedum alfredii* Hance.

Treatment [mM]	Length [cm plant^{-1}]		Surface area [cm^2 plant^{-1}]		Average diameter [mm plant^{-1}]		Volume [cm^3 plant^{-1}]	
	AE	NAE	AE	NAE	AE	NAE	AE	NAE
CK	592.63*ab**	366.70*ab*	78.19*a*	69.17*a*	0.51*a*	0.48*b*	1.24*a**	0.78*a*
0.02	639.61*ab**	438.82*ab*	105.77*a**	65.17*a*	0.45*a*	0.43*b*	1.35*a*	0.95*a*
0.05	507.49*b*	405.52*ab*	103.01*a*	81.70*a*	0.54*a*	0.50*b*	1.47*a**	0.99*a*
0.1	833.50*a**	476.87*a*	108.56*a*	87.27*a*	0.58*a*	0.66*a*	1.51*a**	1.06*a*
0.2	816.51*a*	371.47*ab*	97.51*a**	66.27*a*	0.53*a*	0.66*a*	1.37*a*	1.00*a*
0.4	782.04*a*	322.79*b*	86.33*a*	68.86*a*	0.45*a*	0.52*b*	1.23*a*	0.83*a*

Values are means (n = 3). Different letters indicate significant differences ($P < 0.05$) among the treatments. An asterisk (*) significantly differs AE from NAE plants ($P < 0.05$). CK represents control pots without addition of EDTA.

root morphology, it could be concluded that AE plants are more tolerant to Pb toxicity than NAE plants.

Response of plants to heavy metals generally correlates best with the activity of the free, uncomplexed metal ion in solution. However, there are numerous observations that chelating agents are taken up by plants (Epstein *et al.*, 1999; Geebelen *et al.*, 2002; Wu *et al.*, 1999). In the present study the phytotoxicity of EDTA treatments was quantified by monitoring the shoot desiccation. Application of EDTA at concentrations higher than 0.1 mM was associated with significant water loss from the plant tissues. It could be seen that the water content in the shoots of AE plants increased by 15.1% upon exposure to EDTA at 0.1 mM, while 0.2 and 0.4 mM treatments caused a decrease in the water content by 17.5% and 27.7%, respectively, as compared with CK (Fig. 2A). The same trend was found for the root water content of both ecotypes of *S. alfredii* (Fig. 2B). These results are also supported by the findings that toxicity symptoms in *Indian mustard* exposed to Pb and EDTA were strongly correlated with water loss from plant tissues, demonstrating that increased transpiration is not the mechanism through which EDTA exerts its influence on Pb accumulation (Vassil *et al.*, 1998).

Effects of EDTA on Pb accumulation by both ecotypes of S. alfredii grown in hydroponics culture

EDTA appears to chelate Pb outside the plant, and then the soluble Pb-EDTA complex is transported through the plant xylem and accumulates in the leaves (Kim *et al.*, 2003). The results of the present study showed that EDTA addition at concentrations of 0.02, 0.05 and 0.1 mM, respectively, increased Pb contents in shoots of AE plants significantly (Fig. 3A). However, 0.2 mM EDTA in hydroponics experiments caused a sharp decrease in the shoot Pb contents in AE plants compared with those treated with 0.1 mM. These results are consistent with those of Vassil *et al.* (1998) who also reported that the kinetics of Pb and EDTA accumulation were found to be biphasic. Similar results were obtained for the shoot Pb contents of NAE plants, which were always lower than those of AE plants. Pb contents in roots also decreased significantly ($P > 0.05$) compared with CK at treatment levels up to 0.2 mM; however, there were no significant differences of Pb contents in roots between the two ecotypes of *S. alfredii* ($P > 0.05$) (Fig. 3B). This suggested that a threshold EDTA concentration is required to "induce" the accumulation of high contents of EDTA or Pb-EDTA complex in shoots. The plasma membrane-surrounding root cells are thought to play a major role in forming this barrier. Both Zn^{2+} and Ca^{2+} ions are involved in stabilizing plasma membranes (Pasternak, 1987). Therefore, synthetic chelators may induce the metal chelate uptake and accumulation by removal of stabilizing Zn^{2+} and Ca^{2+} from the plasma membrane.

Effects of EDTA on biomass of AE plants grown in Pb-contaminated soils

The physico-chemical properties of the soil used in the present experiment are listed in Table II. In pot experiments, both shoot and root dry weights

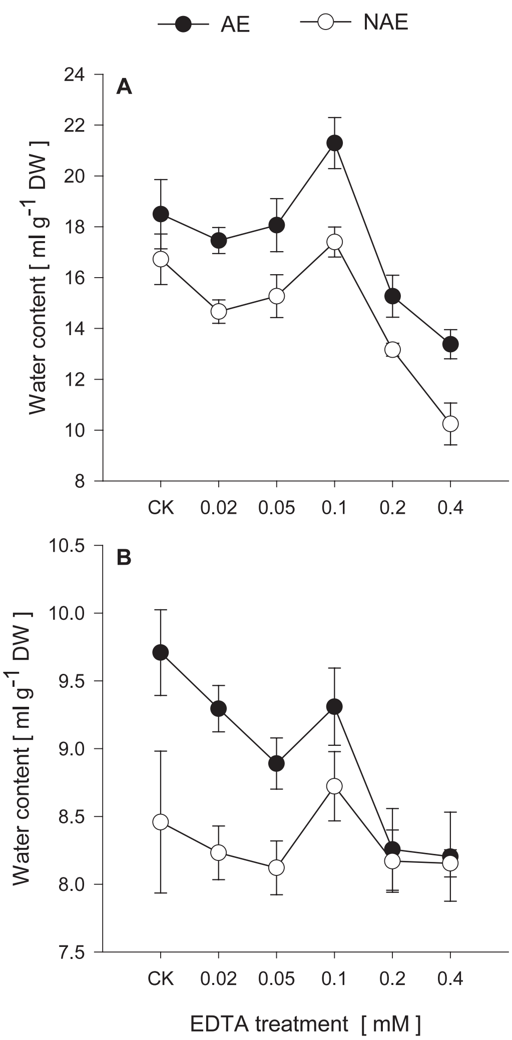

Fig. 2. Water content of shoots (A) and roots (B) of both ecotypes of *Sedum alfredii* Hance exposed to various EDTA concentrations for 12 days in hydroponics experiments. Values are means ± SD (n = 3).

of AE plants grown in soils of low Pb levels were higher than those of high Pb levels ($P < 0.05$), which must be due to the differences of Pb contamination levels in soils. Cooper *et al.* (1999) reported that synthetic chelating agents at high dosages can also be toxic to plants in pot experiments. In the present study, after treating with different dosages of EDTA, the dry weight of AE plants grown in soils contaminated with both levels of Pb decreased significantly compared with CK, showing that EDTA addition significantly increased the contents of available Pb in soils, which might have resulted in the decrease of plant biomass (Fig. 4).

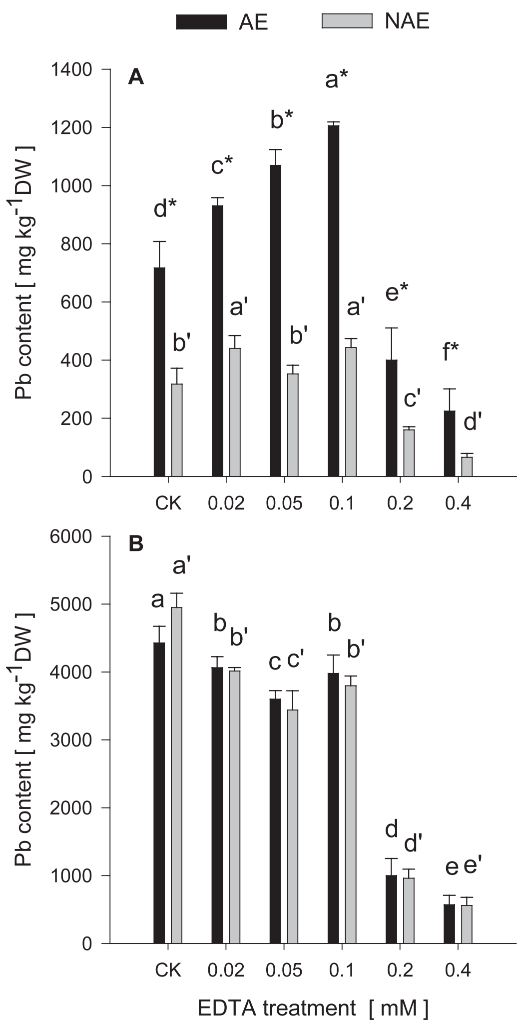

Fig. 3. Pb accumulation in shoots (A) and roots (B) of both ecotypes of *Sedum alfredii* Hance exposed to various EDTA concentrations for 12 days in hydroponics experiments. Values are means ± SD (n = 3). Different letters indicate significant differences ($P < 0.05$) among all the treatments. An asterisk (*) significantly differs AE from NAE plants ($P < 0.05$).

Effects of EDTA on Pb accumulation by AE plants grown in Pb-contaminated soils

After treating with EDTA, Pb contents in shoots grown in soils contaminated with both levels of Pb increased as compared with CK (Fig. 5A). However, no differences in shoots of *S. alfredii* were found among the treatment with 5 and 10 mM EDTA in the soils of low Pb level, showing that the dosage of 5 mM EDTA was sufficient enough for Pb solubility. In contrast, shoot

Table II. Physico-chemical properties of the soils used in the study.

pH	7.12
Organic matter [g kg^{-1}]	22.55
Total N [g kg^{-1}]	1.05
Water-soluble N [mg kg^{-1}]	62.8
Total P [g kg^{-1}]	0.52
Water-soluble P [mg kg^{-1}]	6.5
Total K [g kg^{-1}]	14.6
Water-soluble K [mg kg^{-1}]	66.3
Total metal content [mg kg^{-1}]	
Pb	37.84
Zn	105.33
Cu	15.68
Cd	0.53
Water-soluble metal content [mg kg^{-1}]	
Pb	0.082
Zn	0.151
Cu	0.231
Cd	0.002

Pb contents of the plants grown in soils with high Pb level increased significantly ($P < 0.05$) after treatment with 10 mM EDTA as compared with those treated with 5 mM. It can be concluded that the dosage of 5 mM EDTA might be the optimum dose for soils with low Pb level as compared with the dose of 10 mM. Pb contents in roots were significantly higher ($P < 0.05$) for plants grown in soils with high Pb levels as compared with those grown in soils contaminated with low Pb levels, and the addition of EDTA also effectively increased the Pb contents in roots of *S. alfredii* (Fig. 5B).

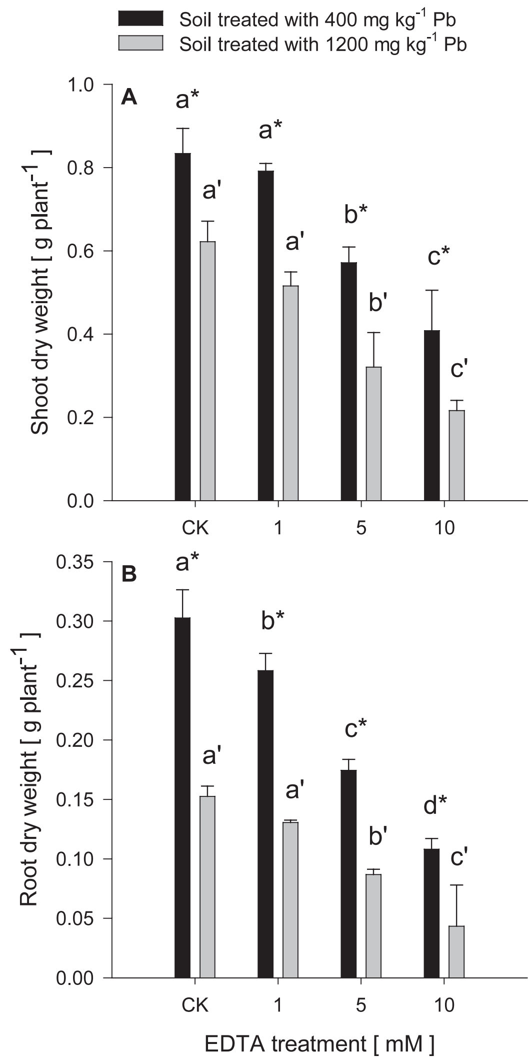

Fig. 4. Dry weight of shoots (A) and roots (B) of accumulating ecotype of *Sedum alfredii* Hance grown in soils contaminated with two levels of Pb exposed to various EDTA concentrations for 10 days. Values are means ± SD ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among all the treatments. An asterisk (*) significantly differs the dry weight of plants grown in soil with low Pb content from those grown in soil with high Pb content ($P < 0.05$).

Post-harvest effects of EDTA on the concentration of water-soluble Pb in Pb-contaminated soils

After application of chelators in the soil, only a limited fraction of mobilized metals was effectively absorbed by the plants (Gupta, 1975; Miller *et al.*, 1986). The concentrations of metals extracted using distilled water were equal to the water-soluble form in the contaminated soil; the metals which were easily leached into the groundwater along with percolating rainwater. Due to the environmental concerns associated with chelators use, post-harvest effects of chelators must be studied (Lai and Chen, 2006; Liphadzi and Kirkham, 2006; Luo *et al.* 2006; Rodriguez *et al.* 2006). It could be seen that the concentrations of water-soluble Pb in soils of both Pb contamination levels increased sharply on the harvest day as compared with CK. At the same time, there was no significant decrease during the first week, and then after 2 weeks the watersoluble Pb content began to decrease significantly compared with before, which might be due to leaching effects and the degradation of EDTA (Fig. 6A). In soils of low Pb level, there were no significant differences of the contents of available Pb between treatments with 5

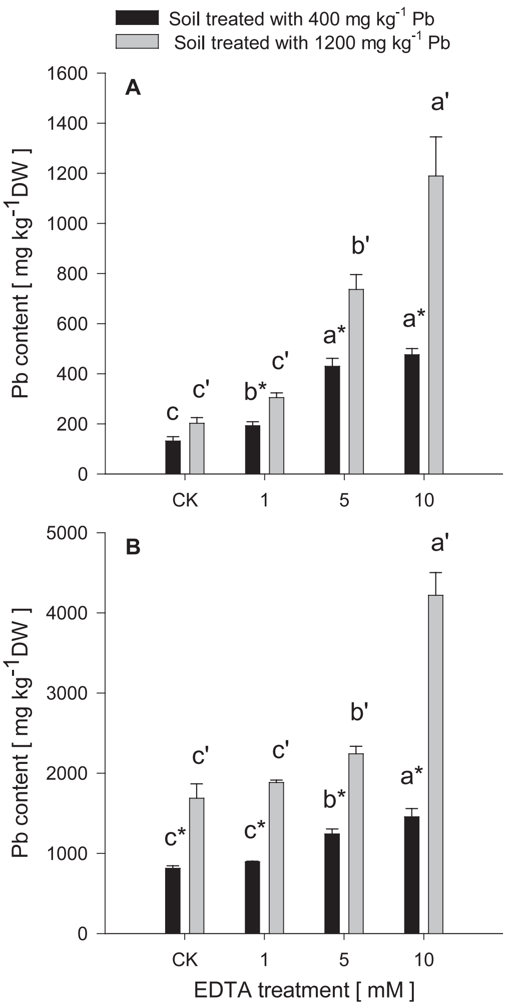

Fig. 5. Pb accumulation in shoots (A) and roots (B) of accumulating ecotype of *Sedum alfredii* Hance grown in soils contaminated with two levels of Pb exposed to various EDTA concentrations for 10 days. Values are means ± SD ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among all the treatments. An asterisk (*) significantly differs the Pb accumulation of plants grown in soil with low Pb content from those grown in soil with high Pb content ($P < 0.05$).

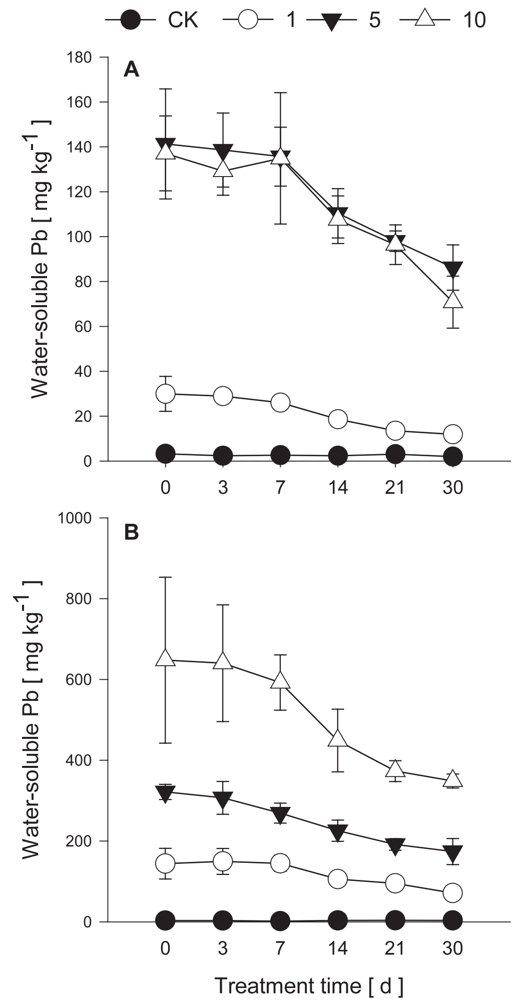

Fig. 6. Post-harvest effects of EDTA on the concentration of water-soluble Pb in soils contaminated with low and high Pb contents along with time. Values are means ± SD ($n = 3$). CK, control; 1, 1 mM; 5; 5 mM; 10, 10 mM.

and 10 mM EDTA showing that the dose of 5 mM EDTA was optimum for phytoremediation, and this result was consistent with the outcome of Pb accumulation in shoots of the pot experiments. In contrast, along with the increase of the EDTA dosage, the contents of available Pb in soils of high Pb level increased accordingly (Fig. 6B). Considering environment risks, it could be concluded that the chelate-assisted technique is more suitable for soils contaminated with low Pb levels.

Acknowledgement

The present research was financially supported by a grant from the National Natural Science Foundation of China (No. 20477039), the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0536), and "973" project from the Science and Technology Ministry of China (No. 2002CB410804).

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