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Optimization of Chelator-Assisted Phytoextraction, Using EDTA, Lead and *Sedum alfredii* Hance as a Model System

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Abstract Pot and leaching column experiments were conducted to optimize chelator-assisted phytoextraction of lead (Pb) from contaminated soils. Optimum phytoextraction occurred at added EDTA concentration of 5 mM in single dose for 10 days in low Pb soil (treated with 400 mg kg^{-1} soil), while it would be better for high Pb soil (treated with 1,200 mg kg^{-1} soil) with five intermittent doses of 10 mM EDTA for 7 days. Combined with column experiment, it could be inferred that chelator-assisted phytoextraction is more suitable for the slightly contaminated soils.

Keywords EDTA · Pb · Phytoextraction · *Sedum alfredii* Hance

Heavy metal pollution of soil is a widespread global problem (Tandy et al. 2006), and this issue has been a major environmental concern over the past several decades. Among all the heavy metals, Pb is considered to be one of the most toxic metals of environmental concern. The major sources of Pb contamination in soil include Pb mining and smelting activities, disposal of Pb-based paints and Pb battery reclamation. These alarming concentrations of Pb posed a critical concern to human health and environmental issues (Diels et al. 2002) and are subjected to most of the current remediation studies.

Phytoremediation of heavy metal-contaminated soil is an emerging technology that aims to extract or inactivate heavy metals in soils (Salt et al. 1998). It has grabbed increased attention for the low cost of implementation and environmental benefits. In spite of the elevated heavy metal concentrations present in contaminated soils, only a fraction of soil heavy metal content is readily available for plant uptake. The bulk of soil heavy metal commonly found as insoluble compounds is unavailable for transport into roots, restricting absorption by hyperaccumulating plants (Pulford and Watson 2003).

Recently many synthetic chelators, such as EDTA, DTPA, HEDTA, EDDS, CDTA and EGTA have been applied in metal-contaminated soils to increase the mobility and bioavailability of heavy metals, thereby increasing the amount of heavy metals accumulated in the aerial parts of phytoextracting plants (Turgut et al. 2005). Among these chelators, EDTA has been found to be the most efficient in increasing water-soluble Pb concentrations (Salt et al. 1998), but its use causes potential heavy metal toxicity in crop plants as well as enhances both leaching of heavy metals to groundwater and promotion of off-site migration (Cooper et al. 1999). Thus, effective implementation of phytoextraction must consider measures to minimize environment risks. However, many studies in the past mainly focused on the chelator types and dosages

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in the application to enhance heavy metal uptake (Meers et al. 2005), and the optimization of chelator-assisted phytoextraction was ignored.

Sedum alfredii Hance which grows in the old Pb/Zn mined areas of southeast China, has been reported to be a Zn/Cd hyperaccumulating plant species (Yang et al. 2004), and later studies by He et al. (2002) showed that it can also accumulate considerable amount of Pb. Previous studies on *Sedum alfredii* H. were mainly focused on the accumulation and transportation mechanisms (Yang et al. 2004, 2006) and less attention was paid to the chelator-assisted phytoremediation techniques.

In the present study, EDTA was used at different concentrations, treatment duration and addition methods to optimize the model of chelator-assisted phytoextraction by *S. alfredii* from soil having two levels of Pb contamination. At the same time, leaching column experiment was conducted to study the concentration of available Pb and residual EDTA denoted with DOC at different depths of soil layer. The major objectives of this study were to explore optimum treatment conditions for chelator-assisted phytoremediation techniques by using *S. alfredii* and provide references for the application of synthetic chelators in the phytoremediation of contaminated soils.

Materials and Methods

Contaminated farm soil was procured from a dumping site in Huajiachi campus of Zhejiang University, China. The samples were sieved through a 2 mm sieve and air-dried for 3 days. The soils were artificially contaminated with Pb at the concentration of 400 (low Pb soil) and 1,200 mg kg^{-1} soil (high Pb soil), respectively. Source of Pb was $Pb(NO_3)_2$. At the same time, 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 days, undergoing five cycles of saturation with distilled water and air-drying.

After equilibrating the soil for 15 days, 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; water-soluble N; water-soluble K; total Pb, Zn, Cu and Cd contents (mixed acid digestion with concentrated HNO_3, HCl, and HF = 3:1:1, v/v); and water-soluble metal contents (solid distilled water 1:2.5, w/v) (Liu et al., 2007). The selected physicochemical properties of the soil are presented in Table 1.

The accumulating ecotype of *S. alfredii* H. was collected from an old Pb/Zn mined area, Zhejiang province of China

Table 1 Physico–chemical properties of the soils used in the study

Physico–chemical properties	
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 concentrations (mg kg^{-1})	
Pb	37.84
Zn	105.33
Cu	15.68
Cd	0.53
Water-soluble concentration (mg kg^{-1})	
Pb	0.082
Zn	0.151
Cu	0.231
Cd	0.002

(Yang et al. 2004). After pre-culturing for 3 weeks in hydroponics, three seedlings of *S. alfredii* were transferred to the pots containing 1.5 kg soil. The soil moisture content was maintained at 60% (w/w) of the water-holding capacity by adding de-ionized water after every 2 days. Plants were grown in a greenhouse at day/night temperature of 30/25°C and day/night humidity of 70%/90% (Liu et al. 2007). After 2 months of growth, three experiments were conducted which are as follows. Experiment 1 (concentration dependent experiment): EDTA was added to the contaminated soil at the concentration of 1, 5, 10 mM respectively, and plants were harvested after 10 days of treatment; Experiment 2 (treatment time dependent experiment): plants were treated with 5 mM EDTA firstly, and then harvest at 7th, 10th and 14th day, respectively; Experiment 3 (addition method dependent experiment): a single dose of 5 mM EDTA, three successive doses of 1.67 mM EDTA and five successive doses of 1 mM EDTA were added to the contaminated soil, respectively, for 10 days. Control pots were those planted with *S. alfredii* without amendments of chelators using three replications. EDTA was purchased in reagent grade and stored at appropriate temperature recommended by the manufacturer.

In leaching column experiment, PVC columns (18 cm inner diameter and 70 mm in length) with three holes (1 cm in diameter) at every height of 0, 15, 30, 45 and 60 cm of the columns respectively were used. The columns were washed with dilute HNO_3 prior to treatment in order to eliminate any adsorbed metals. Soils contaminated with two levels of Pb (mentioned above) equivalent to 15 kg

(FW) were added into these PVC columns respectively, and then all the holes were sealed with plastic membrane. At last three seedlings of *S. alfredii* were planted in each column. The moisture and temperature of the column experiments were same as in pot experiments. After 2 months of growth, 5 mM EDTA was added to each column. After 10 days of EDTA application, 5 g soil (fresh weight) was sampled from the holes at each depth of the columns and analyzed for concentration of water-soluble Pb and DOC (Liu et al. 2007).

The oven-dried plant parts were ground using stainless steel mill, and then passed through 0.1 mm nylon sieve. Approximately 0.1 g of the plant sample was digested using HNO_3/$HClO_4$ (3:1 v/v) digestion method. The digested solutions were washed into 50 ml flasks and made volume using de-ionized water. The plant Pb concentrations were determined using ICP–MS (Agilent 7500a) (Liu et al. 2007).

Statistical analysis was performed using the SPSS statistical package (version 11.0). All values are means of three independent experiments. Data were tested at significant levels of $p < 0.05$ by two-way ANOVA.

Results and Discussion

Cooper et al. (1999) reported that synthetic chelators at high concentrations could also be toxic to plants. In present studies, shoot dry weight of *S. alfredii* grown in both levels of Pb contaminated soils decreased significantly along with the increase of EDTA addition, which was consistent with the studies above (Table 2). It could be seen that 5 mM EDTA was the most optimum dose for phytoextraction from low Pb soil, while 10 mM was more suitable for high Pb soil. At the same time, it was noted that after treating with 1 and 5 mM EDTA, although the concentrations of available Pb in high Pb soil were much higher than those of low Pb soil, there were no significant differences of Pb removal amounts between both kinds of soils; and even for those soils treated with 10 mM EDTA, Pb removal by *S. alfredii* from high Pb soil increased just 0.32-fold as compared with low Pb soil. Considering economic expenditure and potential environmental risks, chelator-assisted phytoextraction might be more suitable for slight Pb contaminated soils.

It has been reported that plants should be harvested 1 or 2 weeks after the applications of chelating agents, as timing may be a crucial factor in the effectiveness of phytoextraction. Chiu et al. (2005) reported that Cu intake in votive shoots under HEIDA application reached maximum at the 16th day, whereas the maximum uptake of As and Zn under NTA applications occurred on the 20th day of treatment. In present study, shoot of *S. alfredii* harvested on the 10th day from low Pb soil and on the 7th day from high Pb soil could achieve the highest phytoextraction efficiencies. It can be concluded that EDTA addition will impact plant growth significantly along with increasing of

Table 2 Shoot dry weight, Pb concentration in shoot of *Sedum alfredii* H. and Pb removal in pot experiment

	Low Pb soil				High Pb soil			
Experiment 1								
EDTA treatment (mM kg^{-1})	CK	1	5	10	CK	1	5	10
Dry weight (g $plant^{-1}$)	0.834a*	0.791a*	0.572b*	0.408c*	0.622a	0.516b	0.324c	0.216d
Concentration (mg kg^{-1})	131.7c	192.6b*	429.5a*	475.6a*	202.5c	305.1c	736.7b	1189.7a
Pb removal (g $plant^{-1}$)	0.110c	0.152bc	0.245a	0.194b*	0.126b	0.157b	0.239a	0.257a
Order	4	3	1	2	4	3	2	1
Experiment 2								
Treatment time (day)	CK	7	10	14	CK	7	10	14
Dry weight (g $plant^{-1}$)	0.834a*	0.575b*	0.572b*	0.496b*	0.622a	0.362b	0.324b	0.275b
Concentration (mg kg^{-1})	131.7c*	374.7b*	429.5ab*	460.7a*	202.5c	675.0b	736.7ab	768.1a
Pb removal (g $plant^{-1}$)	0.110b	0.215a	0.245a	0.229a	0.126b	0.244a	0.239a	0.211ab
Order	4	3	1	2	4	1	2	3
Experiment 3								
Addition methods (times)	CK	1	3	5	CK	1	3	5
Dry weight (g $plant^{-1}$)	0.834a*	0.572c*	0.693b*	0.709b*	0.622a	0.324c	0.517b	0.577ab
Concentration (mg kg^{-1})	131.7d*	429.5a*	345.3b*	267.8c*	202.5c	736.7a	686.4ab	637.0b
Pb removal (g $plant^{-1}$)	0.110c	0.245a	0.239a*	0.190b*	0.126c	0.239b	0.355a	0.368a
Order	4	1	2	3	4	3	2	1

Values are means ± SD (n = 3). Different letters among treatment indicate significant differences at $p < 0.05$. An asterisk (*) significantly differ low Pb soil from corresponding high Pb soil ($p < 0.05$)

treatment duration, especially for high Pb soil. Additionally, the results showed that no significant differences of Pb removals between both kinds of soils at all the treatment duration were noted, which supported the conclusion drawn in experiment 1, i.e., chelator-assisted phytoextraction is not suitable for high metal contaminated soil.

Experiment 3 (addition method dependent experiment) showed that if EDTA was added with three or five successive dosages, Pb concentrations in shoots of *S. alfredii* grown in both levels of Pb contaminated soils would decrease significantly in comparison to those treated with a single dose of 5 mM EDTA. These results were consistent with Grcman et al. (2001), who found that a single dose of 2.9 g EDTA kg^{-1} soil enhanced Pb accumulation in *Brassica oleracea* L. grown in a greenhouse 105-fold, as compared with a 44-fold increase if the same amount of EDTA was split and added in four intermittent doses. However, the split dose addition would lessen the decrease of plant biomass. As a result, in present study the order of phytoextraction effects of addition method for low Pb soil was 1 > 3 and >5 times; in contrast, the sequence was contrary for high Pb soil.

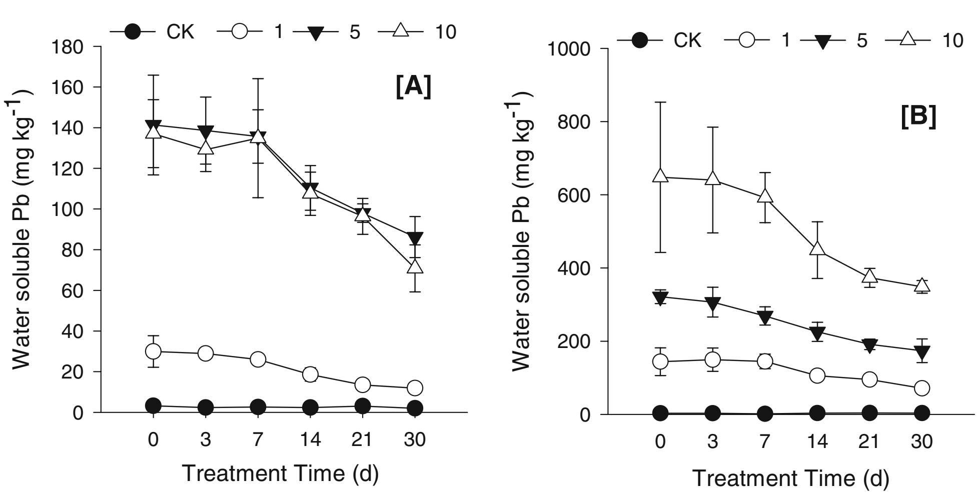
From the results of above three pot experiments, it could be concluded that strong relationship exists between the treatment mode and pollution control for Pb contaminated soils. Therefore, before addition of chelators, the optimum value for chelator-assisted phytoextraction must be prestudied. On the basis of our present results, we can recommend that chelator-assisted phytoextraction is more suitable for the slight-contaminated soils.

After treating the soil with chelator, only a limited fraction of mobilized metals is effectively absorbed by the plants (Miller et al. 1986). Considering the potential environmental risk, post-harvest effects of chelators must be studied. In present study, it could be seen that the concentrations of water-soluble Pb in both low and high Pb soils exposed to different concentrations of EDTA increased sharply on harvest day as compared with CK (Fig. 1). For both levels of Pb contaminated soils, there was no significant decrease of water-soluble Pb during the first week after plants harvest, and then in the following 2 weeks it decreased slowly as compared to CK, which might be due to leaching effects and degradation of EDTA (Wu et al. 2004). At the same time, it was also found that there were no significant differences of water soluble Pb in low Pb soil exposed to 5 and 10 mM EDTA for 10 days, showing that 5 mM EDTA was sufficient enough for application of phytoremediation, and this result was consistent with the outcome of Pb removal amounts in pot experiment 1. In contrast, it could be seen that increasing dosage of EDTA always enhanced the concentrations of soluble Pb in the high Pb soil. It is suggested that considering the potential environment risks, the other degradable chelators might be tried in the future study in the application of chelator-assisted phytoextraction techniques.

In present study, DOC contents in low and high Pb soils without addition of EDTA started to decrease on the 21st day after plants harvest, which suggests that DOC contents in soil were derived from root secretion of plants (Fig. 2). Some studies have indicated that EDTA-metal complexes are resistant to microbial degradation (Romkens et al. 2002). EDTA was found to be biodegraded slowly to CO_2 in soil with only 6.7% degradation after 4 weeks with a lower rate of degradation in the subsoil than in surface soil (Tiedje et al. 1975). In experiment 1, after treating with different dosages of EDTA, DOC contents in soil solution increased significantly at harvest day as compare to CK, and then decreased gradually with the advancement of time after plants harvest. This trend was consistent with the results of available Pb in soil.

Many leaching column studies reported earlier have been conducted to investigate the potential environmental risks produced by the addition of synthetic chelators to soil (Wu et al. 2004). However, the leaching effects at different

Fig. 1 Post-harvest effects of EDTA on the concentration of water-soluble Pb in low Pb soil (**A**) and high Pb soil (**B**). Values are means ± SD (n = 3)



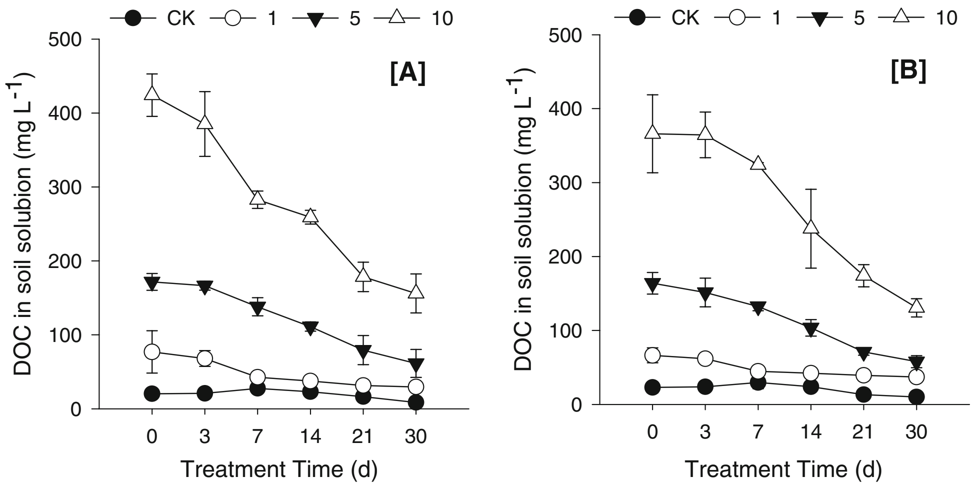


Fig. 2 Post-harvest effects of EDTA on the concentrations of DOC in low Pb soil (**A**) and high Pb soil (**B**). Values are means ± SD (n = 3)

depth of soil layer were ignored, which is quite important to study potential environment risks on groundwater pollution during the application of chelator-assisted phytoextraction technique. In present study, results showed that water soluble Pb in both levels of Pb contaminated soil decreased significantly along with the increasing depth of soil layer, implying that metal solubility activated by EDTA was mostly confined to the surface soil layers (Fig. 3). At the same time, it was noticed that there were no significant differences of available Pb concentrations between the depth of 45 and 60 cm, which means that the leaching effects are present mostly within 45 cm depth of soil.

For DOC, a similar phenomenon could also be traced, demonstrating the existence of strong relationship between soluble heavy metal and residual synthetic chelators (Wu et al. 2004). From the results in present study, it could be concluded that potential environmental risks associated with the application of chelator-assisted phytoextraction must be considered if there is abundant groundwater within such soil depth (Fig. 4).

From present study it could be concluded that chelator-assisted phytoextraction is more suitable for the slightly contaminated soils, and it is better to erect the optimum plant-chelators model system before the application of

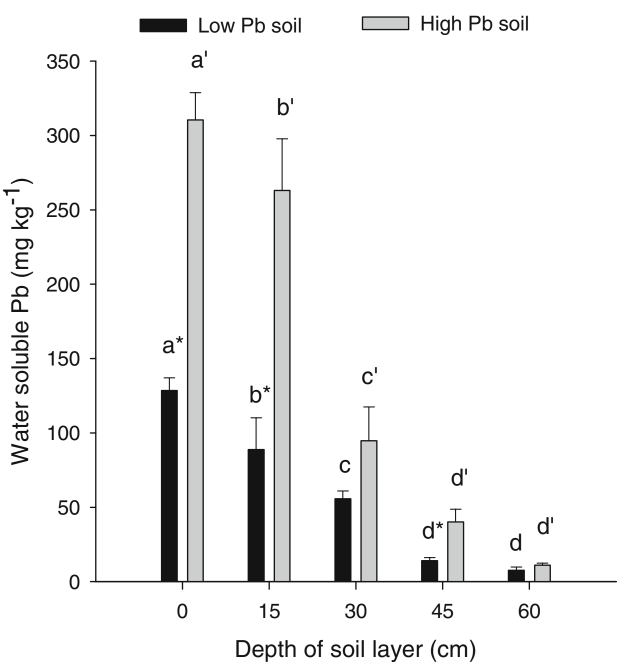


Fig. 3 Water soluble Pb concentration at different level of soil layers in leaching column experiment. Different letters among treatment indicate significant differences at $p < 0.05$. An asterisk (*) significantly differ low Pb soil from corresponding high Pb soil ($p < 0.05$)

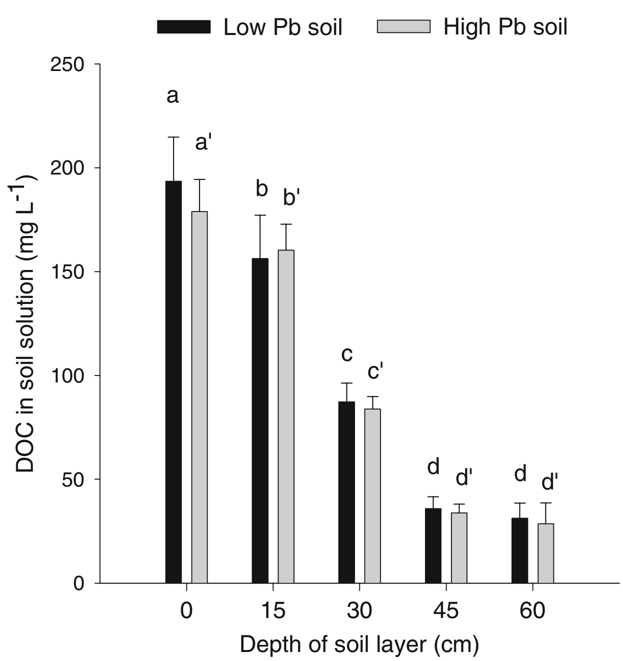


Fig. 4 DOC contents at different levels of soil layers in leaching column experiment. Different letters among treatment indicate significant differences at $p < 0.05$. An asterisk (*) significantly differ low Pb soil from corresponding high Pb soil ($p < 0.05$)

chelator-assisted phytoextraction technique in order to enhance heavy metal removal efficiency and lessen the potential environmental risks, which is dependent on physico-chemical properties and contamination level of the soil.

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