Journal of Hazardous Materials 168 (2009) 530–535

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The effect of EDDS addition on the phytoextraction efficiency from Pb contaminated soil by *Sedum alfredii* Hance

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ARTICLE INFO

Article history:
Received 4 July 2007
Received in revised form 11 November 2008
Accepted 12 February 2009
Available online 23 February 2009

Keywords:
EDDS
Environmental pollution
Pb
Phytoextraction
Sedum alfredii Hance

ABSTRACT

Present study reports the results of three pot experiments, conducted to investigate the chelate-assisted phytoextraction of Pb contaminated soils. The optimum phytoextraction was observed when 2.5 mM ethylene diamine disuccinic acid (EDDS) was added in single dosage for 14 days to low Pb soil (treated with 400 mg kg^{-1} soil). On the contrary, for high Pb soil (treated with 1200 mg kg^{-1} soil), 5 mM EDDS concentration in single dosage for 10 days produced better results. Post-harvest effects of EDDS on the concentrations of available Pb and dissolved organic carbon (DOC) were significantly higher as compared with check (CK i.e. without EDDS addition), and consequently decreased with the passage of time. Our results suggested that chelate-assisted phytoextraction was more suitable for slightly contaminated soils.

1. Introduction

Heavy metal pollution of soil is a widespread global problem [1], and this issue has been a major environmental concern over the past several decades. A wide range of heavy metals has been detected in different biota such as soil, water, and air [2–5]. These metals pose a serious concern to human health and environmental issues due to their abundance as contaminants, low solubility, and the classification as carcinogenic and mutagenic [6]. Among these, Pb is considered as one of the most frequently encountered heavy metals of environmental concern.

Phytoremediation of heavy metal-contaminated soils is an emerging biotechnology that aims to extract or inactivate metals in soils [7]. It has grabbed increased attention currently for its low cost of implementation and other environmental benefits. Metal hyper-accumulator plant species are widely used for the treatment of metal-polluted soils, sediments, and water resources [8]. Though phytoremediation can be applied for the reclamation of elevated concentrations of heavy metals present in contaminated soils, just a fraction of soil metal content is readily available for plant uptake. However, a large portion is generally present as insoluble compounds unavailable for absorption by roots, restricting absorption of hyper-accumulating plants.

Recently, many synthetic chelating agents such as ethylene diamine tetra acetic acid (EDTA), diethylene trinitrilo pentaacetic acid (DTPA), nitrilo triacetic acid (NTA), and citric acid etc., were applied to metal-contaminated soils for increasing the mobility and bioavailability of heavy metals, thereby increasing the heavy metal concentration in the aerial parts of plants used for phytoextraction [1,2,9–12]. Among these chelators, EDTA was found as the most efficient in increasing the concentration of water-soluble Pb [13,14]. However, its persistence in the environment makes it unsuitable for in situ application [15]. Other synthetic chelators such as NTA (nitrilotriacetate) [16,17], have also been used occasionally. (*S*,*S*)-*N*,*N*′-Ethylenediamine disuccinic acid (EDDS) is a biodegradable structural isomer of EDTA [18]. Other stereoisomers of ethylenediamine disuccinic acid are either non-biodegradable (*R*,*R*) or only partially degradable (*R*,*S*, *S*,*R*), so the *SS*-isomer is generally applied. It is now used as a commercial substitute for EDTA in detergents [19] and has the potential to be a substitute of EDTA for chelate-assisted phytoremediation, as it is a strong chelator and unlike EDTA, it is easily biodegradable. Many researchers have recently reported the use of EDDS in chelate enhanced phytoremediation of Pb, Zn, Cu and Cd affected soils [1,20–22]. However, little information is available on the use of EDDS to increase the phytoextraction efficiency before putting it into practical use.

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doi:10.1016/j.jhazmat.2009.02.074

Table 1
Physico-chemical properties of the soils used in study.

Physicochemical properties	
pH	7.12
Organic matter ($g\,kg^{-1}$)	22.55
Total N ($g\,kg^{-1}$)	1.05
Available N ($mg\,kg^{-1}$)	62.8
Total P ($g\,kg^{-1}$)	0.52
Available P ($mg\,kg^{-1}$)	6.5
Total K ($g\,kg^{-1}$)	14.6
Available 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

Sedum alfredii Hance growing in the old Pb/Zn mined areas of southeast China has been reported to be a Zn/Cd hyperaccumulating plant species [23,24], and later it proved as Pb accumulating species [25]. Earlier studies on *S. alfredii* Hance mainly focused on the accumulation and transportation mechanisms [23,24,26–28], and less emphasis was laid on its application for chelate-assisted phytoremediation.

In the present study, EDDS application in relation to chelator dosage, treatment time, and application mode was investigated for the phytoextraction of Pb contaminated soil using *S. alfredii* Hance. Additionally, the post-harvest effects of EDDS were also considered to study the concentration of available Pb and residual chelator denoted with dissolved organic carbon (DOC).

2. Materials and methods

2.1. Soil characterization and preparation

Contaminated farm soil was procured from a ranch at Hua Jia Chi campus of Zhejiang University, Hangzhou city of China (118°56′E, 29°17′N). The samples were sieved through a 2 mm sieve and air-dried for 3 days. The soils were contaminated artificially with Pb as Pb $(NO_3)_2$ at the concentration of 400 (low Pb soil) and 1200 (high Pb soil) $mg\,kg^{-1}$ 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 [29]. After adding heavy metals and fertilizers, the soils were equilibrated for 15 days, undergoing five cycles of saturation with de-ionized water and air-drying. The following parameters were determined prior to the experiments: pH (solid: de-ironed 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 (P) and water-soluble P; water-soluble N; water-soluble K; total As, Zn, and Cu contents (mixed acid digestion with concentrated HNO_3, HCl, and HF = 3:1:1, v/v); and water-soluble metal content (solid: de-ionized water = 1:2.5 w/v) [30]. The selected physicochemical properties of the soil are presented in Table 1.

2.2. Plant culture and treatment levels

In the present study, *S. alfredii* Hance was collected from an old Pb/Zn mined area, Zhejiang province of China, which could accumulate 1182 mg Pb/kg in shoots with the highest growth rate and relatively larger biomass. After pre-culturing for 3 weeks in hydroponics [23], three seedlings of *S. alfredii* were transferred to the pots containing 1 kg soil. The soil moisture content was maintained at 60% water-holding capacity by weight adding de-ionized water after every 2 days. After 2 months of growth, plants were grown in a greenhouse at 30 and 24 °C during the day and night, respectively. Three experiments were conducted: experiment 1 (treatment concentration dependent experiment): EDDS was added to the contaminated soil at the concentration of 1, 2.5, 5 $mM\,kg^{-1}$ soil, respectively, and plants were harvested after 10 days. Experiment 2 (treatment time dependent experiment): plants were treated with 2.5 mM EDDS for 7, 10 and 14 days, respectively; and experiment 3 (addition methods dependent experiment): the same dosage as experiment 2 was used, and EDDS was divided into single, double and five successive doses for each experiment. Finally, plants were harvested after 10 days. Control pots contained *S. alfredii* without amendments of chelating agents using three replicates. All the chemicals were procured as analytical grade from China Chemical Factory, Shanghai, China; EDDS from Sigma Aldrich, USA (purity ≥99%).

2.3. Post-harvest effects of EDDS

For the concentration dependent experiment, soil samples were collected from the pots on 0, 3, 7, 14, 21 and 30 days. For post-harvest study of the concentration of water-soluble Pb and residual EDDS denoted with DOC in soil solution, distilled water was added to give a 1:2.5 (w/v) soil: solution ratio. After shaking for 60 min, tubes were centrifuged and were filtered to collect the supernatants. DOC was determined using a Shimadzu 500 A TOC Analyzer; acidified with HNO_3 and analyzed for different metal concentrations by ICP-MS (Agilent7500a).

2.4. Heavy metal analyses of plant and soil samples

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

To determine the water-soluble Pb in the soil, de-ionized water was added to the soil (soil-to-water ratio of 1:2.5) and the suspension was shaken for 30 min and then centrifuged. The supernatant was filtered through a 0.45 μm paper filter, acidified with HNO_3 and finally analyzed for the Pb concentrations by ICP-MS (Agilent7500a).

2.5. Statistical analysis

Statistical analysis was performed using the SPSS statistical package (version 11.0). All the 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.

3. Results

3.1. Effects of EDDS treatment concentration on the shoot biomass and Pb uptake

It was evident that the dosage of 1 and 2.5 mM did not affect the shoot dry weights of *S. alfredii* significantly ($P < 0.05$) grown in low and high Pb contaminated soil, while dry weights decreased by 27.7% and 42.1% respectively, as compared with respective controls when treated with 5 mM EDDS (Fig. 1A). Similarly, it was noted that the shoot biomass of *S. alfredii* grown in low Pb soil was always significantly lower ($P < 0.05$) than that grown in high Pb soil.

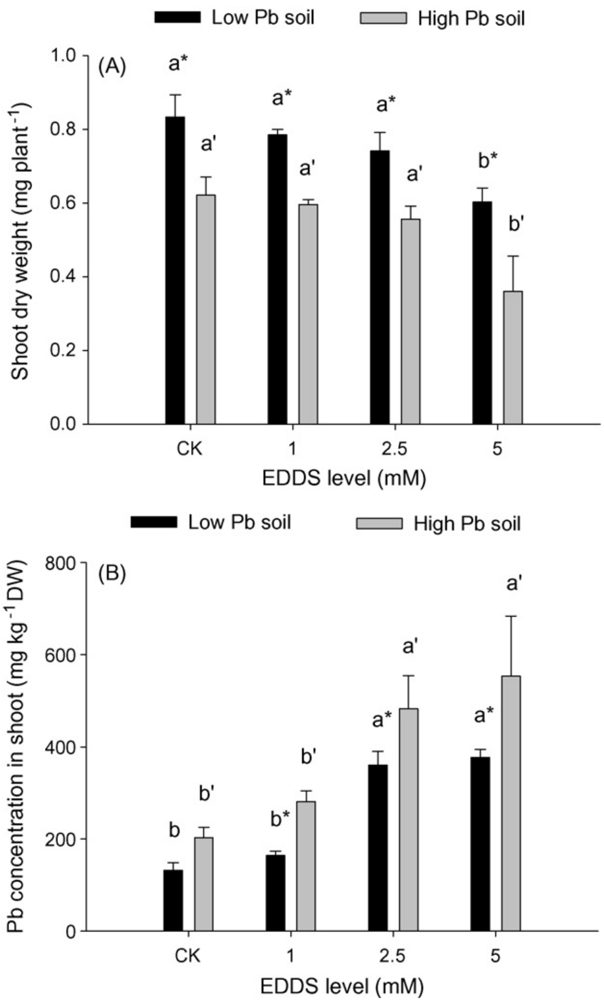

Fig. 1. Effects of treatment dosage of EDDS on the shoot biomass (A) and Pb uptake (B). Values are means ± SD (n = 3). Different letters among treatment indicate significant differences according to Duncan's multiple range test at P < 0.05. An asterisk (*) shows that low Pb soil significantly differs from corresponding high Pb soil (P < 0.05).

In the treatment concentration dependent experiment, Pb concentration in shoots of *S. alfredii* enhanced with the increasing treatment dosage (Fig. 1B). For the low Pb soil, it was evident that no significant (P < 0.05) increase in Pb concentration was observed for the shoots of *S. alfredii* upon the addition of 1 mM EDDS, the increase was 2.74 and 2.86 times over CK. The same trend was noted for the plants grown in high Pb soil.

3.2. Effects of EDDS treatment time on the shoot biomass and Pb uptake

When treated with 2.5 mM EDDS, the shoot dry weight of *S. alfredii* grown in both level of Pb contaminated soils was affected considerably (Fig. 2A). For low Pb soil, there was no significant (P < 0.05) decrease in shoot biomass, while it decreased by 11.1% and 17.1% for the treatment time of 10 and 14 days, respectively, over CK (P < 0.05). Conversely, the shoot dry weight of *S. alfredii* grown in high Pb soil did not show any significant decrease when treated with 2.5 mM EDDS for 7 and 10 days, however, it was 74% of CK. Pb concentration in shoot of *S. alfredii* grown in low Pb contaminated soils increased significantly (P < 0.05) by 2.49, 2.73 and 2.95 times, after treating with 2.5 mM EDDS for 7, 10 and 14 days, respectively; in contrast to 2.22, 2.38 and 2.66 times increase for high Pb soil (Fig. 2B).

3.3. Effects of EDDS treatment methods on the shoot biomass and Pb uptake

It was evident that various application methods had no significant effects on the shoot biomass (P > 0.05) (Fig. 3A) except for the application of single dosage of 2.5 mM EDDS for low Pb soil, which decreased by 11.1% as compared with CK (P < 0.05). However, different application methods influenced the Pb uptake significantly (P < 0.05) (Fig. 3B). It was seen that in case of the low Pb soil, Pb concentration in shoot of *S. alfredii* increased by 2.73, 2.11 and 1.69 times over CK when single dose of 2.5 mM, two successive doses of 1.25 mM and five successive doses of 0.5 mM, respectively, were added. On the contrary, increases for the high Pb soil were 2.38, 1.77 and 1.62 times for high Pb soil under same treatments.

3.4. Post-harvest EDDS effects on the available Pb and DOC contents

To study the post-harvest effects of EDDS in the soil, water-soluble Pb was determined in soils with passage of time after the termination of experiment (Fig. 4). Water-soluble Pb in both levels of Pb contaminated soil did not vary considerably within 30 days. After harvest, the effects of EDDS on available Pb decreased gradually with the passage of time. It could be seen that after 30 days of harvest, water-soluble Pb decreased by 70.7%, 76.3% and 71.4%

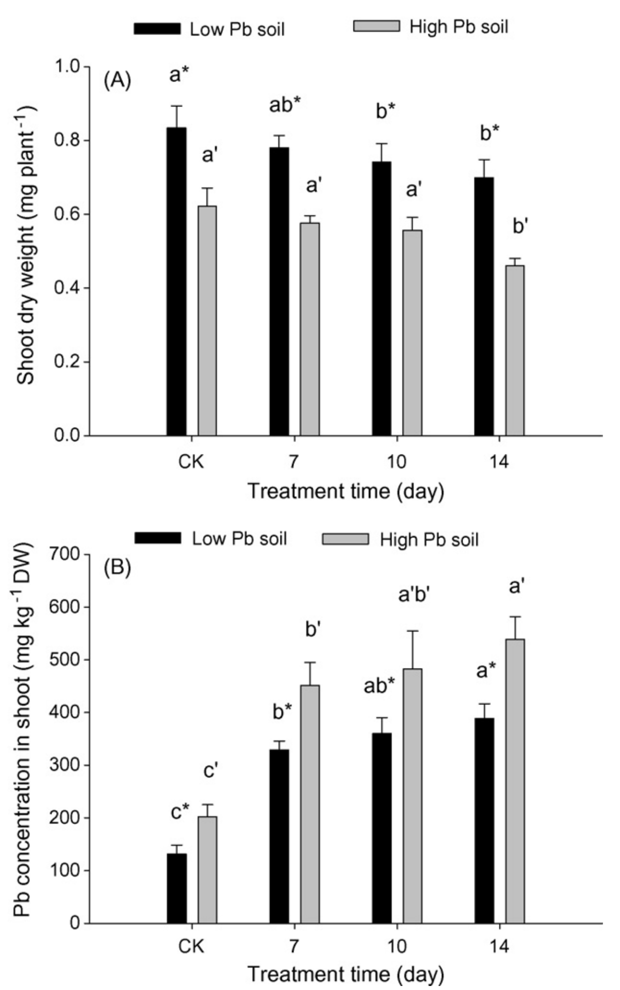

Fig. 2. Effects of treatment time of EDDS on the shoot biomass (A) and Pb uptake (B). Values are means ± SD (n = 3). Different letters among treatment indicate significant differences according to Duncan's multiple range test at P < 0.05. An asterisk (*) shows that low Pb soil significantly differs from corresponding high Pb soil (P < 0.05).

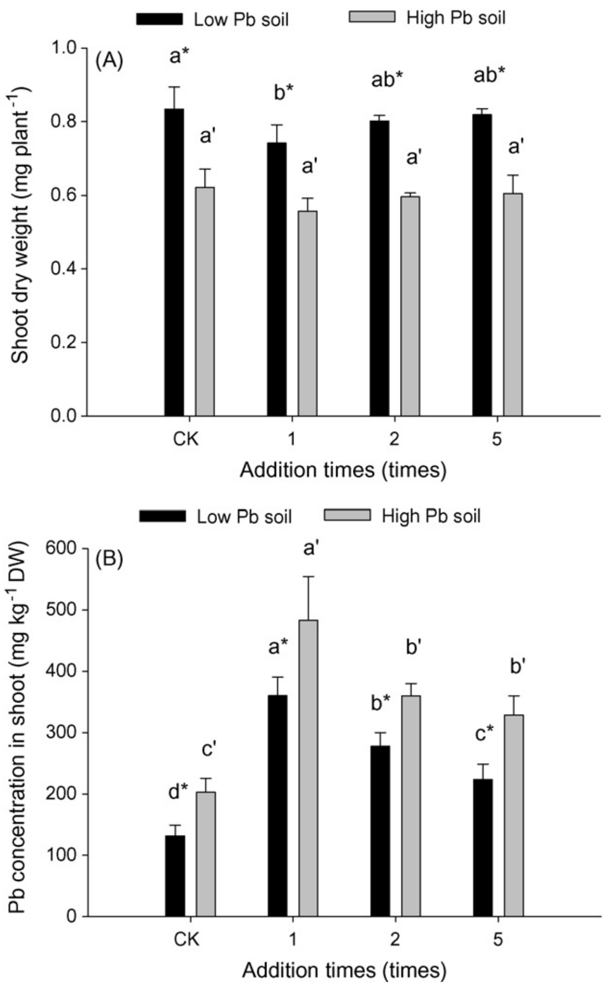

Fig. 3. Effects of addition method of EDDS on the shoot biomass (A) and Pb uptake (B). Values are means ± SD (n = 3). Different letters among treatment indicate significant differences according to Duncan's multiple range test at P < 0.05. An asterisk (*) low Pb soil significantly differs from corresponding high Pb soil (P < 0.05).

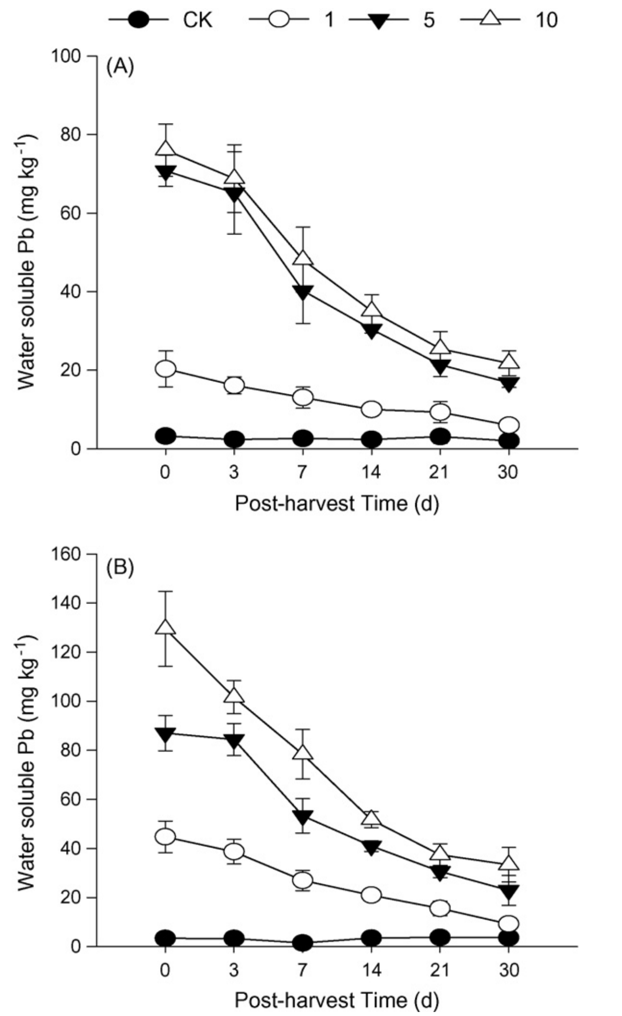

Fig. 4. Post-harvest effects of EDDS on the concentration of water-soluble Pb in low Pb soil (A) and high Pb soil (B). Values are means ± SD (n = 3).

when treated with 1, 2.5 and 5 mM EDDS, respectively, in low Pb soil compared with amount on the harvest day; in contrast, reductions in available Pb for high Pb soil were 79.4%, 73.7% and 74.3%, respectively. Similarly, it was found that there were almost no differences in water-soluble Pb at the treatment levels of 2.5 and 5 mM EDDS in low Pb soil after 30 days of harvest; while for the soil with 1200 mg kg^{-1} Pb, the water-soluble Pb was always higher in soil treated with 5 mM EDDS than in that treated with 2.5 mM EDDS. It was also found that water-soluble Pb concentration in high Pb soil was always higher than that in low Pb soil at the same treatment level on 30th day of harvest.

DOC dynamics in the soil solution after plant harvest have been presented in Fig. 5. It was seen that the DOC concentration in the soil solution fluctuated within the range of 8–30 mg L^{-1} that began to decrease on 21st day, which was 57.8% and 81.1% as compared with 0 day for the low Pb and high Pb soils, respectively, without EDDS addition. Thus, EDDS addition significantly enhanced the DOC concentrations in the soil solution over CK on the harvest day: for the low Pb soil, it increased 2.9, 3.4 and 5.7 times; while it increased 2.4, 3.1 and 5.2 times for high Pb soil in relation to the soil solution without EDDS addition. DOC concentration in soil solution decreased gradually with the passage of time. It was observed that DOC concentration decreased to 42.2%, 26.4% and 29.4% for low Pb soil, while these were 46.3%, 41.9% and 29.4% for high Pb soil on 30th day of treatment with 1, 2.5 and 5 mM EDDS, respectively, in comparison to their respective controls.

4. Discussion

Previous studies indicated that chelator addition could increase the concentration of available heavy metals in the soil, hence their addition may affect the plant growth [14,31]. Chelator addition must be given proper consideration when setting chelator application design for phytoremediation. In experiment 1 of present study, no significant decrease was observed in shoot dry weight of *S. alfredii* grown in both level of Pb contaminated soils with the treatment dosage less than 5 mM showing that low EDDS dosage had little impact on the growth of *S. alfredii*. From Table 2 it could be seen that considering both factors i.e. biomass growth and Pb uptake, 2.5 mM EDDS proved to be the most optimum dosage for the low Pb soil, while 5 mM was more suitable for the high Pb soil aimed at phytoextraction of Pb from contaminated soil. However, based on the expenditure and potential environmental risks involved in reclamation of Pb-contaminated soils, chelate-assisted phytoextraction may be more suitable for slightly contaminated soils.

In general, plants should be harvested one or 2 weeks after the application of chelating agents, as timing may be a crucial factor in the effectiveness of phytoextraction. Chiu et al. [32] reported that Cu intake in vetiver shoots under HEIDA application reached at its

Table 2
The effects of EDDS on the amounts of Pb removal by shoots of *S. alfredii* from soils. Values are means ± SD ($n = 3$). Different letters among treatment indicate significant differences at $P < 0.05$.

	Low Pb soil				High Pb soil			
Experiment 1								
Treatment dosage (mM kg^{-1})	CK	10	50	100	CK	10	50	100
Pb removal (g plant^{-1})	0.110*c*	0.129*c*	0.266*a*	0.206*b*	0.127*b*	0.168*ab*	0.267*a*	0.228*ab*
Order	4	3	1	2	4	3	2	1
Experiment 2								
Treatment time (day)	CK	7	10	14	CK	7	10	14
Pb removal (g plant^{-1})	0.110*b*	0.256*a*	0.266*a*	0.272*a*	0.127*b*	0.260*a*	0.267*a*	0.247*a*
Order	4	3	2	1	4	2	1	3
Experiment 3								
Addition methods (times)	CK	1	3	5	CK	1	3	5
Pb removal (g plant^{-1})	0.110*d*	0.266*a*	0.222*b*	0.183*c*	0.127*c*	0.267*a*	0.214*b*	0.198*b*
Order	4	1	2	3	4	1	2	3

maximum on day 16; whereas, the maximum As and Zn uptake under NTA applications occurred after day 20. At present, treatment time dependent experiment showed that harvesting the shoots of *S. alfredii* on 14th day for low Pb soil and on 10th day for high Pb soil could achieve highest phytoextraction effects (Table 2). It can be concluded that EDDS addition may affect plant growth significantly with the passage of time, especially for high Pb soil because of higher available Pb in soil. As a result, shorter treatment time should be adopted for high Pb contaminated soils.

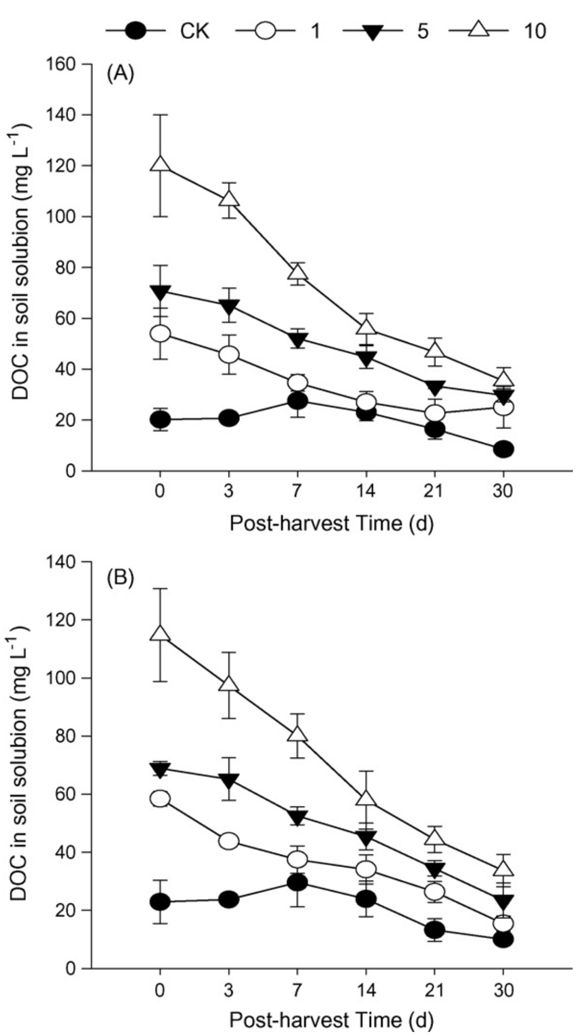

Fig. 5. Post-harvest effects of EDDS on the concentrations of DOC in low Pb soil (A) and high Pb soil (B). Values are means ± SD ($n = 3$).

Grcman et al. [33] reported that single dose of 2.9 g EDTA kg^{-1} enhanced 105-fold Pb accumulation in cabbage (*Brassica oleracea* L.) grown in a greenhouse, as compared with a 44-fold increase if the same amount of EDTA was split and added in four intermittent doses. Our third experiment showed that if the EDDS addition was split into three or five doses, Pb concentration in the shoots of *S. alfredii* grown in both level of Pb contaminated soils decreased significantly in comparison to those treated with a single dosage, which was consistent with the previous results [33]. Table 2 shows that the order of phytoextraction ability for both Pb contaminated soils was 1 time > 2 times > 5 times, which suggested that lower EDDS dosage of about 0.5 or 1.25 mM resulted in weaker Pb solubility in soil resulting in a poor Pb uptake by *S. alfredii*.

After assessing the results of above-mentioned three pot experiments, it could be deduced that strong relationship may exist between the treatment mode and remediation of Pb contaminated soils. The optimum dose of chelators for chelate-assisted phytoextraction must be investigated before the application of this technique. Present study concludes that EDDS should be added at the concentration of 2.5 mM in a single dosage for 14 days in low Pb soils; in contrast, for phytoremediation of high Pb soil EDDS would better be added at the concentration of 5 mM in a single dosage for 10 days.

After chelator amendment of the soil, only a limited fraction of mobilized metals was effectively absorbed by the plant [34,35]. Earlier it was suggested that, the post-harvest effects of chelators must be studied in view of the environmental risk [36,37]. It was noted that the concentrations of water-soluble Pb at both low and high Pb levels in soil increased sharply after treating with different EDDS level on harvest day as compared with CK. Water-soluble Pb in soils gradually decreased with the passage of time, which might be due to leaching effects and degradation of EDDS. Moreover, soil Pb concentration of 400 mg kg^{-1} may have caused non-significant differences when treated with 2.5 and 5 mM EDDS showing of dose of 2.5 mM EDDS was sufficient for phytoremediation of soil with lower Pb, and this result was consistent with the outcome of Pb removal by *S. alfredii* shown in Table 2. However, higher Pb concentration (1200 mg kg^{-1}) resulted in an increased amount of available Pb in the soil. Considering environment risk, it may be concluded that chelate-assisted technique is more suitable for the low Pb contaminated soil.

Present study showed that DOC contents in low and high Pb soils without addition of EDDS began to decrease on 21st day, and even down to 42.3% and 44% of the respective control, which suggested that the DOC content in soil might have derived from root secretions of plants. Tandy et al. [1] reported that EDDS was degraded after a lag phase of 7–11 days with a half-life of 4.18–5.60 days. After treating with 1, 2.5 and 5 mM EDDS, respectively, DOC content in the soil solution decreased gradually with the passage of time.

5. Conclusions

Present study concludes that:

1. Considering biomass growth and Pb uptake, 2.5 mM EDDS proved to be the most optimum dosage for the low Pb soil for 14 days, while 5 mM was more suitable for the high Pb soil for 10 days aimed at phytoextraction of Pb from contaminated soil.
2. Treatment time dependent experiment showed that harvesting the shoots of *S. alfredii* on 14th day for low Pb soil and on 10th day for high Pb soil could achieve highest phytoextraction effects.
3. EDDS addition may affect plant growth significantly with the passage of time, especially for high Pb soil because of higher available Pb in soil. As a result, shorter treatment time may be adopted for high Pb contaminated soils. Strong relationship may exist between the treatment mode and remediation of Pb contaminated soils. Based on the expenditure and potential environmental risks involved in reclamation of Pb-contaminated soils, chelate-assisted phytoextraction may be more suitable for slightly contaminated soils.

Acknowledgements

The present research was financially supported by a grant from the National Natural Science Foundation of China (No. 20477039), Zhejiang Forestry College Science and Technology Foundation (No. 2007FR059).

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