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Comparison of synthetic chelators and low molecular weight organic acids in enhancing phytoextraction of heavy metals by two ecotypes of *Sedum alfredii* Hance

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Abstract

Lab scale and pot experiments were conducted to compare the effects of synthetic chelators and low molecular weight organic acids (LMWOA) on the phytoextraction of multi-contaminated soils by two ecotypes of *Sedum alfredii* Hance. Through lab scale experiments, the treatment dosage of 5 and 10 mM for synthetic chelators and LMWOA, respectively, and the treatment time of 10 days were selected for pot experiment. In pot experiment, the hyperaccumulating ecotype (HE) was found more tolerant to the metal toxicity compared with the non-hyperaccumulating ecotype (NHE). EDTA for Pb, EDDS for Cu, and DTPA for Cu and Cd were found more effective to enhance heavy metal accumulation in the shoots of *S. alfredii* Hance. Compared with synthetic chelators, the phytoextraction ability of LMWOA was lesser. Considering the strong post-harvest effects of synthetic chelators, it is suggested that higher dosage of LMWOA could be practiced during phytoextraction, and some additional measures could also be taken to lower the potential environmental risks of synthetic chelators in the future studies.

Keywords: Heavy metals; Low molecular weight organic acids; Phytoextraction; *Sedum alfredii* Hance; Synthetic chelators

1. Introduction

In the recent years more and more agricultural soils have been contaminated by heavy metals mainly due to the mining activities, industrial emissions, or application of the sewage sludge [1]. Heavy metals pose a critical concern to human health and environment for their prevalence as a contaminant, low solubility in biota, and the classification of several heavy metals as carcinogenic and mutagenic agents [2,3].

Phytoextraction is an emerging technology which aims to remove heavy metals from contaminated soils [4], and has grabbed attention in recent years for the low cost of implementation and environmental benefits. Moreover, the technology is likely to be more acceptable to the public compared with other traditional methods [5,6].

In most of the soils only a fraction of heavy metal is readily available for plant uptake, and the potential for application of hyperaccumulators in phytoremediation studies is limited by deficiency of available heavy metals [7]. Synthetic chelators and low molecular weight organic acids (LMWOA) are the most common chemical amendments used in chemical assisted phytoextraction of heavy metals from soils. Such substances are capable of forming chemical complexes with metal ions; therefore, modifying the bioavailability of heavy metals in soils [2,8]. Although synthetic chelators, such as EDTA, DTPA and EDDS, etc. have shown positive effects on the enhancement of phytoextraction of metals from soil, their usage can also be disadvantageous as: most of synthetic chelators are non-selective

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in extracting metals [9], have poor biodegradability and could decrease plant growth rigorously even at very low concentrations [10]. Therefore, a number of studies were conducted using LMWOA, because of their degradable characteristics [3], however most of the studies have reported lower effectiveness of aliphatic LMWOA such as citric and oxalic acids in inducing metals accumulation in plants compared with synthetic chelators [8,11,12]. Therefore, it is important to compare the application of synthetic chelators with LMWOA in enhancing phytoextraction, and to select suitable chelator types for a particular contaminated soil.

Sedum alfredii Hance growing in old Pb/Zn mined areas of southeast China has been reported to be a Zn-hyperaccumulating plant species [13], and later proved to be a Cd-hyperaccumulating and Pb accumulating species [14,15]. However, these studies on *S. alfredii* Hance were mainly focused on the accumulation and transportation mechanism [14–16], and less attention was paid to the application of chelate-assisted phytoremediation.

In present study, we compared the performance of synthetic chelators (EDTA, DTPA and EDDS) with LMWOA (citric, oxalic and tartaric acid) in metal solubility and enhancing phytoextraction of Pb, Zn, Cu and Cd by *S. alfredii* Hance in a multi-metal contaminated soil. The specific objectives of the study were: (i) to study the mobililization of soil heavy metals under chelator application (ii) and to compare the phytoextraction potential of synthetic chelators and LMWOA.

2. Materials and methods

2.1. Soil characterization and preparation

Soil samples were collected from Fuyang county of Hangzhou in Zhejiang Province, China, where soil has been severely contaminated due to the mining activities and is not appropriate for crop growth. The samples were sieved through a 2 mm sieve and air-dried for 3 days. The basal fertilizers were applied to the soil at the rate of 80 mg P kg^{-1} and 100 mg K kg^{-1} of dry soil as KH_2PO_4 [17]. Total and water-available concentrations of heavy metals in soil were determined by Atomic Absorbance Spectrophotometer, Shimadzu AA-6800 (AAS) after mix acid digestion [1:4 concentrated HNO_3 and $HClO_4$ (v/v)] and extraction with deionized water (soil-to-water ratio of 1:5), respectively. The selected physicochemical properties of the soil are presented in Table 1.

Table 1
Physicochemical properties of the soils used in study

pH	7.03
Organic matter (g kg^{-1})	20.6
Cation exchange capacity (cmol kg^{-1})	8.72
Total N (g kg^{-1})	2.43
Available N (mg kg^{-1})	74.72
Total P (g kg^{-1})	1.11
Available P (mg kg^{-1})	19.81
Available K (mg kg^{-1})	20.6
Total metal concentrations (mg kg^{-1})	
Pb	1015.58
Zn	2209.47
Cu	2013.84
Cd	20.09
Water-available metal concentration (mg kg^{-1})	
Pb	5.33
Zn	68.62
Cu	42.54
Cd	0.32

2.2. Plant material

The hyperaccumulating ecotype (HE) of *S. alfredii* Hance was collected from an old Pb/Zn mined area in Zhejiang province, China, and its non-hyperaccumulating ecotype (NHE) was collected from a tea garden of Hangzhou suburbs in Zhejiang province of China. Healthy and equal-sized shoots of both the ecotypes were selected and grown for 3 weeks in the green house using basic nutrient solution [18].

2.3. Extraction of soil heavy metals by chelators

For concentration dependent extraction experiment, synthetic chelators with different dosages of 1.0, 2.0, 4.0 and 5.0 mM, and of LMWOA, 2.0, 4.0, 8.0 and 10.0 mM, in a 2 mL solution were added to 4 g of soil. After 2 days, deionized water was added to the soil (soil-to-water ratio of 1:5) and the suspension was shaken for 30 min and centrifuged. The supernatant was filtered through a 0.45 μm paper filter, acidified with HNO_3 and analyzed for different metal concentrations by the inductively coupled plasma mass spectrometry (ICP-MS) [19].

For the time dependent extraction experiment, both synthetic chelators and LMWOA with dosages of 5 and 10 mM (obtained from the above concentration dependent extraction experiment), respectively, were added to the 4.0 g of soil in a 50-mL polypropylene centrifuge tube. After 0.5, 1, 2, 4, 6, 8, 10, 14 and 20 days, 20 mL deionized water was added to the soil (soil-to-water ratio of 1:5) and the suspension was shaken for 30 min. The suspensions were then centrifuged and the filtration and chemical analyses conducted were same as mentioned above.

2.4. Pot experiment

After pre-culturing for 21 days in hydroponics, the plants were transferred to the pots containing about 2.5 kg (dry weight) of contaminated soil. Three plants were transplanted in each pot and each treatment was replicated three times. Soil moisture content was maintained at 60% (w/w) of the soil water-holding capacity by adding de-ionized water under the pot in plate after every 2 days. Plants were grown in a greenhouse having natural light and temperature of 30/24 °C, during the day and night, and day/night humidity of 70/85%, respectively.

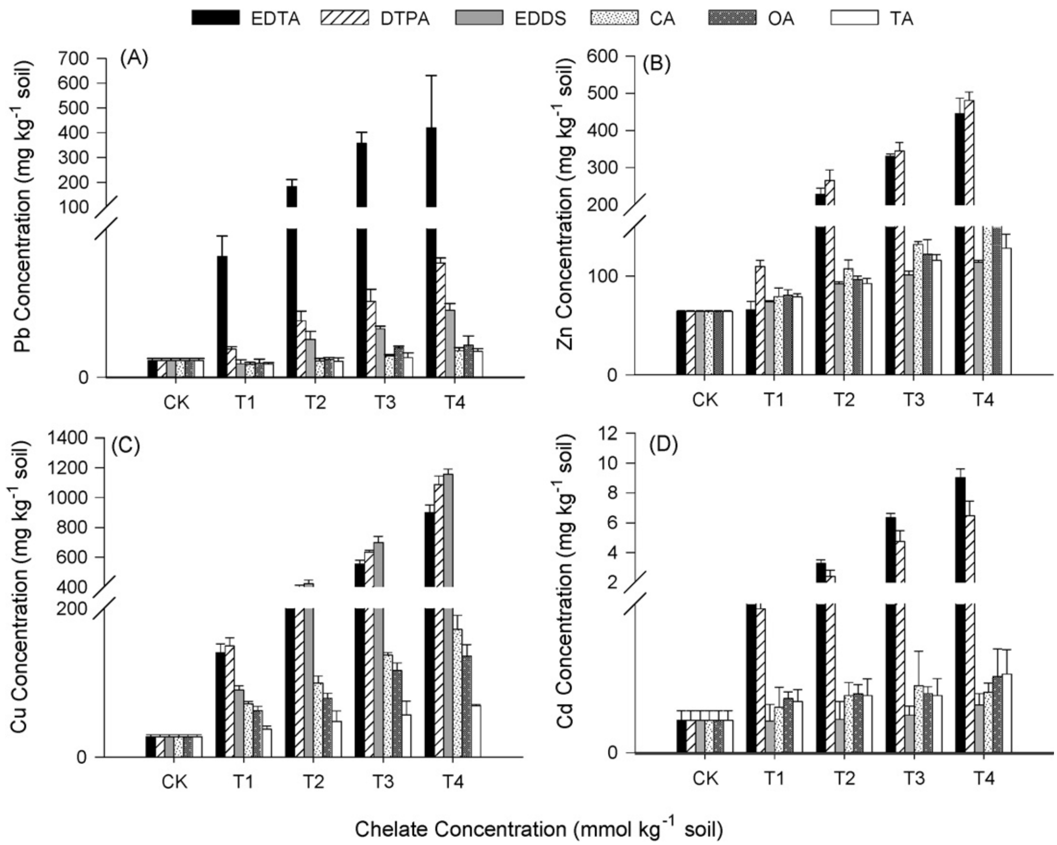

Fig. 1. Effects of various treatment concentrations of synthetic chelators and LMWOA on the solubility of Pb (A), Zn (B), Cu (C) and Cd (D) in contaminated soil. Values are means ± S.D. ($n = 3$). For the synthetic chelators treated soil, T1, T2, T3 and T4 refer to the treatment concentration of 1, 2, 4 and 5 mM kg^{-1}, respectively; while for the LMWOA treated soil, which refer to the treatment concentration of 2, 4, 8 and 10 mM kg^{-1}, respectively.

After 2 months of growth, 5 mM kg^{-1} soil synthetic chelators and mM kg^{-1} soil LMWOA were added to the surface of soil, respectively, in a single application which was according to the dosage used in the concentration dependent experiment, keeping a control (CK) without any chelator application. Both ecotypes of *S. alfredii* Hance were harvested on 10th day after the chelator application.

2.5. Post-harvest heavy metals extraction

Soil was sampled from the pots immediately after the harvest and analyzed for water-soluble metals by extraction with deionized water (soil-to-water ratio of 1:5) [20]. After shaking for 60 min, tubes were centrifuged and filtered to collect the supernatants, acidified with HNO_3 and analyzed for different metal concentrations by ICP-MS.

2.6. Chemical analyses

Harvested plants were thoroughly washed with tap and distilled water and separated into leaves, stems and roots, and then oven dried at 65 °C for 72 h. Dried plant materials were (100 mg) powdered and wet digested in a 10:1 mixture of HNO_3:$HClO_4$ at 160 °C. Digested material was diluted with deionized water and heavy metal concentrations were determined using ICP-MS. Total concentrations of Pb, Zn, Cu and Cd in the contaminated soil were estimated by digesting with mix acid digestion [1:4 concentrated HNO_3 and $HClO_4$ (v/v)], and then analyzed using the AAS.

2.7. Statistical analysis

Statistical analyses were performed using the SPSS statistical package (version 11.0). All values reported here are the means of three independent replications. Data means were tested at significance levels of $P < 0.05$ using one way ANOVA.

3. Results

3.1. Effects of synthetic chelators and LMWOA on solubility of Pb, Zn, Cu and Cd in the contaminated soil

In order to study the effects of synthetic chelators and LMWOA on solubility of heavy metals, both concentration and time dependent extraction experiment were conducted. In the concentration dependent extraction experiment, Pb concentration increased remarkably after treatment with different

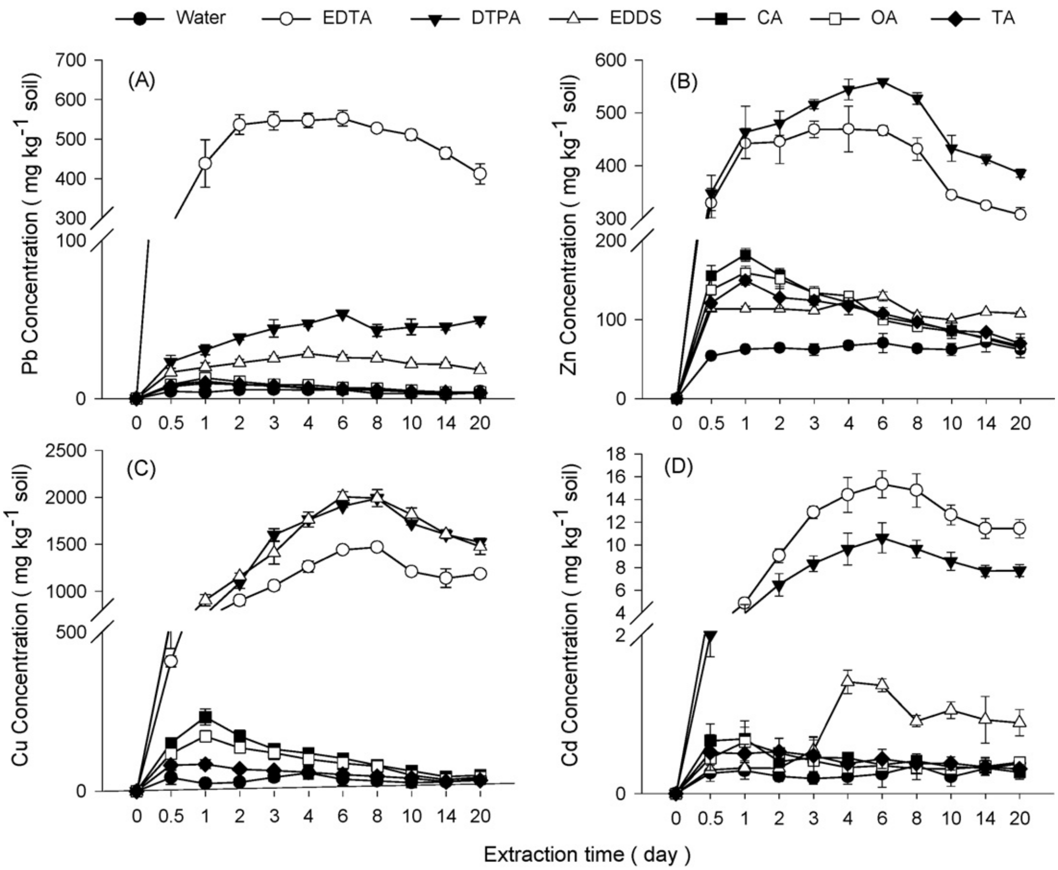

Fig. 2. Effects of various treatment time of synthetic chelators and LMWOA on the solubility of Pb (A), Zn (B), Cu (C) and Cd (D) in contaminated soil. Values are means ± S.D. ($n = 3$).

dosages of EDTA (420.3 mg kg^{-1} at the highest dosage of EDTA) as compared with the other chelators (Fig. 1A). Both EDTA and DTPA had significant effects on solubility of Zn and Cd, which enhanced sharply with increasing the treatment dosage, as compared with EDDS and LMWOA (Fig. 1B and D). In the case of Cu, effects of synthetic chelators were significantly stronger than those of LMWOA, and among the three synthetic chelators, EDDS was the most effective one (Fig. 1C). Although concentrations of soluble heavy metals were different after treating with both synthetic chelator and LMWOA, all of them increased gradually along with increasing chelator dosages.

Variable concentrations of Pb, Zn, Cu and Cd were noted in the extracted solution with different treatment time. After treating with synthetic chelators and LMWOA at the concentration of 5 and 10 mM, respectively, the increasing trend of solubility for Pb, Zn, Cu and Cd differed evidently (Fig. 2). It could be noted that the concentration of available heavy metals increased with advancement of reaction time after treating with synthetic chelators and then started to decline after 6th or 8th day of treatment except for EDDS, which had no significant effects at the first 2 d and then began to increase significantly at the day 3rd and reached the peak at 4th day of treatment. However, the concentration of available heavy metals treated with LMWOA increased slightly at 1st day, and then decreased gradually; on 20th day the concentrations were equal to control (CK).

3.2. Effects of synthetic chelators and LMWOA on plant growths

In pot experiment, hyper-accumulating ecotype (HE) exhibited strong tolerance to the heavy metal toxicity, with erect stem, thicker and dark green colored leaves compared with non-hyper-accumulating ecotype (NHE). There were some necrosis symptoms for both ecotypes of *S. alfredii* Hance after the addition of EDDS and EDTA, but damages were more serious in NHE. The dry weights of both the ecotypes of *S. alfredii* Hance are presented in Fig. 3. Shoot dry weights of HE were always higher than those of NHE plants in both control (CK) and treated plants (Fig. 3A). After treating with EDTA, EDDS and CA (citric acid), shoot dry weight of HE plants decreased significantly by 22.6%, 33.5% and 19.1%, while the other chelators failed to show any significant impact ($P < 0.05$).

For root dry weights, no significant changes were noted for the HE plants after treatment with all the chelators as compared with CK; in contrast, root dry weights of NHE decreased significantly when compared to CK except for DTPA treatment (Fig. 3B).

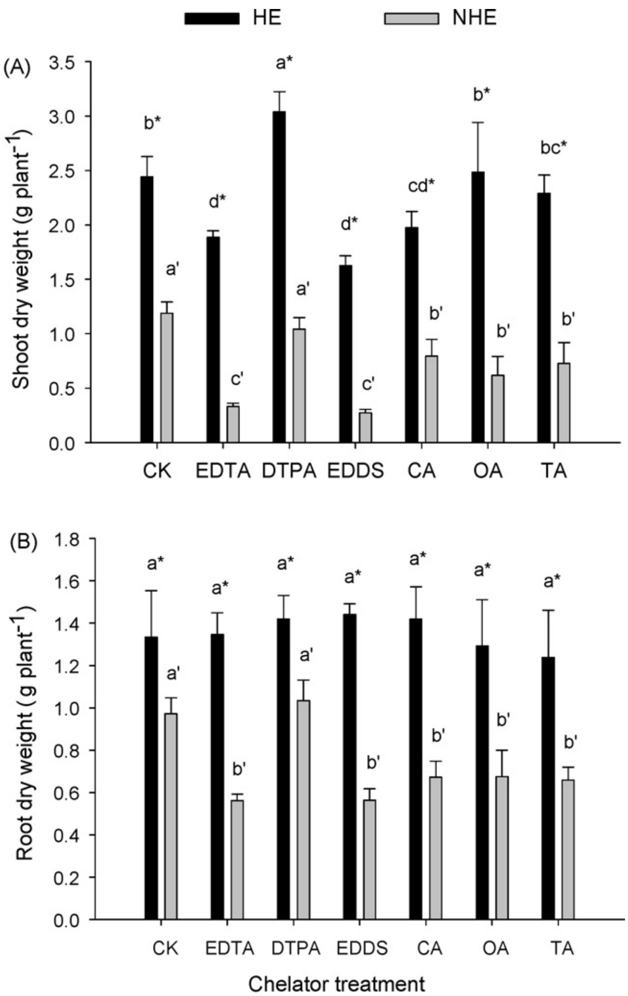

Fig. 3. Effects of synthetic chelators with dosage of 5 mM and LMWOA with the dosage of 10 mM on the dry weights of the shoots (A) and roots (B) of the two ecotypes of *Sedum alfredii* Hance at harvest. Values are means ± S.D. ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among treatments.

3.3. Effects of synthetic chelators and LMWOA on concentrations of Pb, Zn, Cu and Cd in shoots and roots of both ecotypes of Sedum alfredii Hance

Heavy metal concentrations in shoots and roots of both ecotypes of *S. alfredii* Hance are presented in Table 2. After addition of EDTA, Pb concentration in shoot of HE increased significantly, up to 218.24 mg kg^{-1}, which was 2.69-fold higher as compared with CK ($P < 0.05$). CA and OA showed more effectiveness in increasing the Pb concentration in shoots than DTPA and EDDS. At the same time, the results showed that Pb concentration in roots of both ecotypes were always significantly higher than those in shoots. Zn and Cd concentrations in shoots of HE reached to 11238.2 and 193.1 mg kg^{-1}, respectively without chelator addition, which was 9.82- and 10.33-fold higher than that of NHE plants. After treating the plants with CA and OA, Zn concentrations in shoot of HE increased by 21.2% and 11.4%, respectively, but the synthetic chelator showed minimal effects. For Cd, both DTPA and EDDS were effective in enhancing its concentration in shoot of HE plants, which increased by 85% and 136% as compared with CK ($P < 0.05$). Significant increase in Cd concentration was also noted with application of CA and OA. Addition of EDDS increased Cu concentration significantly, which was 7.68-fold higher than CK. Application of CA and OA increased Cu concentration by 1.4- and 0.68-fold in the shoots of HE plants. However, minimal effects of chelator were noted on increasing the metal concentration in shoots of NHE plants, except for the concentrations of Pb after application of EDTA, showing that chelators were more effective in HE plants than those of NHE. Compared with shoots, Pb, Zn and Cu concentrations in roots of both ecotypes increased significantly after treating with LMWOA.

3.4. Effects of synthetic chelators and LMWOA on heavy metal removals by shoots of both ecotypes of Sedum alfredii Hance

After applying EDTA, DTPA and OA at the concentration of 5, 5 and 10 mM respectively, for 10 days, Pb removal by the shoots of HE plants increased significantly by 107.5%, 48.4% and 33.2%, respectively, as compared with CK ($P < 0.05$), while the other chelators had no significant effects (Fig. 4A). It could be noted that DTPA had highest effectiveness on the removal of Zn and Cd, which increased by 20% and 130%, respectively as compared with CK ($P < 0.05$). However, application of OA significantly increased the Zn and Cd removal as compared with CK (Fig. 4B and D). Both DTPA and EDDS showed the highest Cu removal by shoots of both ecotypes, especially in HE plants. Cu removal from the soil was also significantly enhanced by application of CA and OA. Comparing the both ecotypes of *S. alfredii* Hance revealed that Pb, Zn and Cd removal amounts by shoots of HE were always extremely higher than that of NHE, regardless of treatment.

3.5. Post-harvest effects of synthetic chelators and LMWOA on soluble heavy metals in soil

To study the post-harvest effects of chelators on soluble heavy metals in soil, the concentration of water-soluble Pb, Zn, Cu and Cd at the time of harvest were determined (Fig. 5). The results showed that the amounts of water extracted Pb from soil treated with EDTA, increased significantly compared with the other chelators (Fig. 5A). After treating with EDTA and DTPA, the concentrations of soluble Zn and Cd were still remarkably higher as compared with CK (Fig. 5B and D). In case of Cu, post-harvest effects of three synthetic chelators were significantly stronger than that of LMWOA (Fig. 5C). The post-harvest effects of LMWOA on water soluble Pb, Zn, Cu and Cd were less obvious as compared to the synthetic chelators, and most of them decreased to the level of control (CK).

4. Discussion

The investigation of metal bioavailability requires routinely soil extraction studies. Results from present study showed that synthetic chelators were more effective than LMWOA on

Table 2
Effects of synthetic chelators and LMWOA on Pb, Zn, Cu and Cd concentrations in the shoots and roots of both ecotypes of *Sedum alfredii* Hance

Heavy metal	Treatment	HE		NHE	
		Shoot concentration ($mg\,kg^{-1}$ DW)	Root concentration ($mg\,kg^{-1}$ DW)	Shoot concentration ($mg\,kg^{-1}$ DW)	Root concentration ($mg\,kg^{-1}$ DW)
Pb	CK	80.9^{cd}	582.2^{c}	50.7^{c}	351.4^{d}
	EDTA	218.2^{a}	563.2^{c}	145.1^{a}	615.9^{c}
	DTPA	97.2^{b}	441.9^{c}	35.5^{c}	334.8^{d}
	EDDS	102.8^{b}	613.4^{c}	104.8^{b}	374.7^{d}
	CA	112.4^{b}	1373.4^{a}	58.3^{c}	815.0^{b}
	OA	108.4^{b}	1010.8^{b}	62.7^{c}	1242.6^{a}
	TA	61.0^{d}	1353.7^{a}	45.5^{c}	759.6^{b}
Zn	CK	11238.2^{bc}	1374.2^{b}	1144.5^{a}	893.9^{b}
	EDTA	10014.5^{c}	1377.4^{b}	1092.6^{a}	984.9^{b}
	DTPA	10838.7^{bc}	1087.8^{b}	750.3^{b}	575.6^{b}
	EDDS	11734.7^{bc}	1642.5^{b}	1174.9^{a}	931.8^{b}
	CA	13627.5^{a}	3263.0^{a}	892.0^{b}	2581.1^{a}
	OA	12520.5^{ab}	2804.6^{a}	902.6^{b}	2543.0^{a}
	TA	8009.0^{d}	3143.3^{a}	873.8^{b}	2441.5^{a}
Cu	CK	20.5^{e}	869.0^{c}	95.3^{d}	576.8^{c}
	EDTA	62.8^{c}	1003.4^{c}	310.7^{b}	951.4^{b}
	DTPA	78.3^{b}	711.9^{c}	176.4^{c}	468.0^{c}
	EDDS	157.3^{a}	799.1^{c}	420.9^{a}	512.9^{c}
	CA	68.0^{bc}	2785.1^{a}	97.6^{d}	1840.3^{a}
	OA	42.8^{d}	2181.6^{b}	104.9^{d}	2048.1^{a}
	TA	30.4^{de}	2249.9^{b}	78.8^{d}	1948.8^{a}
Cd	CK	193.1^{de}	27.2^{c}	18.7^{a}	8.0^{c}
	EDTA	238.0^{cd}	29.7^{c}	14.3^{b}	7.0^{c}
	DTPA	357.4^{b}	27.6^{c}	11.4^{c}	3.5^{d}
	EDDS	455.4^{a}	30.1^{c}	14.1^{b}	10.0^{c}
	CA	267.5^{c}	87.4^{a}	12.6^{bc}	15.6^{b}
	OA	282.5^{c}	31.3^{c}	14.3^{b}	22.2^{a}
	TA	180.9^{e}	48.5^{b}	11.9^{bc}	17.0^{b}

Note: Values are means ($n = 3$). Different letters among treatment indicate significant differences at $P < 0.05$.

increasing the solubility of Pb, Zn, Cu and Cd, which were consistent with the earlier studies [7,21,22]. The soluble Pb, Zn, Cu and Cd concentrations increased significantly with increasing synthetic chelator dosages, in contrast, for LMWOA treated soil, the soluble heavy metals concentration did not increased significantly at lower treatment dosages. It is suggested that the addition of LMWOA must be up to 10 mM kg^{-1} otherwise there would be no obvious effects on the solubility of soil heavy metals. As a conclusion, the treatment dosage of 5 mM for synthetic chelators and 10 mM for LMWOA were selected for both time dependent extraction and pot experiments.

Many studies reported that low molecular weight organic acids were less effective on the solubility of heavy metals as compared with synthetic chelators [7,22–24]. Similar results were obtained in this context i.e. the concentrations of soluble Pb, Zn, Cu and Cd increased sharply at initial days, then decreased gradually along with advancement of treatment time. Synthetic chelators reached a plateau between 3 and 7 days and LMWOA reached the maximum at 1 day and then decreased with time. However, considering the durable effects of chelators and the maximizing their efficiency, longer treatment time might be more suitable for application. Additionally, by referring results of some earlier reports [1,25,26], 10 days treatment time was selected in the pot experiment.

Some studies indicated that though chelator addition could increase the accumulation of heavy metals significantly in plants, whereas it would also bring some negative effects on the plant biomass [27,28]. In present study, both EDTA and EDDS remarkably affected shoot dry weights of both the ecotypes of *S. alfredii* Hance as compared with the other chelators, which shows that these two synthetic chelators were more toxic to plant growth than the other chelators. At the same time, the results indicated that shoot dry weight of HE were always higher than that of NHE in all the treatments showing that HE had more tolerance to the metal toxicity as well as to application of chelators.

It has been reported that various chelators such as EDTA, DTPA and HEDTA, etc. could assist plants in extracting high percentages of heavy metals from contaminated soils [8,10,19,25,29]. Present study showed that the EDTA was most effective among all the chelators in accelerating Pb uptake, which was consistent with the earlier investigations [8,26]. At the same time, it could be seen that Pb concentration in shoots of HE plants were always higher than those of NHE grown in both CK and treated soils, demonstrating that the HE plants had stronger Pb uptake ability than NHE [15]. Pb concentrations in roots of both ecotypes were significantly higher than those in shoot of both control (CK) and treated plants, and the addition

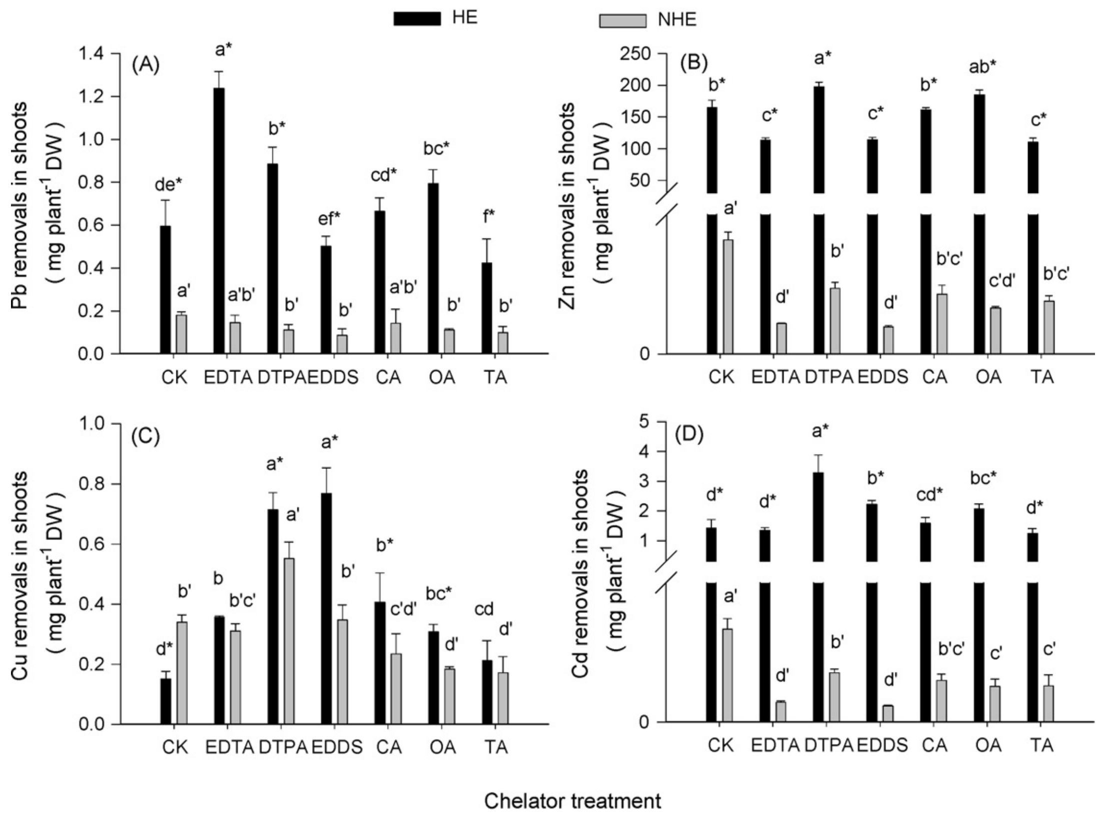

Fig. 4. Pb (A), Zn (B), Cu (C) and Cd (D) removal by shoots of two ecotypes of *Sedum alfredii* Hance at harvest after treating with both synthetic chelators and LMWOA. Different letters indicate significant differences ($P < 0.05$) among treatments.

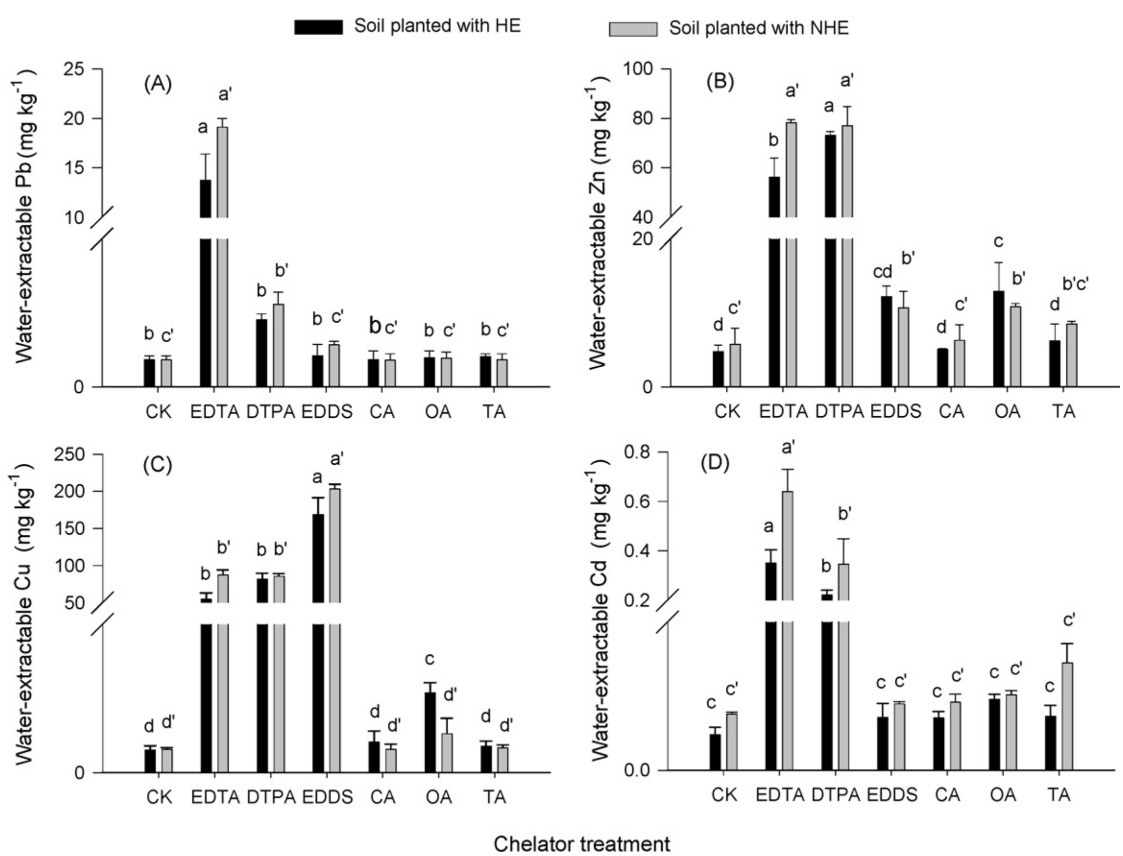

Fig. 5. Post-harvest effects of synthetic chelators and LMWOA on water-soluble Pb (A), Zn (B), Cu (C) and Cd (D) of two ecotypes of *Sedum alfredii* Hance. Different letters indicate significant differences ($P < 0.05$) among treatment.

of EDTA significantly increased the shoot/root ratio of Pb. Due to the Zn/Cd hyper-accumulating characteristics of *S. alfredii* Hance [13,14], both Zn and Cd concentrations in shoot of HE were significant higher than those in roots and were also significantly higher than those in the shoots of NHE plants. The results showed that the addition of synthetic chelators seemed to have no significant effects on increasing the shoot concentrations of Zn. Furthermore, Zn concentrations in the shoots of HE treated with both CA and OA just increased by 21% and 11.4% as compared with CK, showing that concentrations of available Zn were already sufficient in the soil, therefore addition of chelators showed little effects on the enhancement of Zn concentration in shoots of plants. In the case of Cu, it could be found that EDDS was the most effective among all the synthetic chelators and LMWOA to increase Cu concentrations in shoot of both ecotypes, while the effects were weak for the roots.

Heavy metals removal by shoots of plants is an important index which is useful for the practical application of chelators. Pb removal by the HE plants increased by 107% after treating with EDTA, while those of NHE decreased by 20%, as compared with CK, which might be the toxic effects of EDTA on plant growth. In the case of Cu, both DTPA and EDDS had significant effects on enhancing metal removal as compared with CK, and it could also be seen that heavy metal removals by HE plants were higher than that of NHE. The amount of Zn phytoextracted with OA and DTPA application was significantly greater as compared to the control. Cd removal by the HE was significantly enhanced with application of DTPA, EDDS and OA. It could be seen that the removal amounts of Cd was higher than that of Zn after the addition of DTPA but the total concentration of Cd in Fuyang soil was significantly lower than Zn, which shows that Cd could be easily phytoextracted from the contaminated soil after three or five successive harvests of *S. alfredii* Hance under chelators treatment, while the phytoextraction of Zn would need so many years. These results signified that the technique of chelate-assisted phytoremediation is more suitable for slightly-metal contaminated soil.

Among the previous studies on contaminated soils, a major part of these were focused on the spiked soils [22], and few used the naturally contaminated soils [1], which is more closed to the contamination in reality. [25] compared the effects of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals from a dredged sediment derived surface soil, and found that: Zn mobilization was comparable for both substances, Cu was mobilized more by EDDS than by EDTA, Cd and Pb were mobilized more by EDTA than by EDDS. In the present study, we used the naturally contaminated soil due to the mining activities And found that although EDTA for Pb, EDDS for Cu and DTPA for Zn and Cd were effective in removing heavy metals from soil, there were no such remarkable effects reported before [2,8,22], which maybe due to the differences of physicochemical properties of soils and the uptake ability of plants. It could be seen that the concentrations of available Cu and Zn were quite high in the soil, which made the bioavailability effects of chelators become weak. Additionally, although the addition of chelators significantly enhanced the bioavailability of heavy metals in the soil, they would also impact the biomass of plant. So, it is suggested that combination of chelators with some additional chemicals *e.g.* antibiotics, dissolved organic carbon, etc. could be practice in the future studies. The results of the present study showed that although LMWOA had mild effects on the heavy metals removal when compared with synthetic chelators, their advantages of being cheaper and safe in phytoremediation practices, higher dosages of LMWOA could be tried in further studies.

It has been reported that during the application of chelate-assisted technology, plants only absorbed a limited fraction of mobilized metals in soil; hence the post-harvest effects of chelators must be studied considering the potential environmental risks [2,25,26]. Post-harvest concentrations of water-extractable Pb, Zn, Cu and Cd in synthetic chelators treated soil increased sharply compared with CK, while those in LMWOA treated soil had almost no changes implying that the LMWOA were degraded more quickly than synthetic chelator, which is consistent with the earlier reports [30]. The concentrations of heavy metals extracted using deionized water are regarded as the water-soluble metal forms in the contaminated soil [31], which can be easily leached into the groundwater with percolating rainwater and causes threats to the human life. Considering the environmental risks, though synthetic chelators could be more effective in accelerating heavy metals availability in the soil, their stronger post-harvest effects must be taken into account.

5. Conclusions

From present study it could be concluded that:

- To enhance the solubility of heavy metals in soil, EDTA is the most effective chelator for Pb, DPTA and EDTA are more suitable for Zn and Cd, and EDDS, EDTA and DTPA are effective for Cu, as compared to other chelators or LMWOA.
- For phytoextraction by the *S. alfredii* Hance, EDTA for Pb and DTPA for Cd, is most effective among all the other chelators.
- As LMWOA were less effective on enhancing phytoextraction of Cd, Zn, Cu, and Pb by *S. alfredii* Hance as compared to synthetic chelators, therefore higher dosage could be investigated in further studies.
- Post-harvest effects of synthetic chelator are still stronger as compared with LMWOA, so it is necessary to adopt effective measures to lower the environmental risks of heavy metal leaching to the groundwater, during the application of chelators in the contaminated soil.

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