Environmental Science and Pollution Research (2019) 26:28319–28327
https://doi.org/10.1007/s11356-019-06041-w

RESEARCH ARTICLE

Removal of cadmium, lead, and zinc from multi-metal–contaminated soil using chelate-assisted *Sedum alfredii* Hance

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Received: 23 February 2019 / Accepted: 22 July 2019 / Published online: 1 August 2019

Abstract

Biodegradable chelator–assisted phytoextraction is an effective method to enhance remediation efficiency of heavy metals. A greenhouse experiment was conducted to investigate the effects of *S*,*S*-ethylenediamine disuccinic acid (EDDS), citric acid (CA), and oxalic acid (OA) application before planting on the biomass and physiological characteristics of hyperaccumulator *Sedum alfredii* Hance, and its cadmium (Cd), lead (Pb), and zinc (Zn) uptake. The results showed that EDDS and CA slightly inhibited the plant growth, while the 1.0 mmol kg^{-1} (OA-1) and 2.5 mmol kg^{-1} OA (OA-2.5) addition produced 55.3% and 35.2% greater shoot biomass compared with the control, which may be related to that OA can produce higher leaf chlorophyll and soluble protein contents, as well as lower concentrations of malondialdehyde. At the same time, the concentrations of Pb and Zn in leaf after OA-2.5 treatment significantly increased by 127% and 28.4%, and the Cd, Pb, and Zn uptake by shoot was obviously enhanced by 21.5%, 117%, and 44.9% for OA-1 addition and by 39.1%, 80.0%, and 58.3% for OA-2.5 addition, respectively, in comparison with the control ($P < 0.05$). The reductions in available contents of Cd, Pb, and Zn in soil were observed after phytoextraction by *Sedum alfredii* Hance when OA was treated. These findings imply that OA was suitable for facilitating *Sedum alfredii* Hance to remove Cd, Pb, and Zn in co-contaminated soil.

Keywords Phytoextraction · Chelators · Heavy metals · Hyperaccumulator · Co-contamination

Introduction

Human activities such as mining, wastewater irrigation, and application of chemical fertilizers have led to the excessive accumulation of metals in many agricultural soils (Xiao et al. 2008; Han et al. 2018; Feng et al. 2018). Cadmium (Cd) often co-existed with lead (Pb)–zinc (Zn) ores; thus, soils contaminated with Cd, Pb, and Zn compounds were frequently observed in agricultural fields near Pb/Zn smelting areas (Liu et al. 2010), where these heavy metals could be preserved for a long time owing to their non-degradable nature and may adversely affect soil quality and human health (Liu et al. 2017; Huang et al. 2018). Therefore, it is essential to develop an efficient and eco-friendly method for remediation of Cd, Pb, and Zn co-contaminated soils.

Phytoextraction is an ascendant remediation technology due to its less destructive nature and low cost as an in situ technology to remove contaminant from large-scale soil (Xiao et al. 2008; Zeng et al. 2019a, b; Liang et al. 2019). A deciding factor of phytoextraction is the screening of suitable plants with large biomass as well as high contents of toxic metals in its aboveground part (Wei et al. 2010). Hyperaccumulators can take up high levels of heavy metals in shoots, while most are not suitable for practical phytoextraction applications because they are specific to a particular metal, which makes them impractical in the case of multi-contaminated soil (Quartacci et al. 2007). Multi-metal hyperaccumulators such as *Thlaspi*, *Sedum* species, and *Potentilla griffithii* have been investigated to solve this problem (Papoyan et al. 2007; Qiu et al. 2011; Zhang et al. 2011; Wan et al. 2017). The lower bioavailability of heavy metals in contaminated soils and translocation of some metals

Responsible editor: Gangrong Shi

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to the shoots are also the limit factors for phytoremediation (Yang et al. 2012). Hence, some studies have focused on increasing the bioavailability of elements in soil through chelator addition measure, thereby increasing heavy metal uptake by aboveground part of plants used for phytoextraction (Bilal Shakoor et al. 2014; Song et al. 2016). The chelators include synthetic chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and *S*,*S*-ethylenediamine disuccinic acid (EDDS), and weak organic acids, such as oxalic acid (OA) and citric acid (CA) (Sinhal et al. 2010; Li et al. 2014). EDTA and the formed EDTA complexes are low biodegradable in soil (Zhao et al. 2011); therefore, they may lead to a high risk of groundwater contamination through metal leaching (Sillanpää et al. 2011). In the recent past, EDDS has been widely used in phytoremediation of Pb, Cd, Cu, and Zn–polluted soil due to its high metal-chelating ability and fast biodegradability (Lan et al. 2013; Luo et al. 2015; Song et al. 2016). CA and OA are low molecular weight organic acids in plant root exudates, which can minimize Cd stress of plants and facilitate Cd phytoextraction by hyperaccumulators such as *Brassica napus* L. and *Boehmeria nivea* (L.) Gaud. (Sun et al. 2009; Ehsan et al. 2014; Li et al. 2014). Therefore, EDDS, CA, and OA are alternative chelators for enhancing phytoremediation.

Sedum alfredii Hance growing in the old Pb/Zn mining areas of Southeast China was a Cd/Zn hyperaccumulator (Yang et al. 2001) and Pb-accumulating plant (He et al. 2002). Thus, it has great potential for phytoremediation of Cd, Pb, and Zn–co-contaminated soil. Some researchers have focused on chelator-assisted phytoremediation of heavy metals by *S. alfredii*. Liu et al. (2008) found that EDTA is effective for the removal of Pb by *S. alfredii*; however, it has the environment risk of heavy metal leaching to the groundwater. The addition of 8 mmol kg^{-1} CA achieved the maximum accumulations of Cd and Zn, while it inhibited the growth of *S. alfredii* with an 85% reduction in shoot dry biomass (Sun et al. 2009). Wang et al. (2009) found that 2.5 mmol kg^{-1} EDDS was the optimum level for Pb phytoextraction by *S. alfredii* grown in 400 mg kg^{-1} Pb–polluted soil, especially when shoot Pb concentration increased as the experiment proceeded. As shown, the effects of chelator addition, before harvesting, on phytoextraction by *S. alfredii* have been investigated; moreover, soils involved in these works were mainly contaminated with individual metals. So far, knowledge regarding the optimization of chelator-assisted *S. alfredii* simultaneous remediation of Cd, Pb, and Zn in contaminated soil is still limited. In this study, a greenhouse experiment was conducted using EDDS, CA, and OA to mobilize Cd, Pb, and Zn in co-contaminated soils before planting *S. alfredii* with the aims (1) to study plant growth and phytotoxicity characteristics affected by chelator addition and (2) to investigate the effects of chelators on concentrations, translocation, and accumulation of heavy metals in plants. The outcomes of the study will be helpful to compare the effects of different levels of chelators as potential soil amendments in simultaneously facilitating the phytoextraction of Cd, Pb, and Zn by *S. alfredii*.

Materials and methods

Soil sample and plant seedling

The soil samples (0–20 cm depth) were collected from an abandoned paddy field (latitude of 27° 52′ 17.44″, longitude of 113° 03′ 48.84″) near a Pb/Zn smelting plant in Hengyang City of Southern Hunan Province, China. After removing crop residues and debris, the soil was air-dried, ground, and passed through a 2-mm nylon screen for pot experiments. The physicochemical characteristics of soil was as follows: pH 5.02, organic matter content of 27.3 mg kg^{-1}, available nitrogen (N) of 95.4 mg kg^{-1}, available phosphorous (P) of 3.2 mg kg^{-1}, and available potassium (K) of 20.2 mg kg^{-1}. The total Cd, Pb, and Zn content was 10.4 mg kg^{-1}, 358 mg kg^{-1}, and 561 mg kg^{-1}, respectively, which was 34.7, 4.48, and 2.81 times greater than the recommended levels of 0.3 mg kg^{-1} for Cd, 80 mg kg^{-1} for Pb, and 200 mg kg^{-1} for Zn in acid soil, as described in the risk control standard for soil contamination of agricultural land (GB15618-2018) (grade II for soil pH < 5.5) (MEPPRC 2018). The available contents of Cd, Pb, and Zn were 4.71 mg kg^{-1}, 58.8 mg kg^{-1}, and 114 mg kg^{-1}, respectively. Healthy *S. alfredii* seedlings of similar size were collected from Zhejiang Province and planted in unpolluted farmland soil.

Greenhouse experiment

A total of three kg of air-dried soils was put into a plastic container with a diameter of 18 cm and a height of 16 cm. Next, urea ($CO(NH_2)_2$), potassium dihydrogen phosphate (KH_2PO_4), and potassium nitrate (KNO_3) were added to soil in each pot with 0.2 g kg^{-1} N, 0.1 g kg^{-1} P_2O_5, and 0.2 g kg^{-1} K_2O as a base fertilizer, respectively. The soil was fully watered with deionized water to maintain equilibrium for two weeks. Three chelators (EDDS, CA, and OA) as solutions were applied to the individual pots at doses of 1.0 mmol kg^{-1} and 2.5 mmol kg^{-1} soil based on previous studies (Yang et al. 2012; Liang et al. 2019), respectively, which were denoted as EDDS-1, EDDS-2.5, CA-1, CA-2.5, OA-1, and OA-2.5. In addition, a treatment with no chelator was used as a control (CK). One day later, four uniform seedlings of *S. alfredii* (about two cm in height) were transplanted into each pot. Each treatment consisted of four replicates, and the pots were arranged at random in a greenhouse maintained at 30 °C/20 °C (day/night) with a 14-h light period. During the incubation period, the plants were sprayed evenly with deionized water

to maintain the soil moisture at 70% of the field water-holding capacity. The plants were harvested after 90 days of cultivation.

The harvested plants were washed with tap water to remove soil particles then rinsed with deionized water. Each plant was cut into leaf, stem, and root. Some of leaves were stored in fresh at 4 °C for determination of the contents of chlorophyll, malondialdehyde (MDA), and soluble protein, while the remaining parts were oven-dried at 105 °C for 30 min then dried at 60 °C until constant weight. After biomass determination, the plant samples were ground and passed through a 1-mm nylon sieve. The soil samples in each pot after harvesting were collected for analysis of available contents of metals.

Sample analysis

Soil basic physicochemical characteristics

The physicochemical characteristics of the soil samples were determined based on the methods described by Lu (1999). Soil pH was measured after mixing the soil at a soil-to-water ratio of 1:2.5. The available N content was determined by an alkaline hydrolysis and diffusion method. The available P content was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by the molybdenum antimony–ascorbic acid colorimetric method. The available K content was extracted with 1.0 mol L^{-1} NH_4OAc and analyzed by atomic absorption spectrometry. The soil organic matter content was determined using $K_2Cr_2O_7$ oxidation.

Heavy metal content in soil and plant

The soil samples were digested in the mixture of HNO_3–H_2O_2 (USEPA 1996), and plant samples were digested with a solution of HNO_3–$HClO_4$ (Yang et al. 2012). The available Cd, Pb, and Zn content was extracted by the diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell 1978). The contents of Cd, Pb, and Zn in digested and extracted solutions were analyzed using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo, USA). Blank and standard reference materials for plants (GBW07603) and soil (GBW08303) obtained from the China National Center for standard reference materials were used for quality assurance/quality control.

The contents of chlorophyll, malondialdehyde, and soluble protein in leaf

The contents of chlorophyll, MDA, and soluble protein in leaves were analyzed using the methods described by Yang et al. (2012) and Heath and Packer (1968). Briefly, the chlorophyll and carotenoids were extracted by continuous shaking with 95% (v/v) aqueous ethyl alcohol solution in the dark until the leaf color disappeared completely. Light absorbance at 665 nm, 649 nm, and 470 nm was determined using a spectrophotometer. Lipid peroxidation level in leaf was determined based on the MDA content measured by the thiobarbituric acid reaction. The content of soluble protein was measured from the same supernatant using a dye of Coomassie Brilliant Blue G-250 and a standard of albumin.

Statistical analysis

The translocation factor (TF) is described as the ratio of the concentration of heavy metals in shoots to that of heavy metals in roots. Statistical analyses were performed using Microsoft Excel 2010 and SPSS 16.0. Statistically significant differences between treatments were tested by analysis of variance (ANOVA). P values < 0.05 were considered significant.

Results and discussion

Effect of chelators on plant biomass

The biomass of *S. alfredii* was affected by the application of chelating agents (Fig. 1). The addition of EDDS-1, CA-1, CA-2.5, and OA-2.5 had no obvious effects on root biomass compared with control plants, but a significant decrease of 57.8% was observed after EDDS-2.5 treatment. This was in agreement with the results reported by Epelde et al. (2008), who found that 5 mmol kg^{-1} EDDS resulted in 43.0% lower root biomass values of *Cynara cardunculus* in the 2500 mg kg^{-1} Pb–polluted soils. Similarly, the rhizoid biomass of *Pteris vittata* in soil contaminated with heavy metals after 1 mmol kg^{-1} and 2.5 mmol kg^{-1} EDDS treatments was decreased by 45.6% and 27.7%, respectively (Liang et al. 2019). The stem biomass from treatments with EDDS-1, EDDS-2.5, and CA-1 was slightly decreased, while that of the CA-2.5 treatment was being significantly inhibited by 38.2% compared to the control ($P < 0.05$). At the same time, the addition of EDDS and CA led to a significant decrease in leaf biomass of 31.5–46.0% and shoot biomass of 30.2–37.4% in comparison with CK, indicating that EDDS and CA could hinder plant growth. Concurrent to our findings, the shoot biomass of *S. alfredii* grown on mined soil decreased significantly by 33.5% after the 5 mmol kg^{-1} EDDS addition (Liu et al. 2008), indicating that *S. alfredii* was sensitive to the phytotoxicity of EDDS. Wu et al. (2007) have reported that EDDS itself or EDDS-metal complexes showed adverse effects on plant growth. The reductions in growth probably were owing to the combination of heavy metal concentration with the levels of chelator addition that exceeded the ability of plants to activate defense systems (Sun et al. 2009). There were various effects on the growth of plants exposed to CA. The application of 5 mmol kg^{-1} and

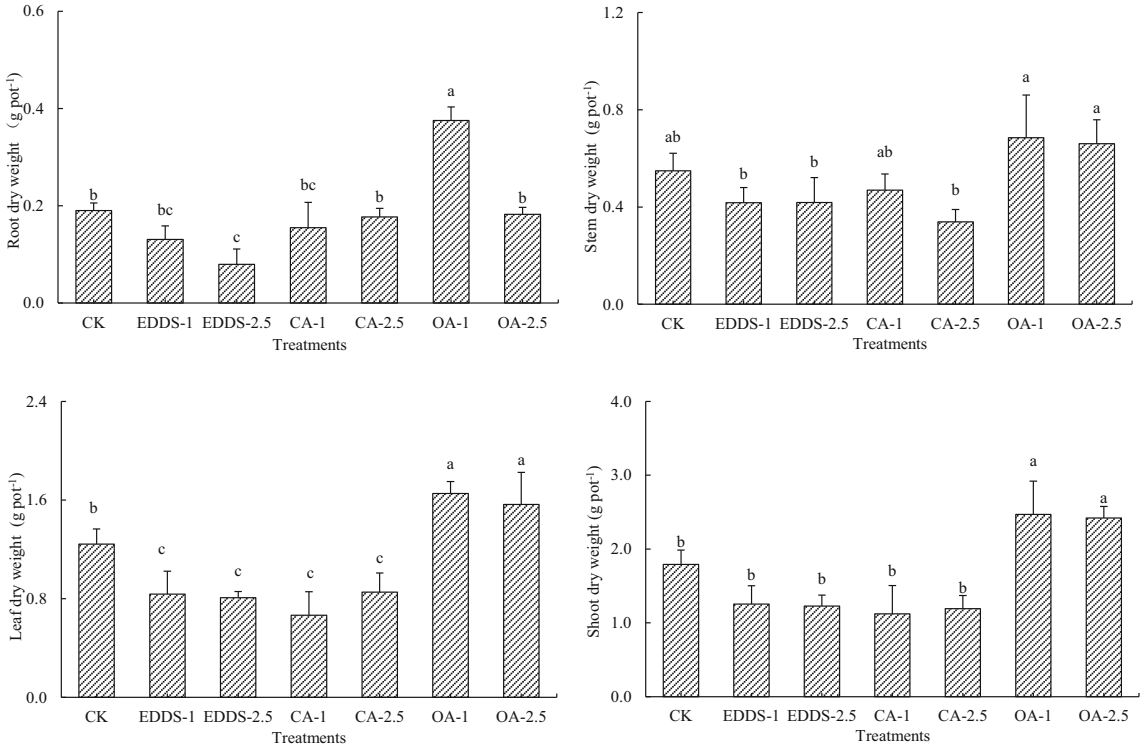

Fig. 1 Effects of chelators on the root, stem, leaf, and shoot dry biomass of *S. alfredii*. Bars marked with different letters are significantly different among treatments with chelators ($P < 0.05$). Values are means ± SD ($n = 4$)

8 mmol kg^{-1} CA induced a significant decrease in leaf biomass of 55.3% and 45.4% for *S. alfredii*, respectively (Sun et al. 2009). This may be related that CA may dissolve the carbonates and induce soil compaction with drastic changes in the physicochemical environment (Lesage et al. 2005), which inhibits plant growth. However, Anwar et al. (2017, 2019) found that the biomass of *Zea mays* increased in response to the 1 mmol kg^{-1} CA application. Najeeb et al. (2011) and Ehsan et al. (2014) also found that CA enhanced the growth of *Juncus effuses* and *Brassica napus* L. stressed by Mn and Cd, respectively. This suggested that the dosage, timing, and method of chelator addition had different phytotoxic influences on the growth of plant. Interestingly, the root, leaf, and shoot biomass was significantly enhanced by 100%, 33.1%, and 25.8% for the OA-1 treatment, and the OA-2.5 addition also induced leaf and shoot biomass increase by 38.0% and 35.2%, respectively, compared to CK ($P < 0.05$) (Fig. 1). This may be related that OA, which is a predominant root exudate of *S. alfredii*, will be used as a carbon source in many plant processes including metal detoxification and nutrient acquisition through mobilization of weakly soluble essential nutrients (Tao et al. 2016). Previous study has been reported that a hyperaccumulating ecotype (HE) of *S. alfredii* had nearly two-fold higher OA secretion than a non-hyperaccumulating ecotype (NHE), and OA was the only dominant organic acid that responded dramatically to Cd exposure in the HE of *S. alfredii* (Zhu et al. 2011), indicating that extraneous OA was a suitable chelator to enhance the growth of *S. alfredii* under heavy metal stress.

Effect of chelators on physiological characteristics of plant

The chlorophyll and carotenoid contents reflect the photosynthesis ability of plants (Yang et al. 2012), which are important to plant growth and biomass production (Tang et al. 2015). The content of chlorophyll in the leaf of plants from the chelator treatments (except for OA-1) showed no significant difference from the control plants, while that in the OA-1 treatment significantly increased by 31.4% ($P < 0.05$) (Table 1). This was in agreement with previous findings that 3 mmol kg^{-1} OA alleviated the adverse Cd influence on the photosynthetic activity of *Boehmeria nivea* (L.) Gaud. (Li et al. 2014). An increase in the concentration of photosynthetic pigments was observed in the OA-1 treatment compared with CK, which was consistent with the increase in biomass (Fig. 1), suggesting that the OA-1 application did not damage the photosynthetic parameters of plant (Yang et al. 2012). Lipid peroxidation is the oxidative degeneration of lipids containing a lot of carbon–carbon double bonds that generate the final product of MDA. Thus, the increase of MDA level is generally considered to be an indicator of oxidative stress (Bilal Shakoor et al. 2014). EDDS-2.5 induced an increase in the MDA content of 36.4%, which was in line with the results of a

Table 1 The contents of chlorophyll, carotenoid, MDA, and soluble protein in *S. alfredii* treated with chelators

Treatments	Chlorophyll content (mg g^{-1} FW)	Carotenoid content (mg g^{-1} FW)	MDA content (nmol g^{-1} FW)	Soluble protein content (mg g^{-1} FW)
CK	1.21 ± 0.15b	0.12 ± 0.02a	2.39 ± 0.50b	26.1 ± 1.94c
EDDS-1	1.13 ± 0.16b	0.11 ± 0.01a	2.50 ± 0.22b	27.0 ± 2.49bc
EDDS-2.5	1.09 ± 0.12b	0.13 ± 0.04a	3.26 ± 0.47a	28.8 ± 1.15abc
CA-1	1.12 ± 0.22b	0.11 ± 0.03a	2.78 ± 0.18ab	28.4 ± 1.83abc
CA-2.5	1.04 ± 0.18b	0.11 ± 0.02a	2.70 ± 0.29ab	27.5 ± 1.88abc
OA-1	1.59 ± 0.05a	0.13 ± 0.01a	2.22 ± 0.46b	30.4 ± 1.42ab
OA-2.5	1.27 ± 0.09b	0.12 ± 0.02a	2.22 ± 0.18b	31.3 ± 1.04a

Data are presented as mean values ± SD ($n = 4$). Means followed by the same letter within the same column are not significantly different ($P > 0.05$) according to Duncan's new multiple range test

previous study that the EDDS addition induced more oxidative damage to plants under Ni stress attributed to the production and accumulation of H_2O_2 and MDA (Sidhu et al. 2018). In contrast, the OA addition prevented MDA accumulation compared with the control and other chelators (Table 1), indicating that it provided a greater ability to alleviate lipid peroxidation leading to mitigation of metal-induced oxidative stress in plant due to the formation of less toxic organic acid–metal chelates. Tao et al. (2016) have reported that oxalate chelated with Cd had the greatest stability constant to form the complex with less toxicity when compared with other organic acid anions such as CA, lactate, and tartrate. A significant increase in soluble protein contents by 16.5% and 19.9% was detected in response to the addition of OA-1 and OA-2.5 (Table 1), which was consistent with a previous study (Li et al. 2014). This may have been because of the increase in antioxidant activity and the decrease in H_2O_2 production and electrolyte leakage (Ehsan et al. 2014). Therefore, OA could alleviate the toxic effects of Cd, Pb, and Zn on photosynthesis and biochemical characteristics of *S. alfredii*.

Heavy metal concentration and translocation factor in plant

There were no significant differences in the root Cd concentration between treatments with chelator addition and CK (Table 2). The Cd concentration in the stems and leaf of plants in the OA-1 group significantly decreased by 37.5% and 24.4% compared with CK, respectively. This might be associated with the increase in shoot biomass, leading to a dilution effect (Chen et al. 2017). The leaf Cd concentrations in plants treated with EDDS-1 and EDDS-2.5 was slightly increased by 16.5% and 6.06% relative to the control, respectively, which was in keeping with the findings reported by Liu et al. (2008) that EDDS was more effective at stimulating Cd uptake than weak organic acids. Similarly, the significant differences in root Pb concentrations between chelator addition treatments and CK were not observed, while root Pb concentration from treatments with EDDS-2.5 and OA-2.5 increased by 23.0% and 20.4% compared with CK, respectively (Table 2). Generally, the stem Pb concentrations in plants from the EDDS, CA, and OA treatments were significantly enhanced by 25.5–74.7%, and leaf Pb concentrations in the chelator addition treatment (except for EDDS-2.5) increased significantly by 54.0–101% when compared with CK. CA and OA had greater effectiveness at enhancing the shoot Pb concentration of *S. alfredii* than EDDS, which was similar to the results reported by Liu et al. (2008). The root Zn concentration in plants from the CA-2.5 and OA-2.5 group was significantly increased by 26.9% and 17.7% compared with CK ($P < 0.05$). The unstirred layer surrounding the root surface due to the existence of negatively charged free radicals can be thinner in the presence of the chelator, making it much easier for plant roots to extract elements (Kinraide et al. 2005). OA was a dominant organic acid in the root exudates of the Cd hyperaccumulator *S. alfredii*, which is most effective in heavy metal mobilization to the plants (Tao et al. 2016; Wu et al. 2018). The concentrations of Zn in the stem and leaf from the OA-2.5 treatment were increased by 9.77% and 28.4% relative to the control, indicating that it was an optimum level for increasing the shoot Pb and Zn concentration of *S. alfredii*.

Generally, the TF_{shoot} value of Cd in the EDDS treatment increased slightly, while it decreased significantly for the CA treatment compared with CK (Table 2), suggesting that EDDS could effectively transfer Cd from roots to shoots in *S. alfredii*. Quartacci et al. (2007) reported that Cd–EDDS was more mobile in the plant apoplastic system and readily translocated from the underground parts to the aboveground parts of *Brassica carinata* when compared to Cd–NTA. This was related to the extracellular loading among spongy tissues and intracellular sequestration in mesophyll vacuoles that enabled chelators to enhance Cd apoplastic and symplastic transportation from roots to shoots (Yin et al. 2015). The Pb uptake by plant was predominantly retained in the root with Pb TF_{shoot} values below or close to 1.0 in all treatments. The low translocation of Pb to shoots probably can be explained by the fact that Pb has a strong ability to bind with carboxyl groups of galacturonic and glucuronic acids in the cell wall, which limits

Table 2 The concentrations and translocation coefficient of Cd, Pb, and Zn in *S. alfredii* grown on soils contaminated with heavy metals treated with chelators

Treatment	Root (mg kg^{-1})			Stem (mg kg^{-1})			Leaf (mg kg^{-1})			TF_{shoot}		
	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
CK	889 ± 286a	534 ± 171ab	7425 ± 1027bcd	2356 ± 586a	411 ± 50c	13,889 ± 636a	2332 ± 109ab	309 ± 7c	17,231 ± 1291b	2.68 ± 0.5abc	0.89 ± 0.12abc	2.19 ± 0.15a
EDDS-1	810 ± 90a	513 ± 113ab	6934 ± 797 cd	2003 ± 558ab	636 ± 59ab	9518 ± 317d	2807 ± 111a	621 ± 48a	16,769 ± 2382b	2.87 ± 0.42a	0.93 ± 0.15abc	2.02 ± 0.17ab
EDDS-2.5	663 ± 38a	657 ± 66a	6202 ± 160d	1794 ± 424ab	587 ± 114ab	10,782 ± 195bcd	2556 ± 336ab	311 ± 33c	17,733 ± 1802b	2.78 ± 0.84ab	0.62 ± 0.19d	1.67 ± 0.22b
CA-1	816 ± 78a	566 ± 50ab	7255 ± 682 cd	1686 ± 103ab	516 ± 12bc	11,492 ± 1212bc	2295 ± 148ab	476 ± 58b	17,374 ± 128b	2.59 ± 0.21bc	0.87 ± 0.12bcd	2.21 ± 0.11a
CA-2.5	872 ± 82a	499 ± 74ab	9421 ± 257a	1769 ± 362ab	626 ± 116ab	12,312 ± 1001b	2266 ± 285bc	485 ± 107b	17,085 ± 2316b	2.23 ± 0.43d	0.77 ± 0.04 cd	1.95 ± 0.14ab
OA-1	748 ± 42a	452 ± 67b	7820 ± 391bc	1473 ± 122b	540 ± 85bc	10,219 ± 688 cd	1822 ± 192c	483 ± 54b	16,782 ± 1001b	2.47 ± 0.44 cd	1.05 ± 0.07ab	2.24 ± 0.17a
OA-2.5	872 ± 74a	643 ± 73a	8742 ± 841ab	1878 ± 418ab	718 ± 97a	15,246 ± 859a	2219 ± 389bc	702 ± 18a	22,120 ± 2318a	2.41 ± 0.20 cd	1.16 ± 0.19a	2.30 ± 0.32a

Data are presented as mean values ± SD ($n = 4$). Means followed by the same letter within the same column are not significantly different ($P > 0.05$) according to Duncan's new multiple range test

the apoplastic transport of Pb (Amer et al. 2013). The TF_{shoot} of Pb from EDDS-2.5 was significantly lower than that in CK treatment, which slightly increased by 16.0% and 30.3% in response to treatment with OA-1 and OA-2.5. Similarly, the TF_{shoot} value of Zn in the EDDS-2.5 treatment was significantly decreased by 23.7%, while that of plants from the OA treatment increased slightly when compared with CK, indicating that OA could enhance the translocation of Pb and Zn from root to shoot. Chelation of heavy metals with organic acid should be viewed as an essential process for efficiently taking part in metal long-distance translocation in the xylem while avoiding the toxic influence of free metal ions in plants (Ghnaya et al. 2013).

Heavy metal uptake by plant shoot

Total metal uptake was based on the biomass multiplied by the metal's concentration. The Cd, Pb, and Zn uptake by shoots of *S. alfredii* were inhibited by the addition of EDDS and CA, while they were significantly enhanced by the OA addition compared to the control (Fig. 2). The Cd uptake in response to treatment with EDDS-2.5 and CA-2.5 was reduced significantly by 36.8% and 35.7%, respectively, when compared with CK ($P < 0.05$). The shoot uptake of Pb from EDDS and CA addition treatments was slightly decreased, and the Zn uptake in the EDDS-1 treatment was significantly decreased by 50.0% when compared with CK. This may be related to the increase in shoot Cd, Pb, and Zn concentration in response to EDDS and CA treatment which was not high enough to make up for the inhibition in plant biomass. It has been reported that 8 mmol kg^{-1} EDDS limited Cd and Pb uptake because of a significant reduction in aboveground biomass in *S. alfredii* (Sun et al. 2009). This was also in accordance with the results of a study that Ni uptake by *Datura innoxia* after EDDS treatment decreased due to biomass reduction (Jean et al. 2008). However, the Cd, Pb, and Zn uptake by shoot in OA-1 and OA-2.5 treatment significantly increased by 21.5% and 39.1%, 117% and 80%, and 44.9% and 58.3% compared to CK, respectively ($P < 0.05$). As reported previously, the Pb uptake by shoots of *S. alfredii* increased significantly by 33.2% after a high level of 10 mmol kg^{-1} OA treatment when compared with CK (Liu et al. 2008). More bioavailable OA–metal complexes diffusing more quickly in substrate and plant organs compared to free metal ions can be generated, suggesting that exogenous oxalate supply may efficiently promote heavy metal uptake (Lim et al. 2012). In addition, higher uptake of heavy metals by *S. alfredii* was linked to an increase in plant biomass under OA treatments (Fig. 1). Therefore, when conducting heavy metal phytoextraction using plants, the dry biomass reduction and heavy metal concentrations should be considered (Komárek et al. 2008). These results imply that planting *S. alfredii* in soil co-contaminated with multi-metals amended with 1.0 mmol

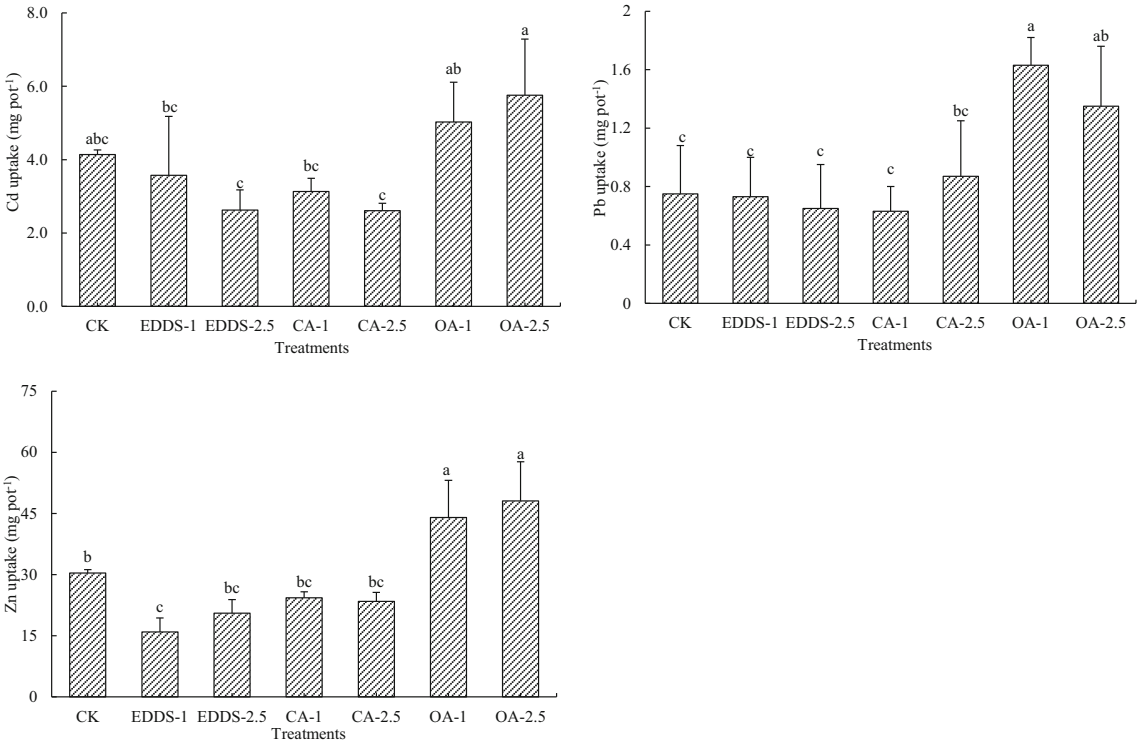

Fig. 2 The Cd, Pb, and Zn uptake by shoot of *S. alfredii* grown on soils treated with different chelators. Bars marked with different letters are significantly different among treatments with chelators ($P < 0.05$). Values are means ± SD ($n = 4$)

kg^{-1} and 2.5 mmol kg^{-1} OA before planting was the most beneficial treatment for the uptake of Cd, Pb, and Zn.

Available contents of heavy metals in soil after phytoextraction

The soil available content of Cd, Pb, and Zn in the soil of CK treatment after phytoextraction was slightly lower than that in the original soil. However, the available Cd content in the soil from EDDS and CA treatments after plant phytoextraction slightly increased, and the available Zn content in the EDDS-2.5 treatment significantly increased by 11.9% when compared with CK (Table 3). Meers et al. (2005) reported enhanced the mobility and availability of Cd, Pb, and Zn in soil under CA application. This may be related to the ability of the chelators to form soluble complexes with heavy metals in soil and thus desorb and solubilize metals from solid soil components (Usman et al. 2013). There are different effects of EDDS on the content of available Cd, Pb, and Zn because metal–chelator complexes have different stability constants. A previous study has reported that the range of stability constants (log *K*) of metal–EDDS complexes is as follows: Zn (13.5) > Pb (12.7) > Cd (10.8) (Bucheliwitschel and Egli 2001). Interestingly, the available contents of Cd, Pb, and Zn in treatments of OA-1 and OA-2.5 decreased by 17.8% and 15.7%, 17.0% and 13.9%, and 26.3% and 20.2% compared with that in the original soil before phytoextraction, and also decreased by 12.2% and 10.0%, 17.0% and 17.5%, and 17.3% and 10.9%, respectively, when compared with CK (Table 3). There was a significant negative relationship between the available contents of Cd, Pb, and Zn in soil and heavy metal uptake by plant (Table 4), which was consistent with the previous research which reported that phytoextraction could reduce the mobility, bioavailability, or toxicity of metals in soil (Zeng et al. 2019a, b). These results showed that OA amended before planting could assist the simultaneous phytoextraction of Cd, Pb, and Zn from multi-contaminated soil by *S. alfredii*.

Table 3 The available contents of heavy metals in soil after phytoextraction

Treatments	Content of available heavy metals (mg kg^{-1})		
	Cd	Pb	Zn
Original soil	4.71 ± 0.77abc	58.8 ± 4.65a	114 ± 5.93a
CK	4.41 ± 0.53abc	56.5 ± 3.81a	102 ± 9.09b
EDDS-1	4.80 ± 0.27abc	56.4 ± 2.53a	103 ± 1.59b
EDDS-2.5	5.12 ± 0.75a	55.4 ± 1.08a	114 ± 4.61a
CA-1	5.06 ± 0.75a	55.1 ± 3.63a	102 ± 7.58b
CA-2.5	4.99 ± 0.34ab	52.1 ± 1.31a	107 ± 3.23ab
OA-1	3.87 ± 0.57c	48.8 ± 1.55b	84 ± 4.77c
OA-2.5	3.97 ± 0.29bc	50.6 ± 2.38b	91 ± 6.89c

Data are presented as mean values ± SD ($n = 4$). Means followed by the same letter within the same column are not significantly different ($P > 0.05$) according to Duncan's new multiple range test

Table 4 Pearson correlation coefficients between available Cd, Pb, and Zn contents after phytoextraction in the soil and Cd, Pb, and Zn uptake by the shoot of plant

Items	Cd uptake	Pb uptake	Zn uptake
Available Cd	− 0.575**	− 0.582**	− 0.641**
Available Pb	− 0.631**	− 0.699**	− 0.839**
Available Zn	− 0.693**	− 0.702**	− 0.724**

**Correlation is significant at the 0.01 level ($P < 0.01$)

Conclusion

The pot experiments showed that EDDS and CA had slight inhibition on the growth of *S. alfredii* and significantly enhanced the concentrations of Cd, Pb, and Zn in plant, while heavy metal uptake was decreased compared with control. The addition of 1 mg kg^{-1} and 2.5 mg kg^{-1} OA alleviated the toxic effects of heavy metals on plant physiological characteristics and significantly enhanced shoot biomass. Moreover, the Cd, Pb, and Zn uptake by shoot significantly increased by 21.5%, 117%, and 44.9% for the OA-1 treatment and 39.1%, 80%, and 58.3% for the OA-2.5 treatment compared with the control, respectively. This study developed a novel phytoremediation technology employing *S. alfredii* assisted with OA before planting for the simultaneous removal of multi-metals in soil.

Funding information This research was supported by the National Key Technology Research and Development Program of China (2015BAD05B02) and Hunan Provincial Natural Science Foundation of China (2018JJ4018).

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