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Effects of zinc and cadmium interactions on root morphology and metal translocation in a hyperaccumulating species under hydroponic conditions

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ABSTRACT

Effects of zinc (Zn) and cadmium (Cd) interactions on root morphology and metal translocation in the hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of *S. alfredii* were investigated under hydroponic conditions. Specific root lengths (SRL), specific root surface areas (SRA) and specific root volumes (SRV) of the HE increased significantly when plant were treated with 500 μM Zn or 100 μM Cd + 500 μM Zn, whereas these root parameters were significantly decreased for the NHE when plant were treated with 100 μM Cd, 500 μM Zn or 100 μM Cd + 500 μM Zn. SRL and SRA of the HE were mainly constituted by roots with diameter between 0.2–0.4 mm (diameter class 3 and 4) which were significantly increased in treatment of 500 μM Zn or 100 μM Cd + 500 μM Zn, whereas in the NHE, metal treatments caused a significant decrease in SRL and SRA of the finest diameter class root (diameter between 0.1–0.3 mm). The HE of *S. alfredii* could maintain a fine, widely branched root system under contaminated conditions compared with the NHE. Relative root growth, net Cd uptake and translocation rate in the HE were significantly increased by adding 500 μM Zn, as compared with the second growth period, where 100 μM Cd was supplied alone. Cadmium and Zn concentrations in the shoots of the HE were 12–16 times and 22–27 times higher than those of the NHE under 100 μM Cd + 500 μM Zn combined treatment. These results indicate strong positive interactions of Zn and Cd occurred in the HE under 100 μM Cd + 500 μM Zn treatment and Cd uptake and translocation was enhanced by adding 500 μM Zn.

1. Introduction

Cadmium is one of the most toxic heavy metal in the environment due to its high mobility and severe toxicity to the organisms [1–4]. Zinc is an essential micronutrient for plants but can be highly toxic when present at excessive concentration [5]. Anthropogenic inputs of Cd and Zn to soils occur via short or long-range of mining, atmospheric depositions, and use of fertilizers/manures, municipal sewage-wastes, compost and industrial sludge [6,7]. Cadmium and Zn are elements having similar geochemical and environmental properties. Zinc ores normally contain 0.1–5% of Cd, and the processing and subsequent release of Zn to the environment is normally accompanied by Cd [8].

Phytoremediation, i.e. the use of green plants to remove pollutants from the environment or to render them harmless has been proposed as an environment friendly and cost-effective technique for soil and water remediation [9,10]. In case of heavy metal contaminated soil, the biological process of phytoextraction includes metal mobilization as well as acquisition and transport [11]. Root system is the main interface of ion exchange between plants and their environment, thus in each process roots play a central role [12]. Excessive metal has marked effects on root growth of plants, however, there is scanty knowledge about the root morphological changes in hyperaccumulators after exposure to heavy metal stress [13]. Various previous studies were limited to toxic effects of metal on the shoot with less attention to root system, and the data about toxic effects of heavy metal on hyperaccumulator roots were mostly limited to root biomass [14,15]. Studies on the interactions between heavy metal conducted so far focused on the conventional plant species, little information is available about the interactions in the hyperaccumulator, especially on root morphological changes [16]. In hyperaccumulators, root length determines the capacity to acquire water and nutrients, and therefore metal uptake capacity is more strongly related to root length than root weight [17,18]. When looking at phytoextraction, not only quantitative root parameters (biomass) should be considered but qualitative root characteristics may also be looked into [19]. Root length, surface area, length–diameter distribution and volume could serve

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as valuable parameters when describing and comparing root systems.

Sedum alfredii grows in the old Pb/Zn mining areas of southeast China, and has been identified as a new Zn/Cd-hyperaccumulator which can accumulate more than 20,000 mg kg^{-1} Zn and 8000 mg kg^{-1} Cd in shoots from hydroponic medium, before showing any symptoms of Zn and Cd toxicity [20,21]. This plant has exceptional abilities to tolerate and accumulate high concentrations of Zn/Cd, and the characteristics of large biomass, rapid growth, asexual propagation, and perennial growth makes it as an ideal plant for studying mechanisms responsible for hyperaccumulation as well as for the phytoremediation practices. Till to date, studies on the mechanism of metal tolerance and hyperaccumulation of *S. alfredii* were mainly focused on the uptake [20,21] and subcellular distribution characteristic [22,23], while little information is available about the effects of Cd and Zn interactions on root morphology. Altered root morphology could directly influence the acquisition of water and nutrients and thus affects plant growth and the efficiency of phytoremediation.

In this paper, we emphasized on the root morphology of *S. alfredii* in response to Zn/Cd interactions. Data obtained from this study will contribute to a better understanding of the mechanism involved in metal hyperaccumulation in *S. alfredii* and to assist in the evaluation of plant species suitable for phytoremediation.

2. Materials and methods

2.1. Plant culture

Seedlings of two contrasting ecotypes of *S. alfredii* were cultivated according to Yang et al. [20]. The hyperaccumulating ecotype (HE) of *S. alfredii* was collected from an old Pb/Zn mine area in Zhejiang Province in China, and the non-hyperaccumulating ecotype (NHE) of *S. alfredii* was obtained from a tea garden in Hangzhou, Zhejiang Province, China. Plants were chosen to grow in noncontaminated soil for several generations to minimize the internal metal contents, then uniform and healthy shoots were selected and cultivated in the basal nutrient solution containing: 2 mM Ca^{2+}, 4 mM NO_3^-, 1.6 mM K^+, 0.1 mM $H_2PO_4^-$, 0.5 mM Mg^{2+}, 1.2 mM SO_4^{2-}, 0.1 mM Cl^-, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 1 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6\ Mo_7O_{24}$, and 100 μM Fe–EDTA. Nutrient solution pH was adjusted daily to 5.8 with 0.1 M NaOH or 0.1 M HCl. Plants were grown under glasshouse conditions with natural light, day/night temperature of 26/20 °C and day/night humidity of 70/85%. The nutrient solution was aerated continuously and renewed every 3 d.

2.2. Effect of zinc and cadmium on root morphology

The seedlings of *S. alfredii* were pre-cultured for 14 d (for the initiation of new roots) prior to Cd^{2+} and Zn^{2+} exposure. The treatments were composed of control (CK), 100 μM Cd, 500 μM Zn, 100 μM Cd + 500 μM Zn, with four replicates for each treatment. Cadmium and Zn were supplied as $Cd(NO_3)_2$ and $ZnSO_4{\cdot}7H_2O$. Plants were harvested after 30 d exposure to metals. At the time of harvest, shoots of each plant were excised at their base and roots were washed with distilled water and stored in 50% isopropyl alcohol at 4 °C until further processing.

Root morphological parameters were determined by using a root automatism scan apparatus (MIN Mac, STD1600$^+$) equipped with WinRHIZO^{TM}2000 software offered by Regent Instruments Inc. WinRHIZO^{TM} 2000 is a software that recognizes digital root images and analyzes root parameters (length, surface area, and volume) for defined root diameters. To improve contrast, roots were stained for 5 min in crystal violet (1 g per 100 ml water) at 50 °C [24]. Root segments were then placed on STD1600$^+$ in a transparent plastic tray filled with 0.01 M NaOH to avoid desorption of the stain. A white plastic plate served as image background. Image record was performed at a resolution of 800 dpi and images were saved as TIFF (tagged image file format). For a better understanding of the root morphological characteristics of *S. alfredii*, 10 root diameter classes were defined with interval width of 0.1 mm for each species, for example, roots with diameter between 0–0.1 mm were defined as diameter class 1 (Table 1). Root length, surface area, volume and average root diameter (ARD) were analyzed for each diameter class. Specific root length (SRL), specific root surface area (SRA) and specific root volume (SRV) were calculated. For each replication, roots of the 10 plants were analyzed.

Table 1
Distribution of diameter classes (d denote root diameter).

Diameter class	Size (mm)
1	$0.0 < d \leq 0.1$
2	$0.1 < d \leq 0.2$
3	$0.2 < d \leq 0.3$
4	$0.3 < d \leq 0.4$
5	$0.4 < d \leq 0.5$
6	$0.5 < d \leq 0.6$
7	$0.6 < d \leq 0.7$
8	$0.7 < d \leq 0.8$
9	$0.8 < d \leq 0.9$
10	$d > 0.9$

2.3. Zinc and cadmium accumulation in S. alfredii

The treatments were same as described in morphology experiment. Plants were harvested after 30 d exposure of metals. The harvested plants were separated into leaves, stem, and roots, oven dried at 65 °C for 72 h, and dry weights were recorded. Dried plant materials were grounded using a stainless steel mill and passed through a 0.25-mm sieve for metal analysis. About 0.1 g of each plant samples was ashed in a muffle furnace at 550 °C for 5.5 h, ash was dissolved in 5 ml of 1:1 HCl:H_2O and the digested material was transferred to a 50 ml volumetric flask. The volume was made using deionized water and then the contents were filtered using a metal free filter paper. The concentrations of Zn^{2+} and Cd^{2+} in the filtrates were determined using Flame Atomic Absorption Spectrophotometer (AA 6800, Shimadzu, Japan).

2.4. Effect of zinc addition on root growth and cadmium uptake

Pre-cultured seedlings (14 d old) of the both ecotypes were used for this experiment. For each ecotype, there were 16 pots with 6 plants in each pot. Prior to the experiment, plants of the 4 pots were harvested for determination of initial root biomass and Zn/Cd concentrations in various plant parts. After 15 d growth in basal nutrient solution (the first growth period, i.e. 0–15 d), 100 μM Cd was added to the basal nutrient solution as nitrate salt. After another 15 d growth in the 100 μM Cd treated solution (the second growth period, i.e. 15–30 d), 500 μM Zn was added to the Cd treated solution as sulfate salt and the plants were harvested after another 15 d growth in Cd + Zn combined treated solution (the third growth period, i.e. 30–45 d). The whole treatment period was of 45 d. For each ecotype of *S. alfredii*, plants from the 4 pots were harvested in each treatment at 15 d (grown in basal nutrient solution without Cd and Zn), 30 d (after 15 d growth under Cd) and 45 d (after 15 d growth under Cd + Zn combined treatment). Root biomass and the concentrations of Zn^{2+} and Cd^{2+} in the harvested plant were determined. For the periods between successive harvests and for the whole treatment period the following indexes were calculated.

Table 2
Dry weights (DW) of the both ecotypes of *S. alfredii* plants treated with 100 μM Cd, 500 μM Zn and 100 μM Cd + 500 μM Zn for 30 d.

Treatments	Root (g plant^{-1} DW)		Stem (g plant^{-1} DW)		Leaf (g plant^{-1} DW)	
	HE	NHE	HE	NHE	HE	NHE
CK	0.106 ± 0.011b	0.102 ± 0.017a	0.521 ± 0.101a	0.534 ± 0.092a	0.911 ± 0.106a	0.811 ± 0.074a
Cd100	0.108 ± 0.018b	0.066 ± 0.007b	0.516 ± 0.097a	0.327 ± 0.061b	0.909 ± 0.084a	0.528 ± 0.061b
Zn500	0.136 ± 0.021a	0.065 ± 0.009b	0.544 ± 0.078a	0.306 ± 0.042bc	0.890 ± 0.081a	0.514 ± 0.032b
Cd100 + Zn500	0.129 ± 0.014a	0.041 ± 0.004c	0.512 ± 0.091a	0.272 ± 0.035c	0.879 ± 0.101a	0.420 ± 0.041c

Data are means ± S.E. of four replicates. Values in each column followed by the same letter are not significant different at $P < 0.05$ as determined by Duncan's Multiple Range Test.

The relative growth rates (RGR) of roots were determined following the methods described by Arduini et al. [3], as

$$\mathrm{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \qquad (1)$$

Where W is the dry biomass at the beginning (W_1) and at the end (W_2) of each period, and $t_2 - t_1$ is the duration of the period.

The net uptake rates of Zn and Cd were determined following the methods described by Engels [25], as

$$\mathrm{NUR} = \frac{P_{c_2} - P_{c_1}}{t_2 - t_1} \frac{\ln(R_{w_2}/R_{w_1})}{R_{w_2} - R_{w_1}} \qquad (2)$$

Where P_c is the Zn/Cd contents in the whole plant and R_w is the dry weight of the root at the beginning (1) and at the end (2) of each period, and $t_2 - t_1$ is the duration of the period.

The net translocation rates (NTR) from root to aerial part were determined following the methods described by Arduini et al. [3], as

$$\mathrm{NTR} = \frac{A_{c_2} - A_{c_1}}{t_2 - t_1} \frac{\ln(S_{c_2}/S_{c_1})}{S_{c_2} - S_{c_1}} \qquad (3)$$

Where A_c is the Zn/Cd contents of the shoot, and S_c is the Zn/Cd contents of the root, at the beginning (A_{c_1} and S_{c_1}) and at the end (A_{c_2} and S_{c_2}) of each treatment period, and $t_2 - t_1$ is the duration of period.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS 11.0. Figures were drawn using Sigma Plot 9.0, and bars in the figures shows standard error (S.E.) of the means with four individual replicates. Means of significant difference were separated at the 0.05 probability level by the least significant difference test [26].

3. Results

3.1. Plant growth

Under various treatments of Cd/Zn, or Cd + Zn, hyperaccumulating ecotype of *S. alfredii* grew normally without showing any toxic symptoms, while in case of non hyperaccumulating ecotype, growth was significantly inhibited and plants showed toxic symptoms like small/wilting leaves and putrescence on root tip after 4 d of treatment with Cd/Zn, or Cd + Zn. Dry weight of NHE plants grown under 100 μM Cd, 500 μM Zn, or 100 μM Cd + 500 μM Zn was significantly decreased compared to the control, in which Cd + Zn combined treatment decreased the biomass of root, stem and leaf by 59.8, 49.1 and 48.2%, respectively (Table 2). However, the dry weight of HE was not decreased at any treatment level of Cd, Zn, or Cd + Zn. An increase in root biomass was recorded in HE under Zn and Cd + Zn treatment where it was increased by 28.3 and 21.7%, respectively, as compared to the control.

No differences were recorded in the relative root growth rate (RGR) in both ecotypes of *S. alfredii* grown in basal nutrient solution (0–15 d), whereas in following growth period (15–30 d), addition of 100 μM Cd reduced the daily increment of root biomass in both ecotypes, but decrease was higher in case of NHE than HE, and the differences were significant as compared with the first growth period (0–15 d). In the third growth period (30–45 d), however, the daily increment of root biomass in HE was increased by adding 500 μM Zn, whereas in case of NHE, it was decreased significantly as compared to the second growth period in 100 μM Cd (Fig. 1).

3.2. Root morphology

Data of the specific root length (SRL), specific root surface area (SRA), specific root volume (SRV) and average root diameter (ARD) are presented in Table 3. The SRL in NHE under the treatment of Cd/Zn, or Cd + Zn was significantly reduced by 50.4, 47.2, and 56.6%, respectively, as compared to the control. However, in case of HE, the SRL was increased under the treatment of 500 μM Zn or 100 μM Cd + 500 μM Zn but was not affected by 100 μM Cd. The effect of Cd and Zn on SRA and SRV on both ecotypes was similar to those on SRL. The SRA and SRV increased significantly in HE under 500 μM Zn or 100 μM Cd + 500 μM Zn, whereas in NHE, the SRA and SRV decreased significantly under the treatment of 100 μM Cd, 500 μM Zn, or 100 μM Cd + 500 μM Zn. For ARD, no such differences were observed among the treatments in NHE, however, in case of HE, ARD was increased by 22.8% in 500 μM Zn treatment as compared to the control (Table 3).

3.3. Root diameter distribution

Figs. 2 and 3 show SRL and SRA under each diameter class. For HE, the SRL was mainly constituted by the diameter classes 3 and 4 (0.2–0.3 and 0.3–0.4 mm) under each treatment (Fig. 2). No such differences were recorded among the treatments in all diameter

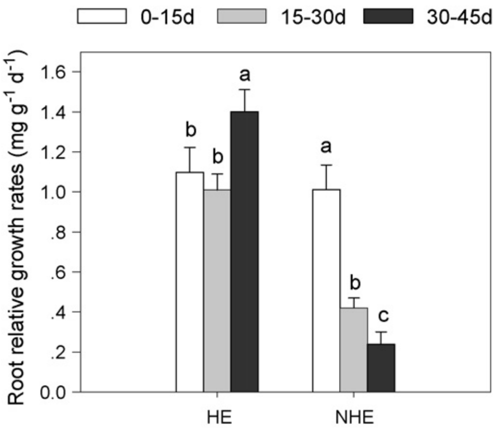

Fig. 1. Relative root growth rate of both ecotypes of *S. alfredii* during the first growth period in basal nutrient solution (0–15 d), second growth period in 100 μM Cd (15–30 d) and third growth period in 100 μM Cd + 500 μM Zn (30–45 d). Bars represent standard error (S.E.) of four replicates. Different letters among growth period indicate significant differences at $P < 0.05$ as determined by the Duncan's Multiple Range Test.

Table 3
Specific root lengths (SRL), specific root surface areas (SRA), average root diameters (ARD) and specific root volumes (SRV) of the both ecotypes of *S. alfredii* treated with 100 μM Cd, 500 μM Zn and100 μM Cd + 500 μM Zn for 30 d.

Treatments	SRL (cm^2 plant^{-1})		SRA (cm^2 plant^{-1})		ARD (mm)		SRV (cm^3 plant^{-1})	
	HE	NHE	HE	NHE	HE	NHE	HE	NHE
CK	468.5 ± 29.4b	438.1 ± 40.3a	149.0 ± 12.8b	150.5 ± 26.7a	0.35 ± 0.04b	0.23 ± 0.04a	2.78 ± 0.20b	2.85 ± 0.38a
Cd100	449.2 ± 34.6b	265.3 ± 20.8b	150.0 ± 20.5b	92.6 ± 11.3b	0.36 ± 0.02b	0.24 ± 0.03a	2.85 ± 0.22b	1.71 ± 0.16b
Zn500	527.2 ± 59.3a	261.0 ± 29.9b	175.8 ± 14.3a	91.9 ± 10.2b	0.43 ± 0.05a	0.27 ± 0.02a	3.48 ± 0.46a	1.67 ± 0.19b
Cd100 + Zn500	507.1 ± 44.6a	190.1 ± 16.4c	168.6 ± 17.4a	66.6 ± 6.3c	0.38 ± 0.03b	0.26 ± 0.03a	3.38 ± 0.31a	1.36 ± 0.08c

Data are means of four replicates. Values in each column followed by the same letter are not significant different at $P < 0.05$ as determined by the Duncan's Multiple Range Test.

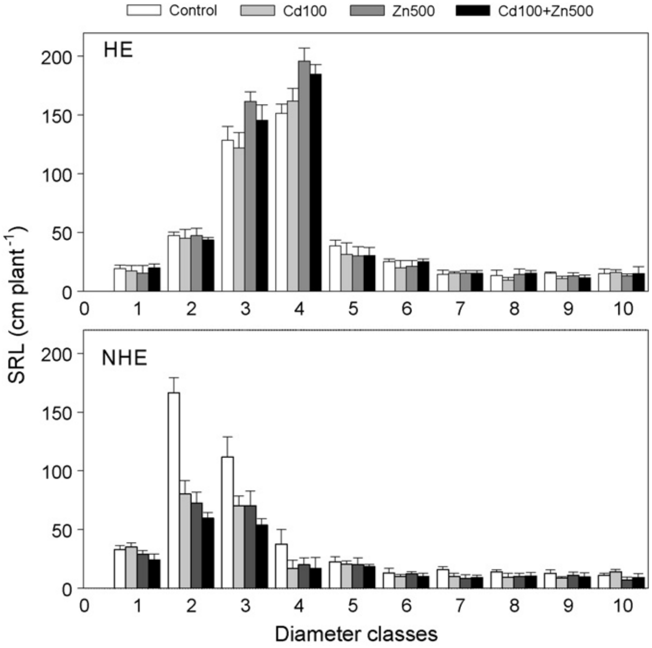

Fig. 2. Root diameter distribution of specific root length (SRL) for both ecotypes of *S. alfredii* treated with 100 μM Cd, 500 μM Zn and 100 μM Cd + 500 μM Zn each for 30 d. Bars represent standard error (S.E.) of four individual replicates.

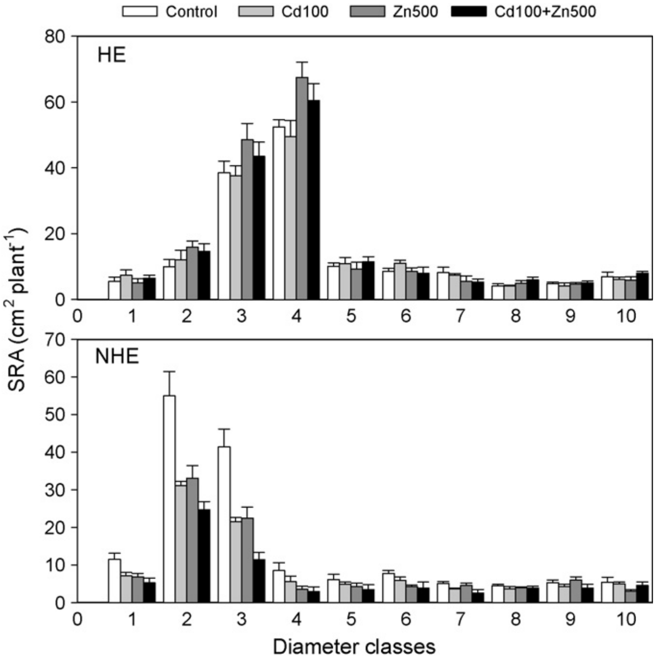

Fig. 3. Root diameter distribution of specific root surface area (SRA) for both ecotypes of *S. alfredii* treated with 100 μM Cd, 500 μM Zn and 100 μM Cd + 500 μM Zn for 30 d. Bars represent standard error (S.E.) of four individual replicates.

classes except in class 3 and 4. In the diameter class 3 and 4, SRL was significantly increased under 500 μM Zn or 100 μM Cd + 500 μM Zn treatment, as compared with control. In case of NHE, the diameter class 2 and 3 (0.1–0.2 and 0.2–0.3 mm) contributed more to SRL under each treatment. In contrast to HE, SRL decreased significantly in the diameter class 2, 3 and 4 respectively, under all treatments in NHE, as compared to control (Fig. 2). In NHE, similar pattern was also recorded for SRA (Fig. 3). In case of HE, the differences in diameter class 3 and 4 were significant and SRA values was much higher under 500 μM Zn treatment than those in the control. However in NHE, the metal treatment affected the SRA negatively and SRA in diameter classes 2, 3 and 4 decreased significantly under all metal treatments, but no such difference were recorded in the remaining classes (Fig. 3).

3.4. *Cadmium and zinc uptake and translocation*

The net uptake rate (NUR) of Cd in *S. alfredii* was very low and there was no difference between the both ecotypes when grown in basal nutrient solution (0–15 d). The Cd NUR markedly increased during the second growth period under the treatment of 100 μM Cd (15–30 d), but increase in HE was higher than NHE. In the third growth period (30–45 d), the Cd NUR in HE was significantly increased by adding 500 μM Zn ($P < 0.05$), whereas in case of NHE it was decreased (Fig. 4a). A similar pattern was recorded for the net translocation rate (NTR) of Cd (Fig. 5a). In HE, Cd NTR was increased by 27.5% by adding 500 μM Zn, as compared to the second growth period of 100 μM Cd addition, whereas in NHE, it was reduced by 33.3% during the third growth period.

The NUR of Zn in the HE was not influenced by adding 100 μM Cd (15–30 d), whereas it was significantly decreased in NHE, as compared to the first growth period in basal nutrient solution. During the third growth period (30–45 d), the NUR of Zn increased markedly in HE, whereas it was slightly increased in NHE by adding 500 μM Zn (Fig. 4b). In case of HE, the NTR of Zn increased significantly by adding 500 μM Zn to the nutrient solution containing 100 μM Cd. However, in NHE the NTR of Zn was reduced by 29.8% during the second growth period under 100 μM Cd, as compared with the first growth period, and was not affected in the third growth period where 500 μM Zn was added to the nutrient solution (Fig. 5b).

3.5. *Metal accumulation in S. alfredii*

Cadmium accumulation in both ecotypes of *S. alfredii* increased significantly with increasing Cd concentrations in the medium. Cadmium concentrations were much higher in both ecotypes grown under 100 μM Cd or 100 μM Cd + 500 μM Zn than those in the control. Cadmium accumulation in leaf and stem of HE were 8.0 and 6.6 times higher under 100 μM Cd treatment, and were 15.6 and 12.4 times higher under 100 μM Cd + 500 μM Zn combined treatment respectively, than those in the NHE (Fig. 6). This indicates that HE had much higher ability to uptake Cd from the growth medium

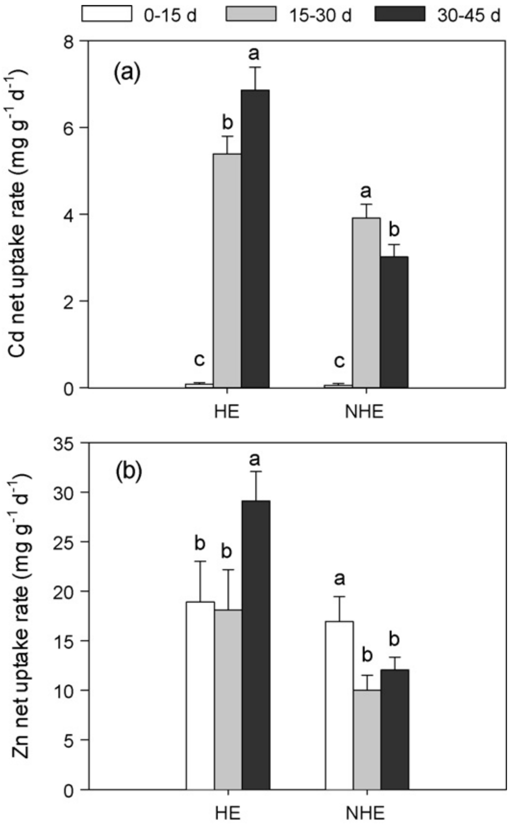

Fig. 4. Cadmium (a) and zinc (b) net uptake rates in both ecotypes of *S. alfredii* during the first growth period in basal nutrient solution (0–15 d), second growth period in 100 μM Cd (15–30 d) and third growth period in 100 μM Cd + 500 μM Zn (30–45 d). Bars represent standard error (S.E.) of four replicates. Different letters among growth periods indicate significant differences at $P < 0.05$ as determined by the Duncan's Multiple Range Test.

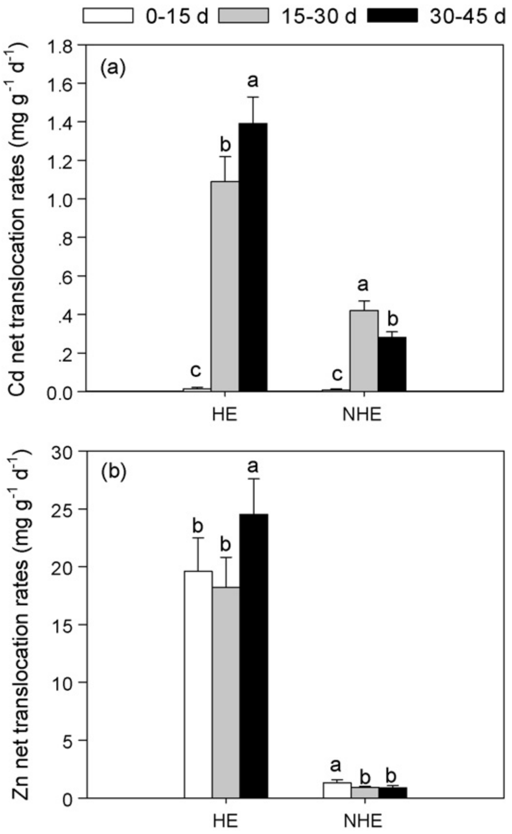

Fig. 5. Cadmium (a) and zinc (b) net translocation rates in both ecotypes of *S. alfredii* during the first growth period in basal nutrient solution (0–15 d), second growth period in 100 μM Cd (15–30 d) and third growth period in 100 μM Cd + 500 μM Zn (30–45 d). Bars represent standard error (S.E.) of four replicates. Different letters among growth periods indicate significant differences at $P < 0.05$ as determined by the Duncan's Multiple Range Test.

and transports it to shoots. Under Cd + Zn combined treatment, Cd accumulation in the stem of the HE was increased significantly as compared to Cd treatment alone which indicates that Cd uptake and transport in the HE is enhanced by Zn addition.

Zinc accumulation in the leaf and stem of HE were 25.5 and 26.4 times higher under 500 μM Zn treatment, and were 22.4 and 26.7 times higher under 100 μM Cd + 500 μM Zn combined treatment, respectively, than those in NHE (Fig. 7). In case of control, Zn accumulation in leaf and stem of the HE were 37.6 and 32.8 times higher than those of the NHE. However, the root Zn concentrations in the HE were lower than that of the NHE when plants were grown under Zn and Cd + Zn combined treatment (Fig. 7). Zinc accumulation in the plant parts was decreased in the order: stem > leaf > root in case of the HE, while the order was: root > stem > leaf for the NHE. The results imply that the hyperaccumulating ecotype of *S. alfredii* has an extraordinary ability to uptake and transport Zn to the shoots.

4. Discussion

The impact of heavy metals on plant growth, particularly root growth, has been reported by various authors [27–30]. Apart from the effects on root biomass production, roots can also respond to metal stress via changes in root growth pattern and morphology. In our previous studies on *S. alfredii*, it was noted that root length, surface-area and volume of the hyperaccumulating ecotype increased obviously under 1224 μM Zn or 200 μM Pb + 1224 μM Zn treatments, whereas in non-hyperaccumulating ecotype these parameter were decreased significantly [16]. Results from the present study showed that the impact of Cd and Zn on root morphology of *S. alfredii* also varied with ecotype. SRL, SRA and SRV in HE increased obviously under Zn or Cd/Zn combined treatments. However, in NHE, SRL, SRA and SRV decreased significantly with all metal treatments (Table 3). Changes in root morphology of *S. alfredii* also became evident in the diameter class distribution of SRL and SRA (Figs. 2 and 3). For the HE, the SRL and SRA were mainly constituted by the relatively coarser roots (0.2 mm < diameter < 0.4 mm), and these coarser roots increased significantly under 500 μM Zn or 100 μM Cd + 500 μM Zn treatment. However, in the NHE, fine roots (0.1 mm < diameter < 0.2 mm) contributed more towards SRL and SRA. Plants of the NHE grown under 100 μM Cd, 500 μM Zn, or 100 μM Cd + 500 μM Zn had considerably shorter fine roots (0.1 mm < diameter < 0.2 mm), as compared to the control. All these contrasting root morphological responses of both ecotypes to metal treatments might be partially responsible for their dissimilar abilities to tolerate and hyperaccumulate Zn and Cd.

Several environmental factors are reported to influence root morphology. These factors include metal stress [16], mechanical impedance [31], soil moisture conditions [32], as well as temperature and nutrient availability [33]. In our study, Zn and Cd concentrations were the main factors that influenced the root morphology. The SRL, SRA and SRV of HE plants treated with 100 μM Cd, 500 μM Zn, or 100 μM Cd + 500 μM Zn were much higher than NHE. As a result, Zn and Cd concentrations in the leaves and stems of HE were much higher than those of NHE. These results indicate that the roots of HE are not susceptible to the toxic effects of heavy metal and root morphological parameters have a significant positive correlation with Cd and Zn concentrations in the leaves and stem of *S. alfredii*, since larger specific root length and sur-

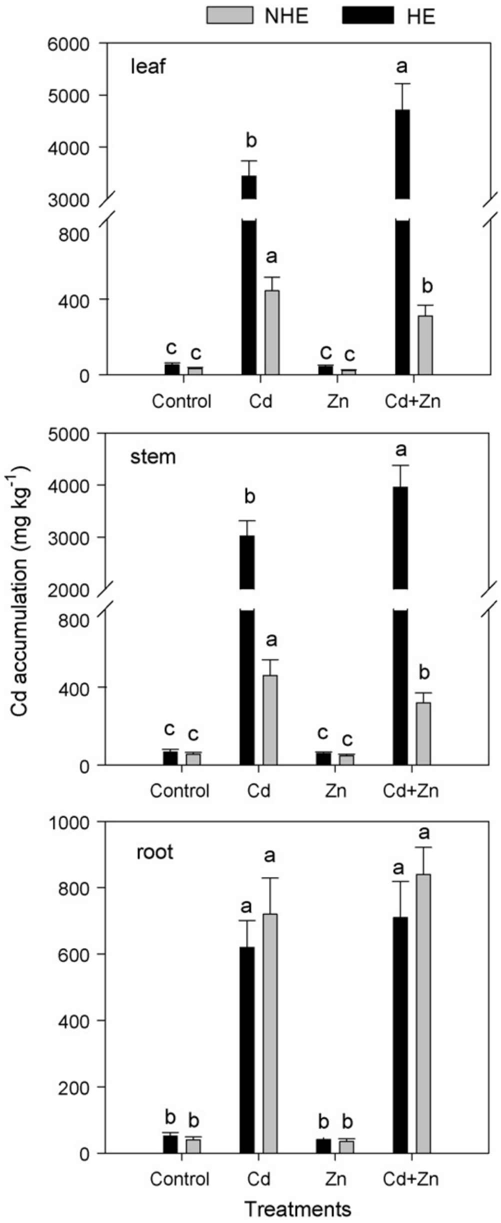

Fig. 6. Cadmium accumulation in both ecotypes of *S. alfredii* treated with 100 μM Cd, 500 μM Zn and 100 μM Cd + 500 μM Zn for 30 d. Bars represent standard error (S.E.) of four replicates. Different letters among the treatments indicate significant differences at $P < 0.05$ as determined by the Duncan's Multiple Range Test.

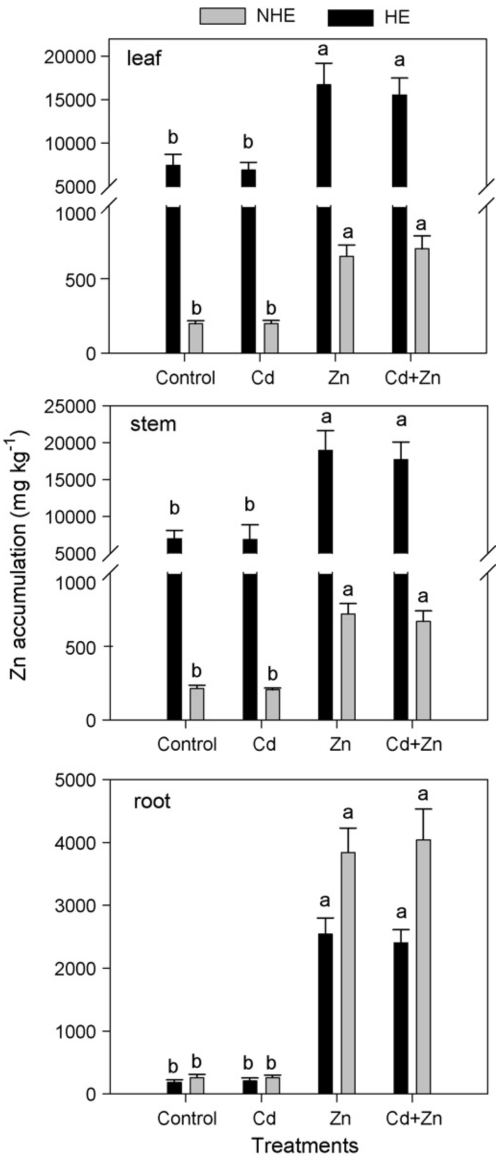

Fig. 7. Zinc accumulation in both ecotype of *S. alfredii* treated with 100 μM Cd, 500 μM Zn and 100 μM Cd + 500 μM Zn for 30 d. Bars represent standard error (S.E.) of four replicates. Different letters among the treatments indicate significant differences at $P < 0.05$ as determined by the Duncan's Multiple Range Test.

face are reported to offer more space for the metal uptake [12]. These results are in agreement with the previous results that Zn and Pb concentrations in *S. alfiedii* were positively correlated with root length, root surface area or root volumes [16]. All these results demonstrate that HE of *S. alfredii* could maintain large and widely branched root system under contaminated conditions and has great potential for its application in the large-scale phytoremediation of complex heavy metal polluted soils. Considering the potential use of *S. alfredii* for phytoremediation, what are the implications of root morphological changes? Roots are capable of producing plant hormones and consequently perturbations in the environment and thus root growth can influence plant development. Roots change physically and physiologically with age and environment, accompanied by great differences in the substances exuded by different root parts [34]. Consequently, changes in root morphology can influence the activation of heavy metals in the rhizosphere and subsequent uptake by roots. A selection criterion for plant species for phytoremediation is a fine, widely branched root system [35]. This trait should be maintained under contaminated conditions as can be expected from the HE *S. alfredii*.

Studies on the interactions between Cd and Zn ions so far were focused on the conventional plant species and little information was available about the Cd–Zn interactions in the hyperaccumulators. Some authors have suggested that interactive pattern is antagonistic, whereas others argued that it is synergistic and Cd toxicity is enhanced by Zn addition [36]. Results from our present study indicate that the interactions between Zn and Cd on the growth of *S. alfredii* largely depend on the plant ecotype. For NHE, root dry weight decreased significantly due to Cd, Zn or Cd + Zn combined treatment, but the decrease was much greater under the combined treatment of 100 μM Cd + 500 μM Zn (Table 2). Furthermore, relative root growth rates were decreased significantly by adding 500 μM Zn, as compared to the second growth period in 100 μM Cd (Fig. 1). These results indicate that the interactive pattern of Cd and Zn in NHE root is synergistic and Cd toxicity is enhanced by adding 500 μM Zn. However, in case of HE, root growth was promoted by 500 μM Zn or 100 μM Cd + 500 μM Zn treatment (Table 2), and relative root growth rates increased significantly by adding 500 μM Zn as compared to the first two growth periods, but it was not influenced under 100 μM Cd treatment compared to the first growth period in basal nutrient solution (Fig. 1). These results indicate that HE has an extraordinary ability to co-tolerate Zn and Cd, but Zn was the dominant factor influence HE growth and the interactions between Cd and Zn has minimal influence on HE growth. Interactions between heavy metals are reported to affect both root growth, ion uptake and its transport from root to shoot [37,38]. In our previous studies, we found that Pb concentrations in the stem of HE plants treated with 200 μM Pb + 1224 μM Zn were much higher than those treated with 200 μM Pb suggesting that Zn stimulated the Pb uptake and subsequent translocation to the shoot [16]. The results of our present study showed that net uptake rate and translocation rate of Cd in HE increased significantly by adding 500 μM Zn (Figs. 4 and 5), and as a result, Cd concentrations in the stem and leaf of HE treated with Cd + Zn were higher (4710 mg kg^{-1} and 3960 mg kg^{-1}, respectively) than those treated with 100 μM Cd alone (3440 mg kg^{-1} and 3020 mg kg^{-1}, respectively) (Fig. 6). These results imply that 500 μM Zn also stimulate Cd uptake and subsequent translocation to shoot in HE, which may be attributed to an altered and specialized transporter of metal ions in the plasma membrane system induced by addition of Zn [5,39–41]. Lasat et al. [42] have cloned a high affinity Zn transporter (ZNTI) that is over expressed in *Thlaspi caerulescens*, and demonstrated that ZNTI mediates Zn uptake as well as that of Cd uptake with a much lower affinity. Recently, Zhao et al. [43] found that *Arabidopsis halleri* is able to hyperaccumulate Cd partly through the Zn pathway. In contrast to HE, Cd net uptake and translocation rate in NHE decreased significantly by adding 500 μM Zn (Figs. 4 and 5), and this treatment caused a significant reduction in the Cd concentrations of NHE shoots (Fig. 6), which might be resulted from the competitive transport and absorption interactions between these two metal ions [37].

The results of present study showed that the interactions of Zn and Cd in *S. alfredii* are different from the normal crop plants and varied greatly between the two ecotypes. Strong positive interactions between Zn and Cd were noted under 100 μM Cd + 500 μM Zn combined treatment in HE, and Cd uptake and translocation was enhanced by adding 500 μM Zn. On the other hand, the effect of Cd and Zn interactions on the root growth in NHE was synergistic and Cd toxicity was exasperated by adding 500 μM Zn. Furthermore, 500 μM Zn inhibited the uptake of Cd, likely due to the competition between Cd and Zn. The hyperaccumulating ecotype of *S. alfredii* could maintain large and widely branched root system and thus has great potential for its application in the large-scale phytoremediation of complex heavy metal polluted soils.

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