



## Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance)

X.E. Yang<sup>1,3</sup>, X.X. Long<sup>1</sup>, H.B. Ye<sup>1</sup>, Z.L. He<sup>1,2</sup>, D.V. Calvert<sup>2</sup> & P.J. Stoffella<sup>2</sup>

<sup>1</sup>College of Natural Resources and Environmental Sciences, Zhejiang University, Huajiachi Campus, Hangzhou 310029, China. <sup>2</sup>University of Florida, Indian River Research & Education Center, Fort Pierce, FL34945, USA.

<sup>3</sup>Corresponding author\*

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### Abstract

*Sedum alfredii* Hance has been identified as a new zinc (Zn) hyperaccumulating plant species. In this study, the effects of cadmium (Cd) supply levels (control, 12.5, 25, 50, 100, 200, 400, 800  $\mu\text{mol Cd L}^{-1}$ ) on the growth and cadmium accumulation and Zn supply on Cd accumulation in *S. alfredii* Hance were studied. The results showed that no reduction in shoot and root dry matter yields were noted when the plants were grown at Cd supply levels up to 200  $\mu\text{mol L}^{-1}$  in nutrient solution. Slight stimulation on shoot growth was noted at relatively low Cd levels (25 to 100  $\mu\text{mol L}^{-1}$ ). Cadmium concentrations in leaves and stems increased with increasing Cd supply levels, and reached a maximum of approximately 9000 and 6500  $\text{mg kg}^{-1}$  (DW) at 400  $\mu\text{mol Cd L}^{-1}$ , respectively. Root Cd concentration increased sharply only at relatively high Cd levels. Cadmium distribution in different parts of the plant was in the order: leaf > stem  $\gg$  root. The amount of Cd accumulated in the shoots reached 2.9 and 3.2  $\text{mg plant}^{-1}$  at external Cd levels of 200 and 400  $\mu\text{mol L}^{-1}$ , respectively. The shoot/root Cd ratios were greater than 2 and more than 95% of the total Cd taken up by *S. alfredii* was translocated to the shoots at the external Cd levels  $\leq 200 \mu\text{mol L}^{-1}$ . The concentrations of P, Ca, Mg, B, Fe, Mn, Cu, and in the shoots and roots were influenced differentially by Cd treatments. High Zn supply (500  $\mu\text{mol L}^{-1}$ ) enhanced Cd concentrations in the leaves and stems at the Cd levels  $\leq 100 \mu\text{mol L}^{-1}$ , and root Cd concentration at the Cd levels  $\leq 50 \mu\text{mol L}^{-1}$ . These results indicate that *S. alfredii* has an extraordinary ability to tolerate and hyperaccumulate Cd and this is the first report of the new Cd hyperaccumulator *S. alfredii* Hance. The finding of Cd/Zn hyperaccumulation in *S. alfredii* Hance provides an important plant material for understanding the mechanisms of Cd/Zn co-hyperaccumulation and for phytoremediation of the heavy metal contaminated soils.

### Introduction

Cadmium (Cd) is a highly toxic metal, and has been ranked number 7 among the top 20 toxins mainly due to its negative influence on the enzymatic systems of cells (Al-Khedhairi et al., 2001; Sanità et al., 1999). Large areas of land in many countries have been contaminated by cadmium and other heavy metals due to the application of sludge or urban composts, pesticides, fertilizers, emissions from waste incinerators,

waste water irrigation, residues from metalliferous mining, and the metal smelting industry (McGrath et al., 2001; Reeves and Baker, 2000; Yang et al., 2002b). Cadmium contamination in agricultural soils is unlikely to affect plant growth, however, as Cd is easily transferred to human food chain from the soils, Cd contamination is a great threat to human health. These effects limit the marketing of agricultural products and reduce the profitability of the agricultural industry. The residence time of Cd in soil is over thousands years (Alloway, 1995). Phytoremediation is a novel technique to clean up contaminated soils using green plants, which offers the benefits of

\*FAX No: +571-8604-9815.

E-mail: xyang@zju.edu.cn; xyang@mail.ifas.ufl.edu

being *in situ*, low cost, and environmentally sustainable (Salt et al., 1998; Long et al., 2002b). Identification of new Cd hyperaccumulators, especially multiple metal hyperaccumulating plant species, is of great importance to the successful phytoremediation of the contaminated soils with Cd and other metals, because contamination is seldom restricted to a single metal.

Plant tolerance to heavy metals has different mechanisms, and may have been through the two basic strategies: metal exclusion and metal accumulation (Baker, 1981, 1987). The term hyperaccumulator has come to mean a plant capable of taking up concentrations of trace metals approximately 100 times greater than normal species. To date, more than 400 species of hyperaccumulators belonging to 45 families have been identified, of which about 18 species are Zn-hyperaccumulators (Reeves and Baker, 2000). Hyperaccumulation of Cd is a rare phenomenon in higher plants. So far, only *Thlaspi caerulescens* J & C. Presl (*Brassicaceae*) (Baker et al., 2000; Zhao et al., 2002) and *Arabidopsis halleri* (L.) O' Kane & Al-Shehbaz (Brooks, 1998; Küpper et al., 2000) have been identified as Cd-hyperaccumulators.

*Sedum alfredii* Hance is a new Zn-hyperaccumulator found in an old Zn/Pb mining area in China (Yang et al., 2002a). Zinc concentration in its shoots can reach over 20 g kg<sup>-1</sup> when grown at 80 mg Zn L<sup>-1</sup> in nutrient solution without showing any toxic symptoms (Yang et al., 2002a). In addition, it has characteristics of fast growth, large biomass, asexual reproduction, and perennial. The plants of *S. alfredii* Hance can grow up to 40 cm height, propagate 3-4 times in a year if the environmental conditions are favorable (Yang et al., 2001). The objectives of this study were to examine the growth response and Cd uptake, distribution, and accumulation at varied Cd supply levels as well as the effects of Zn levels on Cd accumulation in *S. alfredii* Hance in nutrient solution.

## Materials and methods

### Plant culture

*Experiment 1. Growth response and Cd accumulation at different Cd supply levels.* The plant materials of *S. alfredii* Hance were collected from an old Pb/Zn mining area in Quzhou city of Zhejiang province, China. Plant stems were cleaned with tap water and grown in 3-L plastic containers in the greenhouse. The residual Cd concentration in the stems (shoots)

was 456 mg kg<sup>-1</sup>. The composition of the nutrients in solution were (in mmol L<sup>-1</sup>): Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O 2.0, KH<sub>2</sub>PO<sub>4</sub> 0.10, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.50, KCl 0.10, K<sub>2</sub>SO<sub>4</sub> 0.70, and (in μmol L<sup>-1</sup>): H<sub>3</sub>BO<sub>3</sub> 10.00, MnSO<sub>4</sub>·H<sub>2</sub>O 0.50, ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.50, CuSO<sub>4</sub>·5H<sub>2</sub>O 0.20, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> 0.01, Fe-EDTA 100. The treatments were composed of control (without addition of Cd), 12.5, 25, 50, 100, 200, 400, 800 μmol Cd L<sup>-1</sup>, and cadmium was supplied as CdCl<sub>2</sub>. Cadmium concentration in nutrient solution of the control was detected at 0.002 μmol L<sup>-1</sup> from the impurity of the used chemicals. The seedlings of *S. alfredii* Hance were precultured for 14 days for the initiation of the new roots before they were exposed to different Cd levels. The shoot and root dry weights were 361 and 22 mg plant<sup>-1</sup> before the plants were exposed to Cd treatments. Each container had 14 plants. A randomly complete block design was used with each treatment replicated three times. The nutrient solution was aerated and replaced every four days, and the pH was maintained at 5.8 by daily adjustment with 0.1 mol L<sup>-1</sup> HCl or 0.1 mol L<sup>-1</sup> NaOH. Plants were grown under controlled glasshouse conditions at a temperature of 27 ± 3 °C, and relative humidity of 70 ± 3%.

*Experiment 2. Effect of Zn levels on Cd uptake and accumulation of S. alfredii Hance.* The plants of *S. alfredii* Hance were pre-cultured as described in the Experiment 1. Then they were exposed to different Cd/Zn complex levels. The treatments were composed of Cd supply levels of control (without addition of Cd), 25, 50, 100, 400 (μmol L<sup>-1</sup>) and Zn levels of 50, 500 (μmol L<sup>-1</sup>), respectively. Cadmium and Zn were applied as chloride and sulfate, respectively. The nutrient solution, growth conditions and their management are the same as those described in Experiment 1.

### Plant sampling, chemical and data analysis

The experiments were terminated after the plants had been exposed to metal treatments for 21 days. At harvest, the maximum root length and shoot height was recorded. Roots of intact plants of each container were rinsed with tap water, and immersed in 20 mM Na<sub>2</sub>-EDTA (disodium ethylenediaminetetraacetate) for 15 min to remove Cd adhering to the root surfaces (Yang et al., 1996). The plant samples were separated into leaves, stems, and roots. The bases of stems and roots were rinsed thoroughly with deionized water, blotted dry, and then oven dried at

60 °C. Fresh weight (FW) and dry weight (DW) of different plant parts were recorded. Samples of plant dry materials were ground with stainless steel mill and passed through a 0.25 mm sieve prior to analysis.

A portion (0.500 g) of plant samples was wet-digested in a high-purity acid mixture (HNO<sub>3</sub>:HClO<sub>4</sub>, 5:1) at 200–220 °C. Concentrations of Cd, Zn and other elements in the solutions were determined using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; IRIS/AP).

Analysis of variance (ANOVA) was performed on all the data from Experiment 1, and the least significant difference (LSD,  $P < 0.05$ ) was used for multiple comparisons between the treatment means. The data from Experiment 2 were analyzed using T TEST of SAS program.

## Results

### Response of plant growth to different Cd levels

Plant growth of *S. alfredii* Hance was normal at the Cd levels  $\leq 200 \mu\text{mol Cd L}^{-1}$ . However, visual Cd toxicity symptoms with necrosis and browned root tip were observed on the roots of *S. alfredii* Hance grown for 4 days at external Cd level of  $400 \mu\text{mol Cd L}^{-1}$ . The toxicity symptoms became more severe with increasing Cd levels and exposure time. The leaves of *S. alfredii* Hance wilted after the plants were grown for 4 days, and the old leaves began to fall off after grown for 16 days at  $800 \mu\text{mol Cd L}^{-1}$ .

Reduced shoot height was noticed when plants were grown at Cd levels  $\geq 200 \mu\text{mol L}^{-1}$ , whereas, shoot fresh and dry weights slightly increased when plants were grown at the Cd levels  $\leq 100 \mu\text{mol L}^{-1}$  (Table 1). As compared with the control, the increase in shoot fresh and dry weights was statistically significant ( $P < 0.05$ ) at Cd supply levels of 25–100  $\mu\text{mol L}^{-1}$ . No reduced shoot fresh and dry weights were observed even grown at Cd levels of 200  $\mu\text{mol L}^{-1}$ . However, shoot height and shoot dry matter yields decreased gradually with increasing Cd levels from 200 to 800  $\mu\text{mol L}^{-1}$ , as compared with the control (Table 1). Similar response patterns to Cd supply levels were noted between the shoot height and the shoot dry weight. The maximum root length decreased with increasing Cd supply levels from 50  $\mu\text{mol L}^{-1}$  and dramatically decreased at Cd levels higher than 400  $\mu\text{mol L}^{-1}$  (Table 1). The maximum root length grown at 800  $\mu\text{mol Cd L}^{-1}$  was

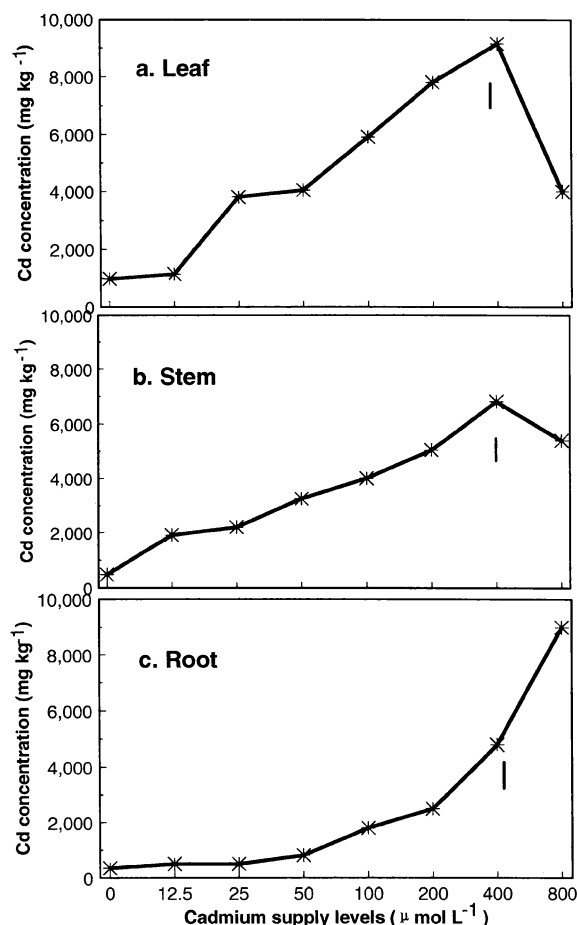


Figure 1. Cadmium concentrations in the leaves, stems, and roots of *Sedum alfredii* Hance grown at different Cd supply levels. Plants were exposed to Cd for 21 days. All the data are means of 3 replications, and the bar depicts the least significant difference (LSD<sub>0.05</sub>).

only about 48% of the control. However, more side roots were grown at Cd levels from 25–200  $\mu\text{mol L}^{-1}$ , and there was no obvious decreases in root fresh and dry weights of *S. alfredii* Hance when the plants were grown at the Cd levels  $\leq 200 \mu\text{mol L}^{-1}$  (Table 1). It appears that *S. alfredii* Hance can maintain optimal growth at Cd levels as high as 100  $\mu\text{mol L}^{-1}$  in term of both shoot and root dry matter production, although the longest (maximum) root length reduced significantly at the Cd levels  $> 50 \mu\text{mol L}^{-1}$ . These results indicate that *S. Alfredii* Hance has high tolerance to Cd toxicity and its shoot growth can be slightly enhanced by Cd supply at suitable levels.

Table 1. Effects of Cd supply levels on the growth of *Sedum alfredii* Hance

Cd levels ( $\mu\text{mol L}^{-1}$ )	Plant height (cm)	Maximum root length (cm)	Fresh weight (g plant $^{-1}$ )		Dry weight (mg plant $^{-1}$ )	
			Shoot	Root	Shoot	Root
CK	18.9	19.4	7.38	0.87	423.45	51.23
12.5	18.9	17.9	8.84	0.77	496.16	52.35
25	19.9	17.6	9.72	0.87	527.38	52.15
50	19.4	15.5	8.98	0.80	498.76	51.89
100	19.5	16.0	9.28	0.79	522.33	56.34
200	17.0	16.4	7.33	0.75	437.35	50.23
400	16.5	15.7	5.89	0.74	391.28	49.89
800	16.1	9.3	5.00	0.20	373.56	19.87
Sig. <sup>#</sup>	**	***	***	***	***	***
LSD <sub>0.05</sub>	1.73	1.90	1.042	0.157	76.09	9.34
LSD <sub>0.05</sub>	2.40	2.61	1.522	0.216	101.46	12.56

Sig.<sup>#</sup>-significant levels: \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

#### Cadmium concentration and accumulation at different Cd supply levels

The Cd concentration in the leaves and stems of *S. alfredii* Hance increased sharply with increasing external Cd supply levels, peaked at  $400 \mu\text{mol L}^{-1}$ , and then decreased with further increasing Cd levels (Figure 1a, b). The maximum Cd concentrations in the leaves and stems reached  $9000$  and  $6500 \text{ mg kg}^{-1}$  (DW), respectively, when the plants were grown at  $400 \mu\text{mol Cd L}^{-1}$  for 21 days. Root Cd concentrations of *S. alfredii* Hance changed little when the plants were grown at the Cd supply levels less than  $50 \mu\text{mol L}^{-1}$ , but increased linearly with further increasing of Cd levels, especially from 200 to  $800 \mu\text{mol L}^{-1}$  in the nutrient solution (Figure 1c). At the Cd levels  $\leq 200 \mu\text{mol L}^{-1}$ , the Cd concentration in various parts of the plant decreased in the order: leaf  $>$  stem  $\gg$  root, whereas the opposite was found when the external Cd levels were higher than  $400 \mu\text{mol L}^{-1}$  (Figure 1). The Cd concentrations were approximately  $7800$ ,  $5600$ , and  $2300 \text{ mg kg}^{-1}$  in the leaves, stems, and roots, respectively, when the plants were grown at the Cd level of  $200 \mu\text{mol L}^{-1}$ . These results indicate that *S. alfredii* Hance has an extraordinary ability to absorb Cd and concentrate it in the stems, particularly in the leaves.

Similarly, Cd uptake by the shoots linearly increased with increasing Cd supply levels, peaked at the Cd level of  $400 \mu\text{mol L}^{-1}$ , and then dramatically decreased with further increasing external Cd levels (Figure 2a). The maximum amount of Cd taken

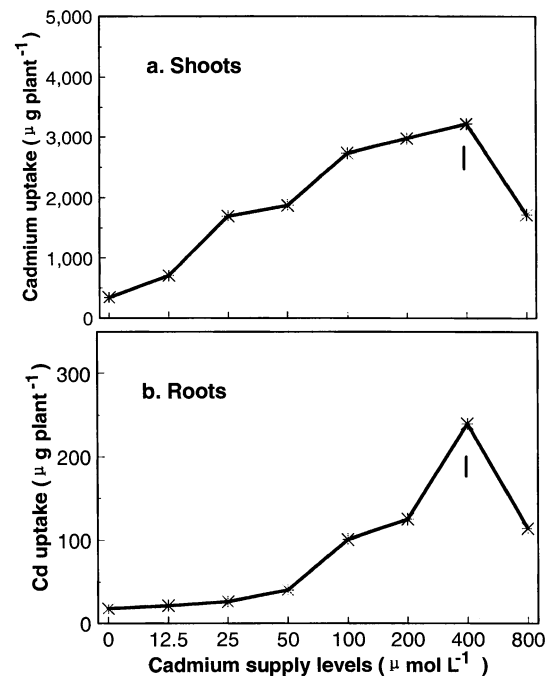


Figure 2. Cadmium uptake by shoots and roots of *Sedum alfredii* Hance at different Cd supply levels. All the data are means of 3 replications, and the bar depicts the least significant difference (LSD<sub>0.05</sub>).

up by the shoots was as high as  $3.2 \text{ mg plant}^{-1}$  grown at the external Cd level of  $400 \mu\text{mol L}^{-1}$ , and  $2.9 \text{ mg plant}^{-1}$  at the Cd level of  $200 \mu\text{mol L}^{-1}$ . The amount of Cd accumulated in the roots was small (only  $18\text{--}26 \mu\text{g plant}^{-1}$ ) when grown at the

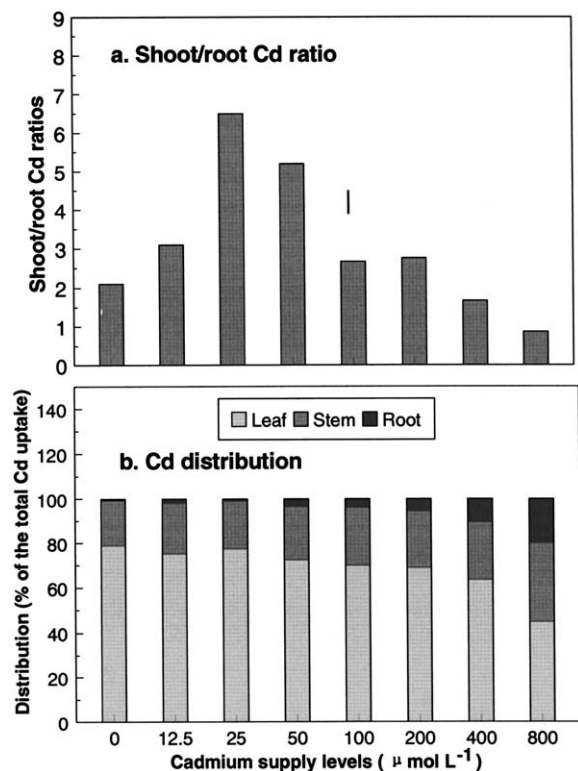


Figure 3. The ratios of Cd concentration of shoot to root (a) and the Cd distribution in various plant parts of *Sedum alfredii* Hance (b) grown at different Cd supply levels. All the data are means of 3 replications, and the bar depicts the least significant difference ( $\text{LSD}_{0.05}$ ).

Cd supply levels  $\leq 25 \mu\text{mol L}^{-1}$ , but increased dramatically with further increasing external Cd levels, and peaked at  $400 \mu\text{mol L}^{-1}$  (Figure 2b). High Cd ( $>400 \mu\text{mol L}^{-1}$ ) treatments considerably reduced Cd accumulation in the roots due to its toxic effects on root growth. The maximum amount of Cd taken up by the roots was  $240 \mu\text{g plant}^{-1}$ , only one tenth of that by the shoots (Figure 2). These results imply that *S. alfredii* Hance has an extraordinary ability to hyperaccumulate Cd in the shoots, and can provide a suitable plant material for phytoextraction of Cd in contaminated soils.

#### Cadmium distribution within the plant at different Cd levels

The distribution of Cd in the leaves, stems, and roots was significantly different among the Cd treatments. The ratios of Cd concentration of shoots to roots varied with Cd supply levels, and the greatest shoot/root Cd ratio was noted at the Cd level of  $25 \mu\text{mol L}^{-1}$

(Figure 3a). The values of Cd concentration ratios of shoots to roots were over 2 at the Cd supply levels  $\leq 200 \mu\text{mol L}^{-1}$ . The distribution of Cd in the leaves, stems, and roots differed also with Cd supply levels. The Cd distribution in the leaves decreased while that in the roots increased with increasing Cd levels from 100 to  $800 \mu\text{mol Cd L}^{-1}$  (Figure 3b). At the Cd levels  $\leq 100 \mu\text{mol Cd L}^{-1}$ , around 70% and 27% of the total Cd taken up by the plant were allocated in the leaves and stems, respectively, but only 3% in the roots. More than 95% of total Cd taken up by *S. alfredii* H. was accumulated in the shoots at the external Cd levels  $\leq 200 \mu\text{mol L}^{-1}$  (Figure 3b). These results indicate that leaf is the major part, followed by stem, for the hyperaccumulation of Cd in *S. alfredii* H.

#### Effects of Cd levels on other nutrient concentrations in the plant

The concentrations of other essential mineral nutrients in the leaves, stems and roots of *S. alfredii* H were significantly influenced by Cd treatments (Table 2). The concentrations increased for phosphorus (P), whereas decreased for manganese (Mn) when the plants were grown at the Cd levels  $\leq 400 \mu\text{mol L}^{-1}$ . Calcium concentration in the leaves was increased at Cd supply levels up to  $400 \mu\text{mol L}^{-1}$ . The concentrations of potassium (K), magnesium (Mg), and sulphur (S) were decreased in the roots, but were not affected in the leaves and stems of *S. alfredii* Hance with increasing Cd supply levels up to  $400 \mu\text{mol L}^{-1}$ . The concentrations of copper (Cu) and boron (B) were dramatically raised in the roots while decreased in the leaves at the external Cd higher than  $50 \mu\text{mol L}^{-1}$ . The root iron (Fe) concentrations increased over 2-fold with increasing Cd levels, whereas the Fe concentrations in the leaves and stems were fluctuated with increasing Cd levels. Zinc concentrations increased in the leaves but decreased in the roots by more than two times with increasing Cd supply levels up to  $400 \mu\text{mol L}^{-1}$ , whereas stem Zn concentrations remained unchanged for different Cd levels (Table 2). Remarkable decreases in the concentrations of all the nutrient elements except for Ca in the leaves and those of K, Mg, S, Cu, and Mn in the roots were noted in *S. alfredii* Hance only at very high Cd level ( $800 \mu\text{mol L}^{-1}$ ). These results imply that the tolerance of *S. alfredii* Hance is closely associated with its exceptional ability of maintaining balanced nutrition of essential elements in the plant. It appears that Cd

Table 2. Effects of Cd levels on the concentrations of K, Ca, Mg, P, S, and Zn ( $\text{g kg}^{-1}$  DW) and Fe, Mn, Cu, B ( $\text{mg kg}^{-1}$  DW) in the leaves, stems, and roots of *Sedum alfredii* Hance

Cd levels ( $\mu\text{M}$ )	K	Ca	Mg	P	S	Zn	Fe	Mn	Cu	B
	Leaves									
0	39.6	5.04	3.78	5.10	10.0	4.85	644	260	9.39	45.8
12.5	39.8	5.12	3.71	5.17	9.32	4.89	668	251	9.46	45.0
25	40.7	5.45	3.90	5.57	9.29	5.01	533	129	9.64	45.8
50	42.8	5.70	4.16	6.02	9.27	5.28	318	137	7.48	36.5
100	42.3	5.88	4.23	6.00	9.18	5.45	288	143	6.33	34.0
200	44.2	5.89	4.02	6.35	10.1	5.68	919	149	6.81	36.8
400	43.2	5.71	4.12	6.22	9.62	5.95	730	164	5.75	27.9
800	28.9	5.53	2.89	3.20	7.22	6.50	565	104	3.92	33.4
Sig. <sup>a</sup>	***	***	***	***	***	***	***	***	***	***
LSD <sub>0.05</sub>	4.24	0.182	0.313	0.563	0.560	0.225	88.5	32.4	1.085	3.12
	Stems									
$\mu\text{M Cd}$										
0	29.2	5.84	4.82	4.98	3.54	7.68	119	259	4.05	33.5
12.5	28.0	5.77	4.70	5.62	3.65	7.58	253	209	4.54	37.7
25	26.8	5.77	4.54	5.39	3.45	7.70	278	172	4.53	36.4
50	33.2	6.14	4.88	6.22	3.70	7.72	112	199	5.53	34.9
100	30.0	5.84	4.89	6.33	3.72	7.65	579	218	5.43	36.5
200	32.6	5.70	4.77	6.04	3.74	7.80	550	229	4.82	31.9
400	31.1	5.61	4.65	5.46	3.96	7.85	734	304	5.16	33.6
800	25.4	5.37	3.88	4.72	3.06	7.90	669	206	3.68	34.4
Sig. <sup>a</sup>	**	**	***	***	***	NS	***	***	***	NS
LSD <sub>0.05</sub>	3.68	0.285	0.236	0.600	0.318	0.387	70.1	57.4	0.691	4.00
	Roots									
$\mu\text{M Cd}$										
0	44.5	4.45	3.22	17.0	12.1	7.01	7940	721	13.5	107
12.5	39.7	5.16	3.22	18.6	10.1	5.51	10485	491	15.0	124
25	37.2	4.38	2.98	18.2	10.2	4.89	9868	421	16.5	103
50	39.4	4.28	2.86	21.8	10.0	3.52	13679	440	21.5	153
100	31.7	4.19	2.70	22.1	9.55	3.46	12952	277	31.7	181
200	34.8	4.44	2.67	23.1	11.6	2.91	14750	110	46.2	171
400	24.3	4.78	2.19	23.5	10.1	2.82	19406	96	78.7	217
800	8.29	6.40	1.87	22.9	7.44	5.01	23840	126	107	253
Sig. <sup>a</sup>	***	***	***	**	***	***	***	***	***	***
LSD <sub>0.05</sub>	2.54	0.515	0.214	3.60	1.30	0.658	1563	54.2	5.78	47.3

<sup>a</sup>Significant levels: NS, not significant, \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

depresses Zn absorption in the roots but enhanced Zn accumulation in the leaves of *S. alfredii* Hance.

#### Effects of Zn levels on Cd concentration and accumulation

The optimal growth and Zn hyperaccumulation in the shoots of *S. alfredii* H. occurred at the external Zn level of 500–1000  $\mu\text{mol L}^{-1}$  (Long et al., 2002a). Increasing Zn supply levels from 50 to 500 significantly increased Cd concentrations ( $P < 0.01$  by *t*-test) in the leaves and stems when the plants were grown at

the Cd supply levels  $\leq 100 \mu\text{mol L}^{-1}$ , and increased root Cd concentration ( $P < 0.05$  by *t*-test) when at the Cd supply levels  $\leq 50 \mu\text{mol L}^{-1}$  (Figure 4). However, there were no significant differences between the Cd concentrations in the stems and roots at 500  $\mu\text{mol Zn L}^{-1}$  and those at 50  $\mu\text{mol Zn L}^{-1}$ , if the overall effect at all Cd supply levels (CK-400  $\mu\text{mol L}^{-1}$ ) was considered. Nevertheless, the overall effect of high Zn treatment on the Cd concentrations of leaves was significant ( $P < 0.01$ ) when plants were grown at all Cd supply levels tested. Similarly, cadmium ac-

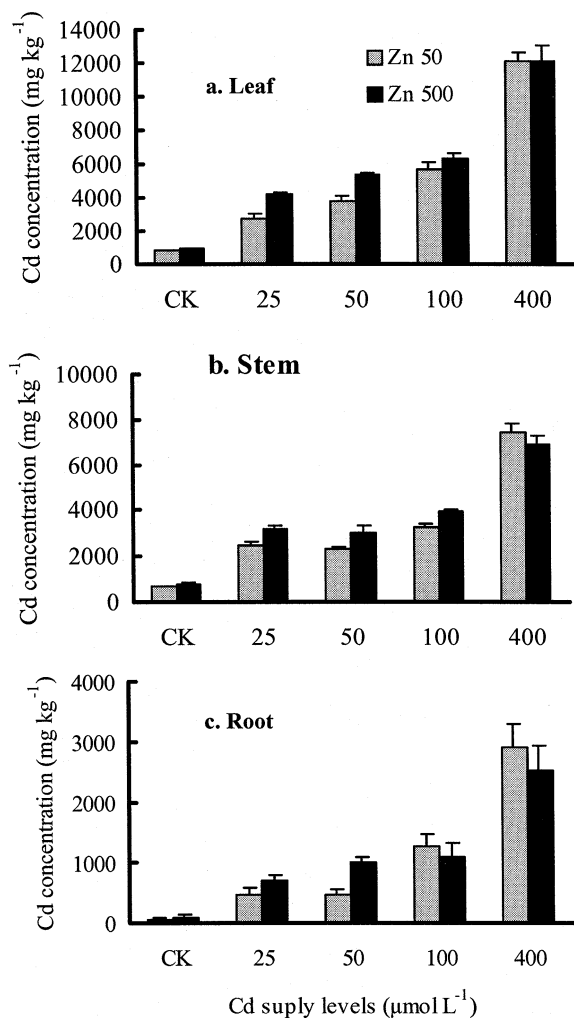


Figure 4. Effects of Zn supply on Cd concentrations in the leaves, stems, and roots of *Sedum alfredii* Hance. Zn50 and Zn500 refer to Zn supply levels of 50 and 500  $\mu\text{mol L}^{-1}$ , respectively. All the data are means of 3 replications, and the error bar depicts the SE.

accumulation in the shoots and roots was enhanced by high Zn supply. For instances, at the external Cd level of 100  $\mu\text{mol L}^{-1}$ , the amount of Cd taken up by the shoots reached to as high as 3.24  $\text{mg plant}^{-1}$  at high Zn (500  $\mu\text{mol L}^{-1}$ ), which was 58% higher than that at the low Zn (50  $\mu\text{mol L}^{-1}$ ) (Figure 5a). These results indicate that the enhancement of optimal Zn supply on Cd accumulation occurred only when plants were grown at sub-optimal Cd supply levels.

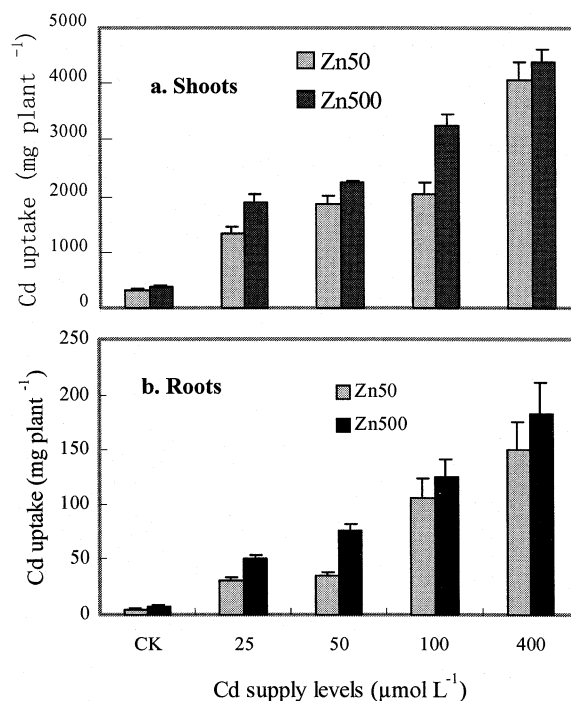


Figure 5. Effects of Zn supply on Cd accumulation in the shoots and roots of *Sedum alfredii* Hance. Zn50 and Zn500 refer to Zn supply levels of 50 and 500  $\mu\text{mol L}^{-1}$ , respectively. All the data are means of 3 replications, and the error bar depicts the SE.

## Discussion

Cadmium is highly toxic to plants. The critical Cd levels in nutrient solution for the conventional crop plants are reported to be 8  $\mu\text{mol L}^{-1}$  for white clover and maize, and 14  $\mu\text{mol L}^{-1}$  for cabbage, and 28  $\mu\text{mol L}^{-1}$  for ryegrass (Yang et al., 1995). The results from this study show that *S. alfredii* Hance has an exceptional ability to tolerate and hyperaccumulate Cd in terms of growth response to external Cd level and Cd concentration in the shoot tissues. The plants showed no visible symptoms of metal-induced toxicity and no reduced shoot and root dry matter yields were observed when plants were grown at the Cd levels up to 200  $\mu\text{mol L}^{-1}$ . Moreover, shoot fresh and dry weights were increased by Cd supply at 25–100  $\mu\text{mol L}^{-1}$  (Table 1). Our further experiments with a wider range of Cd supply levels also showed that shoot growth was enhanced and reached optimal at Cd level of 100  $\mu\text{mol L}^{-1}$  (Yang et al., 2003 unpubl data). Although the maximum root length was reduced significantly by Cd levels higher than 50  $\mu\text{mol L}^{-1}$ , a decrease in root dry weight was only noted at Cd supply levels  $\geq 200 \mu\text{mol L}^{-1}$ , which was probably

due to the fact that more side roots were developed at Cd levels from 25 to 100  $\mu\text{mol L}^{-1}$ . Our previous studies demonstrated that *S. alfredii* Hance could tolerate not only high Zn but also high Pb levels in growth medium. It grew healthy at the external Zn levels of 160  $\text{mg L}^{-1}$  in nutrient solution and at the total and DTPA-Zn levels of 4000 and 300  $\text{mg kg}^{-1}$  in the soils, respectively (Yang et al., 2001). Shoot growth and dry matter yields of *S. alfredii* Hance were not reduced when grown at 320  $\text{mg Pb L}^{-1}$  in nutrient solution and at the total and DTPA extractable Pb levels of approximately 6000 and 1000  $\text{mg kg}^{-1}$  in the soils, respectively (He et al., 2002). The high tolerance of *S. alfredii* Hance to Cd and other metal toxicity is important plant characteristics required for phytoremediation of multiple-metal contaminated soils. The mechanisms behind the co-tolerance of *S. alfredii* Hance to Cd and other metal toxicities need to be further clarified.

Cadmium hyperaccumulator is defined as plant species capable of accumulating more than 100  $\text{mg Cd kg}^{-1}$  in the shoot dry weight (Baker et al., 2000). So far, only few Cd hyperaccumulators have been identified in *Thlaspi caerulescens* and *Arabidopsis halleri* (Brown et al., 1995; Lombi et al., 2000; Küpper et al., 2000). The results from this study showed that concentrations of Cd in the leaves and stems were up to 7800 and 5600  $\text{mg kg}^{-1}$ , respectively at 200  $\mu\text{mol Cd L}^{-1}$  in the nutrient solution, and were as high as 3890 and 2360  $\text{mg kg}^{-1}$ , respectively, even at 25  $\mu\text{mol Cd L}^{-1}$  in the nutrient solution (Figures 1 and 2). Cadmium concentrations in leaf and stem were as high as 890 and 520  $\text{mg kg}^{-1}$ , respectively for the Control, which may be caused by both the great ability of the plant to take up Cd from very low external Cd level and the effect of residual Cd in plant seedlings. The residual Cd concentrations in the shoots (mainly stems) were 456  $\text{mg kg}^{-1}$ , which came from the collected stems from the mined sites. The soil total Cd and DTPA-Cd were 40.4 and 2.77  $\text{mg kg}^{-1}$ , respectively, where *Sedum alfredii* seedlings were collected. The Cd level in the nutrient solution of the control was detected at 0.002  $\mu\text{mol Cd L}^{-1}$  which might have contaminated from the impurity of the used chemicals. Moreover, *Sedum alfredii* Hance possessed a typical ability of Cd hyperaccumulation, characterized by the ratios of shoot/root Cd concentration greater than 2, and an exceedingly large accumulation of Cd in the leaves and stems (Figure 4). The results from our pot experiments showed that Cd concentration in the shoots of *S. alfredii* Hance reached as high as

570  $\text{mg kg}^{-1}$  (DW) after two month's growth on an old Pb/Zn mined soil containing total and DTPA extractable Cd levels of 40 and 10  $\text{mg kg}^{-1}$ , respectively (Yang et al., unpublished data). Our field experiments demonstrated that *S. alfredii* Hance could concentrate Cd in the shoots up to 200  $\text{mg kg}^{-1}$  (DW) from the Cu and other metal contaminated paddy soil with a DTPA-Cd around 2  $\text{mg kg}^{-1}$  soil (Yang et al., unpublished data). In most plant species, Cd concentration is generally lower than 3  $\text{mg kg}^{-1}$ , but may reach 20  $\text{mg kg}^{-1}$  or more in the Cd-enriched soils. A plant concentration of  $>100 \text{ mg kg}^{-1}$  may be regarded as exceptional, even on a Cd-contaminated site (Reeves and Bakers, 2000). These results indicate that *S. alfredii* Hance is a new powerful Cd hyperaccumulator and can offer a useful plant material for phytoremediation of Cd-contaminated soils. This is the first report of Cd hyperaccumulation in *S. Alfredii* Hance.

The mechanisms of uptake, transport and accumulation of Cd in hyperaccumulator plants are not fully understood yet. It is generally believed that Cd uptake by non-accumulator plants may through the same carrier as for other divalent cations such as  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , or  $\text{Fe}^{2+}$ , or via cation channels of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Welch and Norvell, 1999). Inhibitory effects of Cd on the uptake and accumulation of Zn, Cu, Mn, and Ca were noted in conventional crop plants (Yang et al., 1996; Wong et al., 1984). In the Zn/Cd hyperaccumulator the Ganges ecotype of *Thlaspi caerulescens*, Cd uptake was found to be metabolically dependent, and not inhibited by Zn, Cu, Mn, and Fe(II) (Zhao et al., 2002). The Zn transporter (ZNT1) has been recently cloned from the *Thlaspi caerulescens* and shown to mediate low-affinity uptake of Cd (Lasat et al., 2000; Pence et al., 2000). The characteristics of Cd absorption kinetics showed that there may exist a high-affinity Cd transporter system in the root cell plasma membranes of *Thlaspi caerulescens* (Lombi et al., 2001). In the Cd hyperaccumulator *Arabidopsis halleri*, high Zn (500  $\mu\text{mol L}^{-1}$ ) dramatically decreased Cd concentration in both shoots and roots (Küpper et al., 2000), implying that similar uptake and transport systems for Cd and Zn may exist in the Cd-hyperaccumulator of *Arabidopsis halleri*. The results from this study showed that at the Cd levels  $\leq 400 \mu\text{mol L}^{-1}$ , the concentrations of Mn, Cu, and B in the leaves and those of Mg and Zn in the roots considerably decreased, whereas Ca, P, and Zn in the leaves and P, Fe, Cu, and B in the roots significantly increased with increasing Cd levels (Table 1). High Zn level (500  $\mu\text{mol L}^{-1}$ ) significantly increased



Cd concentrations in the leaves and stems at the Cd levels  $\leq 100 \mu\text{mol L}^{-1}$ , and those in the roots at the Cd levels  $\leq 50 \mu\text{mol L}^{-1}$ . These results indicate that there exists synergistic interaction of Cd and Zn for both absorption and transport at the sub-optimal levels. High Zn supply appears to enhance particularly Cd translocation to the leaves. These results suggest that there might exist separate specific transport systems for Cd absorption across the root plasma membrane and Cd translocation across the leaf tonoplast in the hyperaccumulator *S. alfredii* Hance. The finding of the new Cd-hyperaccumulator *S. alfredii* Hance provide an important plant material for understanding the mechanisms of co-hyperaccumulation of Cd and Zn in higher plants.

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