

## Characteristics of Cadmium Uptake and Accumulation by Two Contrasting Ecotypes of *Sedum alfredii* Hance

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### ABSTRACT

The mined ecotype of *Sedum alfredii* Hance has been identified to be a zinc (Zn) hyperaccumulator native to China. In the present article, the characteristics of cadmium (Cd) uptake and accumulation were compared with hydroponic experiments between the mined and the nonmined ecotypes of *Sedum alfredii* Hance. The results indicate that the plants of the mined ecotype (ME) have higher tolerance of Cd than the plants of the nonmined ecotypes (NME) in terms of dry matter yield. The thresholds of external Cd levels for the reduction of plant growth were  $100\ \mu\text{mol L}^{-1}$  for the NME and  $400\ \mu\text{mol L}^{-1}$  for the ME, respectively. Kinetic study showed that the rates of Cd influx into roots (IR) and transport to shoots (TR) were higher in the ME than in the NME, with 5-fold higher for the maximum IR ( $I_{\text{max}}$ ) and 13-fold higher for the maximum TR ( $T_{\text{max}}$ ) in the NME, respectively. Cadmium concentrations increased with increasing external Cd supply levels. Root Cd concentrations in the NME were higher than that in the ME, with a maximum being  $5646\ \text{mg kg}^{-1}$  for the NME and  $2889\ \text{mg kg}^{-1}$  for the ME at  $1000\ \mu\text{mol L}^{-1}$  Cd. On the contrary, shoot

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Cd concentrations of the NME were far lower than that of the ME. Maximum shoot Cd concentrations were  $533 \text{ mg kg}^{-1}$  in leaves and  $935 \text{ mg kg}^{-1}$  in stems at  $1000 \mu\text{mol L}^{-1}$  Cd for NME, whereas, 4933 and  $3874 \text{ mg kg}^{-1}$  at  $400 \mu\text{mol L}^{-1}$  Cd for the ME, respectively. Meanwhile, Cd concentrations in the shoots of both the NME and ME increased with advancing Cd treatment time. At  $100 \mu\text{mol L}^{-1}$  Cd, concentrations of Cd in leaves and stems of the NME sharply increased within initial 8 and 12 days, and those in the ME increased dramatically until D20 and D16, respectively. However, leaf and stem Cd concentrations reached their maximum values on D4 for the NME and D8 for the ME, respectively, when the plants were exposed to  $1 \mu\text{mol L}^{-1}$  Cd. Cadmium accumulation by plant shoots was obvious higher in the ME than in the NME at varied Cd supply levels or Cd treatment time. The maximum Cd taken up by the shoots was  $1032 \mu\text{g plant}^{-1}$  in concentration-dependent uptake, and  $1699 \mu\text{g plant}^{-1}$  in time-course uptake for the ME, with 15-fold and 18-fold higher than those for the NME, respectively. The ratios of shoot/root of Cd ranged from 12 to 39, varying with Cd supply levels, and from 13 to 24 in the varied treatment times for the ME, more than 10 times greater than those for the NME. In addition, Cd distribution in leaves, stems and roots of ME was greatly different from those of NME. The percentage of Cd distribution in shoots was more than 79 at the varied Cd supply levels, or 83 in the varied treatment time for ME, both higher than that for NME. It could be concluded that the mined ecotype of *Sedum alfredii* Hance has a greater ability to tolerate, transport, and accumulate Cd, as compared with the nonmined ecotype.

**Key Words:** Cadmium; Ecotype; Hyperaccumulator; *Sedum alfredii* Hance.

## INTRODUCTION

Cadmium is one of the most toxic heavy metals in the environment due to its high mobility and toxicity at low concentrations in organisms. Moreover, soil Cd pollution in agricultural ecosystem becomes more and more severe due to improper agricultural management and industrial wastes discharge.<sup>[1,2]</sup> A number of hazardous effects on plant are evoked by Cd including inhibition of cell fission and growth, spoiling subcellular structure and membrane selective permeability as well as other physiological metabolisms.<sup>[3-5]</sup> Furthermore, human and animal health may be affected, as Cd is easily transferred to drinking water and food such as corn seeds from Cd contaminated soils.<sup>[1,2,5]</sup>

The Cd polluted agricultural land is reported to be 13,000 ha in China,<sup>[2,6]</sup> and Cd in seriously polluted soils has reached  $5-7 \text{ mg kg}^{-1}$ , where rice seeds contain Cd up to  $1-2 \text{ mg kg}^{-1}$ .<sup>[6]</sup> Therefore, it is imperative to remedy these contaminated soils. However, remediation of large volumes of such soil by conventional technologies, which were developed for small, heavily contaminated site, would be very expensive.<sup>[7]</sup> Phytoremediation emerged as an alternative technique to remove toxic metals from soil offering the benefits of being in situ, cost-effective and environmentally sustainable.<sup>[8]</sup> The basic strategies of the phytoremediation are to grow suitable plants on the polluted soils to take up heavy metals from soil and to remove the metals by successively harvesting the plants.<sup>[9,10]</sup> Successful



implementation of phytoremediation depends on the identification of suitable plant species that are not only capable of growing on soils containing high levels of metals, but also accumulating much higher concentrations of metals in their shoots than normal species.<sup>[9–11]</sup> These plants are termed hyperaccumulator.<sup>[11]</sup> Up to now, more than 450 species of hyperaccumulators belonging to 45 families have been identified. However, most of the metal-accumulating plants identified so far are slowly growing, have small biomass and cannot meet the need of remediation on a large scale.<sup>[7,12]</sup> Few Cd-hyperaccumulators have been reported, including *Thlaspi caerulescens*, *Arabidopsis halleri*, and *Brassica juncea*.<sup>[13–15]</sup> *S. alfredii* H. growing in a Pb/Zn mine area has also been identified as a Zn-hyperaccumulator native to China.<sup>[16,17]</sup> The ability of hyperaccumulating Cd by *S. alfredii* H. has been demonstrated.<sup>[18,19]</sup> It also has characteristics of large biomass, fast growth, asexual propagation, and perennial. So it is an ideal plant to study the mechanism responsible for hyperaccumulation and could be applied for practice of phytoremediation. The objectives of this study were to compare the characteristics of Cd tolerance, uptake and accumulation between the two contrasting ecotypes of *S. alfredii* H.

## MATERIALS AND METHODS

### Plant Culture

#### Experiment 1. Concentration-dependent Cadmium Uptake and Accumulation

The plants of nonmined ecotype of *S. alfredii* Hance (NME) were collected from a tea garden in suburb of Hanzhou city and those of mined ecotype (ME) from an old Pb/Zn mined area in Quzhou, Zhejiang Province, China, respectively. Healthy and equally sized shoot cuttings were grown in nutrition solution for 14 days till the initiation of new roots. Composition of the nutrient solution was (in  $\mu\text{mol L}^{-1}$ ):  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  2000,  $\text{KH}_2\text{PO}_4$  100,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  500,  $\text{KCl}$  100,  $\text{K}_2\text{SO}_4$  700,  $\text{H}_3\text{BO}_3$  10,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  0.5,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  1.0,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0.2,  $(\text{NH}_4)_6\text{M}_2\text{O}_7$  0.01,  $\text{Fe-EDTA}$  100. The plants were treated with 9 Cd levels (i.e., control (without Cd addition), 25, 50, 100, 200, 400, 600, 800, and 1000  $\mu\text{mol Cd L}^{-1}$ ), added as form of  $\text{CdCl}_2$ . Each treatment replicated three times. Eighteen uniform seedlings were transplanted to 3-L plastic container, and then exposed to Cd treatment in nutrient solution after the preculture. The nutrient solution were aerated continuously and renewed every four days. Solution pH was maintained at 5.5 adjusted daily with  $0.1\text{mol L}^{-1}$   $\text{HCl}$  or  $\text{NaOH}$ . Plants were harvested after been treated with Cd levels for 12 days ( $\text{D}_{12}$ ).

#### Experiment 2. Time Course Cadmium Uptake and Accumulation

The preparation of plant seedlings and the nutrient composition were similar to those in Experiment 1. The plants were treated with two Cd levels i.e., 1 and 100  $\mu\text{mol Cd L}^{-1}$ , which contained 7 replications. The plants were harvested after



exposed to the Cd treatments for 0, 1, 2, 4, 8, 12, 16, 20, 28 days. At harvest, plants were washed with tap water, and roots were immersed in  $20 \text{ mmol L}^{-1}$   $\text{Na}_2\text{-EDTA}$  (disodium ethylenediaminetetraacetate) for 15 min to remove metal ions adhering to root surface.<sup>[20]</sup> The plants were then rinsed with deionized water, blotted dried, separated into leaves, stems, and roots, and oven dried at  $60^\circ\text{C}$  to constant weight. Fresh and dry weights (FWs and DWs) were recorded.

### Plant Analysis and Data Processing

The dried plant materials were ground with stainless steel mill and passed through a 0.25 mm sieve. The plant samples were ashed in a muffle furnace at  $550^\circ\text{C}$ . The ash was dissolved in 1:1 HCl. The solution was transferred to a 50 mL volumetric flask, made up to volume and filtered through a Whatman No. 40 filter paper. Concentrations of Cd in the filtrates were analyzed using a flame atomic absorption spectrophotometer (AA 6800, Shimadzu, Japan). Analysis of variance (ANOVA) was performed using a statistics package (SPSS 10.0). Cadmium influx rate (IR) into roots and transport rate (TR) into shoots were calculated following the methods of Baligar<sup>[21]</sup> and Yang.<sup>[22]</sup> Fitted function equation was established with external Cd concentration by using SigmaPlot (8.0)

## RESULTS

### Plant Growth Response to Cd Supply Levels

Plant growth response to Cd between the two ecotypes of *S. alfredii* was obviously different (Fig. 1). For the nonmined ecotype (NME), shoot dry weight (DW), root dry weight (DW), plant height and the maximum root length of NME slightly decreased with increasing Cd supply levels to  $100 \mu\text{mol L}^{-1}$ , and dramatically decreased with further increasing Cd levels from 100 to  $1000 \mu\text{mol L}^{-1}$  (Fig. 1). The shoot DWs, root DWs, plant heights and the maximum root length of the NME grown at  $200 \mu\text{mol Cd L}^{-1}$  decreased by 67, 58, 65, and 59%, respectively, as compared to the control. Furthermore, toxic symptoms such as wilting, etiolating, and root tip putrescence on the plants, were observed on day 4 of the treatment in plants grown at Cd levels higher than  $200 \mu\text{mol L}^{-1}$ , and became more seriously with the advance of treatment time. These results indicate that the NME is very sensitive to Cd toxicity.

In contrast, shoot DW and plant height of the ME slightly increased with increasing Cd supply levels till  $100 \mu\text{mol Cd L}^{-1}$ , and was not significantly inhibited by Cd at  $\leq 400 \mu\text{mol L}^{-1}$  Cd, decreased gradually thereafter (Figs. 1a and c). In comparison with the control, growth of shoot DW of ME, root DW and the maximum root length of ME were not affected by increasing Cd till  $400 \mu\text{mol L}^{-1}$ , but were remarkably reduced by further increasing external Cd (Fig. 1b and d). No Cd toxicity symptoms were observed in the ME grown at Cd levels to  $400 \mu\text{mol L}^{-1}$ . Slight symptoms such as leaf scorching, cast and root color darkening were shown on D6 of the treatment only when external Cd was up to  $600 \mu\text{mol L}^{-1}$ . These results



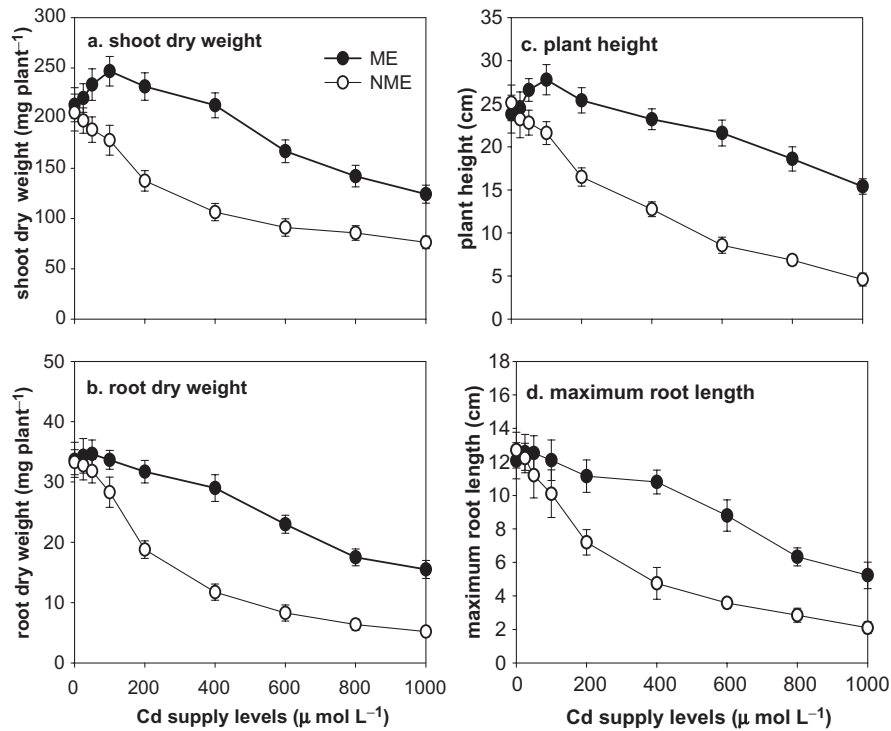


Figure 1. Growth responses of the two ecotypes of *Sedum Alfredii* to different Cd supply levels. (a) shoot dry weight, (b) root dry weight, (c) shoot height, and (d) the maximum root length. All data are means of three replications, vertical bars denote SE.

indicate that the mined ecotype of *S. alfredii* Hance is very tolerant to external high Cd, exhibiting an optimal and critical growth at 100 and 400 μmol Cd L<sup>-1</sup>, respectively.

### Dynamics of Cd Influx and Transport

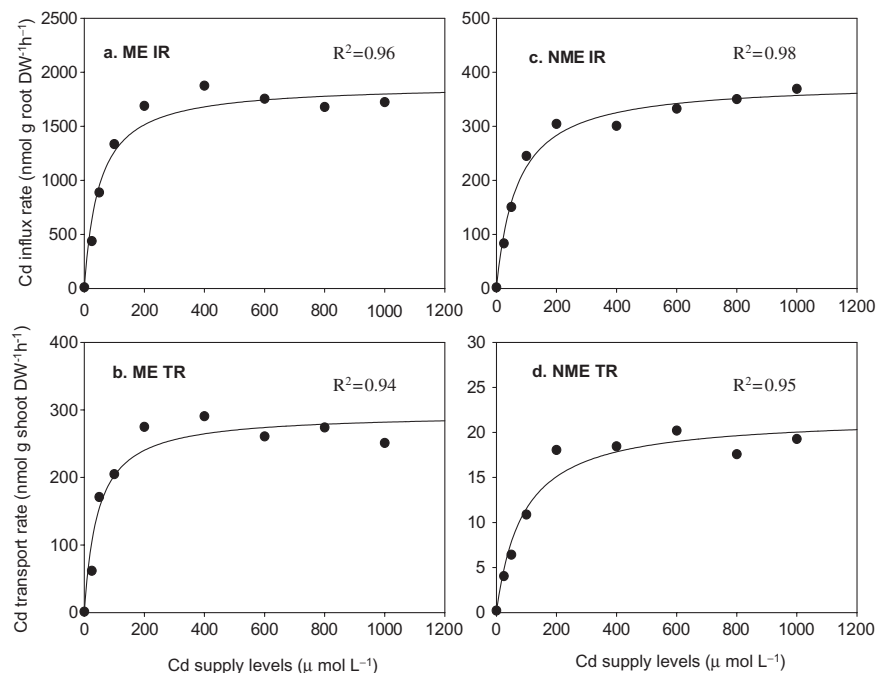
Cadmium influx and transport rates as function of the external Cd concentration were found best fit for Michaelis-Menten equation either for ME or NME. As a result, their concentration-dependent kinetics curves were showed as a component of hyperbola (Fig. 2a, b, c, d). Michaelis-Menten can be described as:

$$IR = I_{\max} \times C / (K_m^I + C) \quad (1)$$

$$TR = T_{\max} \times C / (K_m^T + C) \quad (2)$$

where *IR* and *TR* were the rates of Cd influx into roots and Cd transport into shoots respectively; *I*<sub>max</sub> and *T*<sub>max</sub> were the maximum Cd influx and transport rates,





**Figure 2.** The rates of Cd influx into roots (a and b) and Cd transport into shoots (c and d) of the two ecotypes of *Sedum Alfredii* at different Cd supply levels. Data points represent mean values of three replications. The curves represent the best fit for the data using the Michaelis-Menten kinetic equation.

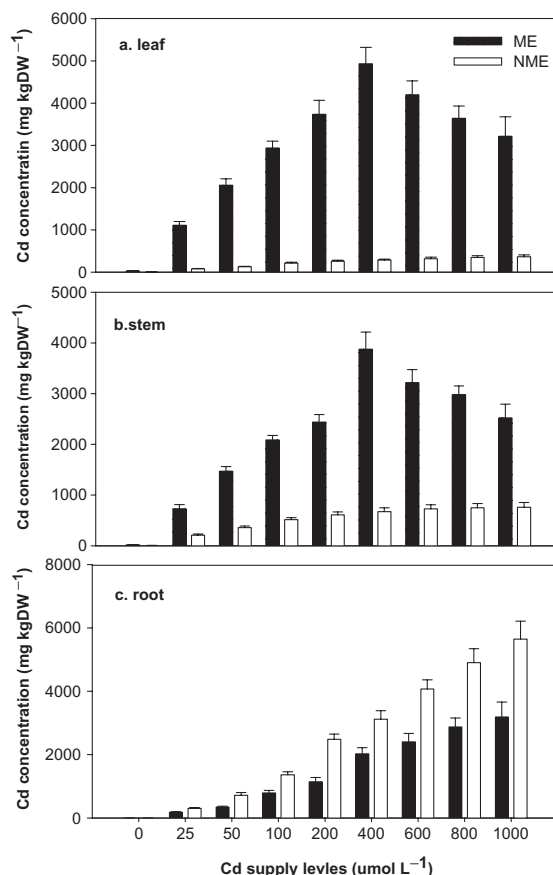
respectively;  $C$  was Cd concentration in solution, and  $K_m^I$  (or  $K_m^T$ ) was uptake constants, which was the Cd concentration in solution where  $IR=1/2I_{\max}$  (or  $TR=1/2T_{\max}$ ).<sup>[23,24]</sup>

Both for ME and NME,  $IR$  and  $TR$  increased dramatically with increasing Cd supply levels till  $200\ \mu\text{mol L}^{-1}$ , thereafter leveled off (Fig. 4). But the values were remarkably higher in ME than in NME at varied Cd supply levels (Fig. 4).  $I_{\max}$  and  $T_{\max}$  were up to  $1936\ \text{nmol g}^{-1}\text{RW h}^{-1}$  and  $294\ \text{nmol g}^{-1}\text{SW h}^{-1}$  in ME, with 5-fold for  $I_{\max}$  and 13-fold for  $T_{\max}$  in NME, respectively. Meanwhile,  $K_m^I$  and  $K_m^T$  were lower in ME than in NME, especially for  $TR$ , which  $K_m^T$  was only  $45\ \mu\text{mol L}^{-1}$ , 51% of that of in NME (Table 3). These results indicate that the rates of influx and transport of Cd were greatly higher in ME than in NME.

### Changes of Cadmium Concentrations with External Supply Level and Exposure Time

Cadmium concentrations in leaves and stems of ME increased with increasing external Cd levels, and peaked at  $400\ \mu\text{mol Cd L}^{-1}$ , whereas those of the NME



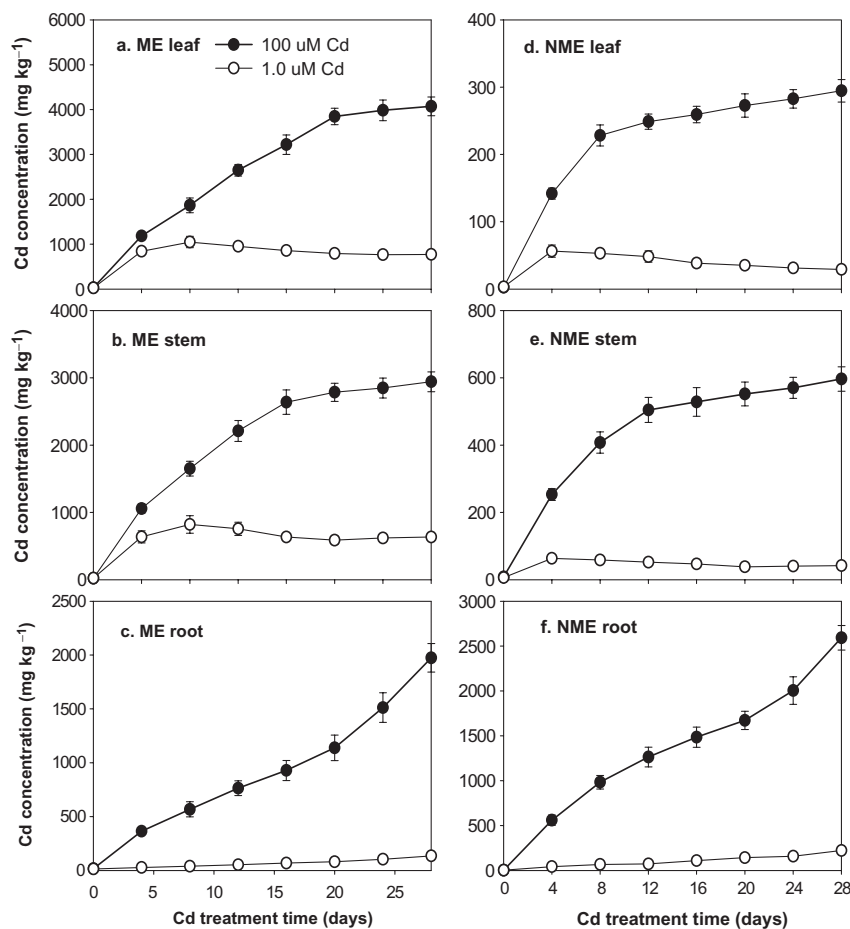


**Figure 3.** Cadmium concentrations in leaves, stems and roots of the two ecotypes of *Sedum Alfredii* grown at different Cd supply levels. All data are means of three replications, vertical bars denote SE.

increased slightly with Cd supply levels, and leveled off at 600  $\mu\text{mol Cd L}^{-1}$ . The maximum Cd concentrations observed in the leaves was 4933  $\text{mg kg}^{-1}$  for the ME but only 533  $\text{mg kg}^{-1}$  for the NME. It was evident that Cd concentrations in the leaves and stems of the ME were extremely higher than those of the NME, with approximately 10-fold or more for the leaf concentration and around 4-fold for the stem concentrations grown at all Cd supply levels (Fig. 3). Root Cd concentrations increased with increasing Cd supply levels till 1000  $\mu\text{mol L}^{-1}$  for both ecotypes. Root Cd concentration was much higher in the NME than in the ME grown at each Cd supply level (Fig. 3c). For instance, root Cd concentration of the NME was 2880  $\text{mg kg}^{-1}$  while that of the ME was only 1040  $\text{mg kg}^{-1}$ , nearly 3-fold higher when grown at external Cd supply level of 200  $\mu\text{mol L}^{-1}$ .

At a low Cd supply level (1.0  $\mu\text{mol L}^{-1}$ ), Cd concentration in leaves and stems of ME increased with treatment time, and reached their maximum on D<sub>8</sub>, 1058 and





**Figure 4.** Cadmium concentrations in leaves, stems, and roots of the two ecotypes of *Sedum Alfredii* in different Cd treatment time when the plants were exposed in 1 and 100 μmol L<sup>-1</sup> Cd, respectively. All data are means of three replication, vertical bars denote SE. Error bars do not outside some symbols.

820 mg kg<sup>-1</sup>, respectively (Fig. 4a and b.). While Cd concentration in leaves and stems of NME increased only in the first four days. The maximum Cd concentration was recorded on D<sub>4</sub>, being 56 mg kg<sup>-1</sup> in leaves and 63 mg kg<sup>-1</sup> in stems, respectively (Fig. 4d and e). At a high Cd supply level (100 μmol L<sup>-1</sup>), leaf and stem Cd concentration in ME increased rapidly until D<sub>20</sub> and D<sub>16</sub>, respectively. Thereafter, they reached a plateau. Leaf Cd concentration was up to 3848 mg kg<sup>-1</sup> on D<sub>20</sub>, 92% of the maximum on D<sub>28</sub> (4072 mg kg<sup>-1</sup>), and stem Cd concentration was as high as 2639 mg kg<sup>-1</sup> on D<sub>16</sub>, 84% of the maximum on D<sub>28</sub> (3043 mg kg<sup>-1</sup>) (Fig. 4a and b.). The rapid increases in leaf and stem Cd concentrations in NME lasted merely for 8 and 12 days, and followed by a slow increase until D<sub>28</sub> when the maximum values





were recoded as 295 mg kg<sup>-1</sup> in leaf and 596 mg kg<sup>-1</sup> in stem, respectively. Root Cd concentrations increased continuously with advancing treatment time for the two ecotypes. Root Cd concentration was much higher in the NME than in the ME for each sampling time either at 1.0 μmol L<sup>-1</sup> Cd or 100 μmol L<sup>-1</sup> Cd. These results indicate that the mined ecotype of *S. alfredii* Hance has much greater capacity to contain and tolerate high concentration of Cd in shoot tissues than the nonmine ecotype, especially in that of leaves.

### Cadmium Accumulation and Distribution with External Supply Level and Treatment Time

Cadmium accumulation by plant shoots increased with increasing external Cd supply in the range of 0–100 μmol L<sup>-1</sup> for NME and till 400 μmol L<sup>-1</sup> for ME, respectively. However, Cd accumulation by the shoots decreased at higher Cd supply levels (Table 1). Cadmium uptake by the roots increased with external Cd levels till 200 μmol L<sup>-1</sup> for the NME while till 400 μmol L<sup>-1</sup> for the ME. The maximum Cd taken up by of the shoots were 67 μg plant<sup>-1</sup> for the NME at 100 μmol Cd L<sup>-1</sup>, whereas 1032 μg plant<sup>-1</sup> for the ME at 400 μmol Cd L<sup>-1</sup> (Table 1). The ratios of shoot/root for Cd taken up were 12–39 at varied Cd supply levels in the ME, more than 10 times greater than that in NME (Table 1). For instances, the maximum ratio of shoot /root Cd accumulation in the ME was 39 grown at 50 μmol Cd L<sup>-1</sup> while that in the NME was only 2.6 grown at 25 μmol Cd L<sup>-1</sup> (Table 1).

The time course accumulation of cadmium by the shoots and roots also differed considerably between the two ecotypes (Table 2). At external Cd supply level

**Table 1.** Cadmium uptake in shoots and roots of the two ecotypes of *S. alfredii* at different Cd supply levels.

Supply levels (μmol L <sup>-1</sup> Cd)	NME <sup>a</sup>		ME <sup>b</sup>	
	Shoot	Root	Shoot	Root
	(μg plant <sup>-1</sup> )			
control	0.86	0.24	2.92	0.30
25	25.53	9.87	223.54	6.23
50	48.36	22.77	483.83	12.35
100	66.92	34.55	668.57	27.09
200	51.58	46.69	811.39	36.26
400	40.44	36.62	1031.62	58.68
600	35.45	33.73	740.19	39.64
800	34.99	31.12	477.24	33.42
1,000	30.92	29.36	366.78	30.33
LSD ( <i>P</i> < 0.05)	8.73	7.30	118.89	6.05
LSD ( <i>P</i> < 0.01)	12.03	10.06	163.81	8.33

<sup>a</sup>NME = the nonmined ecotype.

<sup>b</sup>ME = the mined ecotype.



**Table 2.** Cd uptake in shoots and roots of the two ecotypes of *S. alfredii* exposed to 100  $\mu\text{mol L}^{-1}$  Cd in different Cd treatment time.

Cd supply time (day)	NME		ME	
	Shoot	Root	Shoot	Root
	( $\mu\text{g plant}^{-1}$ )			
0	0.13	0.01	0.43	0.03
4	18.16	4.72	126.89	6.44
8	44.60	12.72	354.06	15.13
12	66.83	34.19	677.58	28.76
16	73.94	54.95	1062.01	49.05
20	83.75	69.60	1348.05	69.13
24	89.49	89.25	1541.13	95.50
28	96.28	107.63	1698.89	132.09
LSD( $P < 0.05$ )	10.50	8.40	111.32	16.07
LSD( $P < 0.01$ )	14.57	11.65	154.50	22.29

**Table 3.** Kinetic parameters for Cd uptake by the two ecotypes of *S. alfredii*.

	$IR^a$		$TR^b$	
	$I_{\max}^c$ ( $\text{nmol g RW}^{-1}\text{h}^{-1}$ )	$K_m^l{}^d$ ( $\mu\text{mol L}^{-1}$ )	$T_{\max}^e$ ( $\text{nmol g SW}^{-1}\text{h}^{-1}$ )	$K_m^{Tf}$ ( $\mu\text{mol L}^{-1}$ )
ME	1,936	54	294	45
NME	382	70	22	89

<sup>a</sup> $IR$  = the rate of Cd influx into roots.

<sup>b</sup> $TR$  = the rate of Cd transport into shoots.

<sup>c</sup> $I_{\max}$  = the maximum rate of Cd influx into roots.

<sup>d</sup> $T_{\max}$  = the maximum rate of Cd transport into shoots.

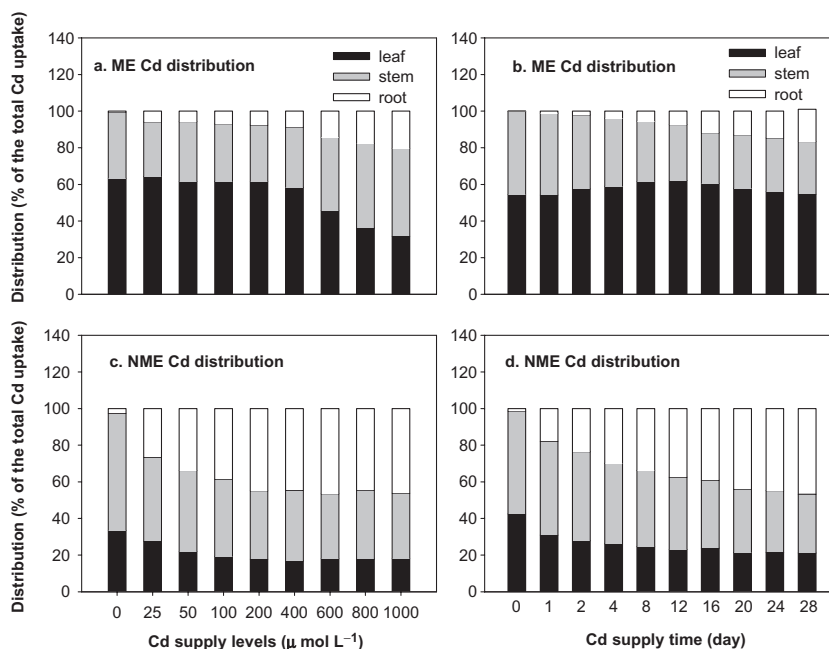
<sup>e</sup> $K_m^l$  = Cd concentration in solution where  $IR = 1/2I_{\max}$ .

<sup>f</sup> $K_m^T$  = Cd concentration in solution where  $TR = 1/2T_{\max}$ .

of 100  $\mu\text{mol Cd L}^{-1}$ , rapid accumulation phase was noted within 12 days for the NME, whereas up to 24 days for the ME. During the rapid uptake time course, the increase of Cd taken up by the shoot reached over 60  $\mu\text{g plant}^{-1} \text{ day}^{-1}$  for the ME, but only about 6  $\mu\text{g plant}^{-1} \text{ day}^{-1}$  for the NME. However, root Cd accumulation increased linearly with advance of Cd treatment time up to 28 days for both ecotypes (Table 2). The ratios of shoot/root Cd taken up decreased with advancing of absorption time, and the mined ecotype had much greater ratios of shoot/root Cd uptake than the nonmined ecotypes (Table 2).

Cadmium distribution in the two ecotypes was remarkably different. For ME, the percentage of Cd distribution in shoots was maintained at high values, with increasing Cd supply levels or advancing treatment time (Fig. 5a and c). The least





**Figure 5.** The ratios of Cd distribution in leaves, stems, and roots of the two ecotypes of *Sedum Alfredii* at different Cd supply levels (a and c) or at different Cd treatment time (b and d).

percentage was recorded as 79% in the concentration-dependent uptake studies (Fig. 5a), or 83% in time-dependent studies in ME (Fig. 5c). In the range of 0–400  $\mu\text{mol L}^{-1}$  Cd supply level, the order of Cd distribution was noted as leaf (50–60%) > stem (30–40%) > root (less than 20%) in ME (Fig. 5a and c). By contrast, the percentages of shoot Cd distribution in NME decreased with increasing Cd supply levels or with advancing treatment time. As external Cd supply exceeded 200  $\mu\text{mol L}^{-1}$  Cd or Cd treatment time was later than D<sub>12</sub>, the order of Cd distribution in different parts of NME was root (40–50%) > stem (30–40%) > leaf (10–30%) (Fig. 5b and d).

These results indicate that the mined ecotype of *S. alfredii* Hance not only has a much greater ability of taking up Cd from growth medium, but also has an extremely higher ability of transporting Cd from roots to shoots, as compared with the nonmined ecotype. Moreover, the translocation of Cd mainly took place in initial 16–20 days after the plant was exposed to Cd treatment.

## DISCUSSION

In this study, marked differences in *IR* and *TR* were demonstrated between the two ecotypes of *Sedum alfredii* H. Compared to NME, ME had greater  $I_{\text{max}}$  and  $T_{\text{max}}$ , and smaller  $K_m^I$  and  $K_m^T$ . Moreover, the differences in magnitude of parameters



were greater in *TR* than in *IR*. These differences appear to attribute to different abilities of absorbing as well as transporting Cd between ME and NME. Previous studies had shown that there are significant differences in Cd uptake ability among different populations for some plant species.<sup>[25]</sup> Lombi et al.<sup>[26]</sup> compared the capacity to accumulate Zn and Cd among four populations of *Thlaispi caerulescens*. Results show that the four populations had the same capacity of accumulating Zn, but significantly different ability of accumulating Cd. These results indicate that the mechanisms responsible for Zn and Cd accumulation are not identical. Further studies on Cd absorption kinetics of two contrasting ecotypes revealed that there may exist high-affinity Cd transporters, which are different from that involved in Zn uptake, in the root cell plasma membrane of the Cd-hyperaccumulator population of *T. caerulescens*.<sup>[25,27]</sup> In this study, both the concentration-dependent and time-course uptake studies showed that gross Cd uptake in ME was obviously greater than in NME at the same Cd supply levels or in same Cd treatment time (Tables 1 and 2). The maximums Cd accumulation of ME was recorded as high as  $1090 \mu\text{g plant}^{-1}$  in the concentration-dependent uptake studies, and  $1830 \mu\text{g plant}^{-1}$  in the time-course uptake studies, being 7-fold and 8-fold higher than those of NME, respectively (Tables 1 and 2). It is possibly attributed to higher ability of absorbing Cd in ME than in NME, as evidenced by the higher  $I_{\text{max}}$  in ME.

Hyperaccumulator plants are characterized by highly efficient translocation of heavy metals from roots to shoots.<sup>[27]</sup> Previous studies demonstrated that root uptake and shoot accumulation of Cd appear to be independent processes.<sup>[28]</sup> In this study, the Cd percentages of shoot were shown significantly higher in ME than in NME either in the concentration-dependent uptake studies, or in time-course uptake studies (Fig. 5a, b, c, d). The ratios of shoot/root for Cd uptake in ME were higher than those in NME (Tables 1 and 2). These results demonstrate that ME has an extremely higher ability of transporting Cd from roots to shoots than NME, besides its more effective absorbing ability. Furthermore, the difference in Cd transport abilities is greater between the two ecotypes, in comparison with the difference in their Cd uptake abilities. This may be the reason that  $T_{\text{max}}$  in ME was 13-fold higher than that in NME. Generally speaking, a significantly larger  $V_{\text{max}}$  value suggests that there is a higher density of transporter per unit membrane area in roots, or these systems are expressed more actively, while a lower  $K_m$  value means there is a higher affinity between the transporter and its substrate.<sup>[24,27,29–31]</sup> In this study, judgment from the great differences in the values of  $I_{\text{max}}$  and  $T_{\text{max}}$  as well as  $K_m^I$  and  $K_m^T$  between the two ecotypes, implying that there is a higher density of Cd absorbing system and (or) Cd transporting system on the root cell membranes of ME than NME. Nevertheless, this primary judgment needs to be further proved.

Plant hyper-tolerance and hyperaccumulation of transition metal ions are somewhat linked,<sup>[25]</sup> and they are associated with intracellular binding and sequestration drive passage across the plasma membrane.<sup>[29]</sup> Phytochelatin synthesis is generically known as principal response to Cd in plants.<sup>[30]</sup> Overexpressing enzymes involving in the synthesis of the phytochelatin precursor glutathione led to an enhancement of Cd tolerance and accumulation in *Brassica juncea*.<sup>[31,32]</sup> On the contrary, the *cad2* mutant of *Arabidopsis thaliana*, deficient in phytochelatin



synthase activity, does not form any Cd-phytochelatin complex, and is consequently sensitive to Cd.<sup>[30,33]</sup> These previous results indicate that phytochelatin play a crucial role in Cd detoxification and sequestration for some plants. In this study, ME showed significantly higher ability to tolerate Cd than NME in terms of their growth response to Cd supply levels and Cd concentrations in shoots. For instance, ME grew normally at  $400\ \mu\text{mol L}^{-1}$  Cd for 12 days without obvious phytotoxicity. Simultaneously, shoot Cd concentrations in the ME were 10-fold higher for the leaf and 6-fold higher for the stem than those of NME, respectively. The shoot Cd concentration is far over the standard of Cd hyperaccumulation ( $100\ \text{mg kg}^{-1}$ ).<sup>[34]</sup> Moreover, the plants even grew better at  $100\ \mu\text{mol L}^{-1}$  Cd than the control. In contrast, NME had severe toxic symptoms induced by heavy metal and obvious reduction in dry shoot and root yields with increasing Cd supply levels, especially at Cd treatment  $\geq 200\ \mu\text{mol L}^{-1}$ . The high tolerance is indispensable characteristics for hyperaccumulation and phytoextraction of Cd for ME. Unfortunately, minimal information is currently available about the mechanism responsible for Cd-hyperaccumulator to uptake, transport and accumulate Cd. Whether there are any phytochelatin in hyperaccumulator is also unclear. Therefore, future studies should focus on clarifying the difference in mechanisms of Cd transport between the two ecotypes as well as whether there be any phytochelatin involving in detoxification and sequestration in ME or NME. The two contrasting ecotypes of *S. alfredii* are valuable materials to study mechanisms of hyperaccumulation of Cd using genetic and molecular approaches, and the mined ecotype of *S. alfredii* is also an excellent candidate plant for phytoremediation.

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