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# Dynamics of zinc uptake and accumulation in the hyperaccumulating and non-hyperaccumulating ecotypes of *Sedum alfredii* Hance

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**Abstract**

*Sedum alfredii* Hance has been identified as a Zn-hyperaccumulating plant species native to China. The characteristics of Zn uptake and accumulation in the hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of *S. alfredii* were investigated under nutrient solution and soil culture conditions. The growth of HE was normal up to 1000 $\mu$M Zn in nutrient solution, and 1600 mg Zn $kg^{-1}$ soil in a Zn-amended soil. Growth of the NHE was inhibited at Zn levels ≥250 $\mu$M in nutrient solution. Zinc concentrations in the leaves and stems increased with increasing Zn supply levels, peaking at 500 and 250 $\mu$M Zn in nutrient solution for the HE and the NHE, respectively, and then gradually decreased or leveled off with further increase in solution Zn. Minimal increases in root Zn were noted at Zn levels up to 50 $\mu$M; root Zn sharply increased at higher Zn supply. The maximum Zn concentration in the shoots of the HE reached 20,000 and 29,000 mg $kg^{-1}$ in the nutrient solution and soil experiments, respectively, approximately 20 times greater than those of the NHE. Root Zn concentrations were higher in the NHE than in the HE when plants were grown at Zn levels ≥50 $\mu$M. The time-course of Zn uptake and accumulation exhibited a hyperbolic saturation curve: a rapid linear increase during the first 6 days in the long-term and 60 min in the short-term studies; followed by a slower increase or leveling off with time. More than 80% of Zn accumulated in the shoots of the HE at half time (day 16) of the long-term uptake in 500 $\mu$M Zn, and also at half time (120 min) of the short-term uptake in 10 $\mu$M $^{65}Zn^{2+}$. These results indicate that Zn uptake and accumulation in the shoots of *S. alfredii* exhibited a down-regulation by internal Zn accumulated in roots or leaves under both nutrient solution and soil conditions. An altered Zn transport system and increased metal sequestration capacity in the shoot tissues, especially in the stems, may be the factors that allow increased Zn accumulation in the hyperaccumulating ecotype of *S. alfredii*.

## Introduction

Successful implementation of phytoremediation depends on 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 do. To date, more than 450 species of hyperaccumulators belonging to 45 families have been identified (Baker et al., 2000); however, most of them grow slowly and produce small amounts of shoot biomass annually. An understanding

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of physiological mechanisms of metal tolerance and hyperaccumulation is necessary for exploring new metal hyperaccumulating plant species that are tolerant of high levels of metal or multiple metals, have large biomass, extensive adaptation, and easy propagation (Salt et al., 1998).

Zinc hyperaccumulator is defined as plants that can accumulate Zn in shoots above 10,000 mg $kg^{-1}$. *Thlaspi caerulescens* and *Arabidopsis halleri* are plants that have been reported to hyperaccumulate Zn and Cd (Bert et al., 2000; Brown et al., 1995a, b). Uptake dynamics of Zn in the hyperaccumulating plant species have not been extensively studied. General aspects of Zn uptake in roots and translocation to shoots have been reported (Kochian, 1991, 1993). In time-dependent kinetic studies of root Zn absorption in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.), an initial rapid component of accumulation occurred, followed by a slower linear phase of accumulation (Santa Maria and Cogliatti, 1988; Veltrup, 1978). Certain populations of *T. caerulescens* have been reported to accumulate and tolerate Zn up to 40,000 mg $kg^{-1}$ in their shoots (Brown et al., 1995b). In nutrient solution, *T. caerulescens* accumulated more than 25,000 mg $kg^{-1}$ Zn in their shoots before any yield reduction was observed (Brown et al., 1995a, b). Studies on $^{65}Zn^{2+}$ uptake kinetics by *T. caerulescens* indicated that the concentration-dependent Zn influx yielded non-saturable kinetic curves (Lasat et al., 1996), and maximum velocity for Zn influx in *T. caerulescens* root cells was 4.5-fold higher than the non-hyperaccumulator *Thlaspi arvense*. Time-course studies of Zn accumulation revealed that *T. caerulescens* exhibited a 10-fold greater Zn translocation to the shoot than *T. arvense* (Lasat et al., 1996). The root vacuoles of *T. caerulescens* sequestered less Zn than *T. avense*, rendering Zn more readily available for xylem loading in the hyperaccumulator plants (Lasat et al., 1998). *Arabidopsis halleri* has the ability of Zn hyperaccumulation with normal growth at 1000 $\mu$M Zn in nutrient solution with subsequent accumulation of 32,000 mg $kg^{-1}$ DW in the shoots (Küpper et al., 2000; Zhao et al., 2000). Under field-grown conditions, *A. halleri* shoots accumulated Zn up to 15, 000 mg $kg^{-1}$, which is considered a constitutive trait (Bert et al., 2002).

*Sedum alfredii* Hance has been identified as a new Zn-hyperaccumulator plant species native to China (Yang et al., 2002). It has an exceptional ability to tolerate and accumulate high concentration of Zn, and characteristics of large biomass, fast growth, asexual propagation and perennial (Yang et al., 2001, 2002). Therefore, it is an ideal plant for studying mechanisms responsible for hyperaccumulation and phytoremediation practice. Tolerance to elevated Zn in nutrient solution and high Zn accumulation in *S. alfredii* have been reported (Long et al., 2002; Ni et al., 2004; Yang et al., 2001, 2002, 2004;). Comparison between the hyperaccumulating ecotype (HE) and the non-hyperaccumulating ecotype (NHE) of *S. alfredii* under nutrient solution culture condition showed that Zn concentration were over 19-fold higher in leaves and stems of the HE than the NHE, when grown with external Zn 20–160 mg $L^{-1}$, and Zn uptake by shoots (or accumulated in shoots) of the HE was over 20-fold higher than that of the NHE (Ni et al., 2004; Yang et al., 2001). Results from short term Zn uptake kinetic study in *S. alfredii* indicated that the maximum Zn influx velocity was 3.5 times greater in the HE than in the NHE *S. alfredii* (Li et al., 2005a), implying a higher density of Zn transporters on the plasma membranes in the HE root cells. However, the mechanisms of uptake and translocation that result in Zn hyperaccumulation in *S. alfredii* are not fully understood. In this study, the characteristics of concentration-dependent and time-course dynamics of Zn uptake and accumulation in the HE *S. alfredii* were evaluated in both nutrient solution and soil experiments, as compared with the NHE of *S. alfredii*.

## Materials and methods

### *Plant materials*

The HE *S. alfredii* was obtained from an old Pb/Zn mine area in Zhejiang province of China, and the NHE was obtained from a tea garden in Hangzhou, China. Healthy and uniform stems of the two ecotypes of *S. alfredii* were chosen and grown for 2 weeks in the basic nutrient solution containing (in mM) $Ca(NO_3)_2 \cdot 4H_2O$, 2.00; $KH_2PO_4$, 0.10; $MgSO_4 \cdot 7H_2O$, 0.50; KCl, 0.10; $K_2SO_4$, 0.70; and (in $\mu$M) $H_3BO_3$, 10.0;

$MnSO4 \cdot H_2O$, 0.50; $ZnSO_4 \cdot 7H_2O$, 1.0; $CuSO_4 \cdot 5H_2O$, 0.20; $(NH_4)_6\ Mo_7O_{24}$, 0.01; and Fe-EDTA, 100 (Yang et al., 2002). The nutrient solution pH was adjusted daily to 5.5 with 0.1 M NaOH or 0.1 M HCl. The nutrient solution was continuously aerated and renewed every 4 days.

*Experiment I: concentration-dependent dynamics of Zn uptake by the two ecotypes of S. alfredii grown in nutrient solution*

Seedlings of *S. alfredii* were precultured for 14 days (initiation of the new roots occurred) prior to exposure to Zn. The long-term concentration-dependent uptake of Zn was studied at external Zn supply levels of 1–5000 $\mu$M. Zinc was applied as zinc sulfate. Each treatment was replicated three times. Each 3-L container contained 18 seedlings. Nutrient solution was aerated continuously and renewed every 4 days. The pH was maintained at 5.5. Plants were harvested after exposed to the metal treatments for 24 days. At harvest, roots were rinsed in 20 mM $Na_2$-EDTA for 15 min to remove metal ions adsorbed to root surfaces (Yang et al., 1996) and then the plants were separated into leaves, stem, and roots before fresh weights were recorded. They were oven dried at 65 ° and dry weights were then recorded.

*Experiment II: long-term time course dynamics of Zn uptake and accumulation in different parts of the two ecotypes of S. alfrdedii*

Two Zn treatments (1.0 and 500 $\mu$M Zn) were used in this experiment. Each treatment had three replications at each harvest time. Plants were harvested after exposure to Zn treatments for 0, 1, 2, 4, 8, 16, 24, and 32 days. Nutrient solution was replaced every 2 days, and pH was maintained at 5.5. All the other procedures are identical to those described in Experiment I.

*Experiment III: short-term time course of $^{65}Zn^{2+}$ uptake and accumulation*

Twenty-day-old seedlings were used in all radiotracer studies. The preculture of the seedlings was the same as described in Experiment I. One day prior to the uptake experiment, intact roots were immersed in aerated pretreatment solution consisting of 2 mM MES (2-[*N*-morpholino]-ethanesulfonate)-Tris buffer (pH 6.0) and 0.5 mM $CaCl_2$.

Roots of the HE and NHE *S. alfredii* were immersed in 3 L of aerated uptake solution containing 2 mM MES-Tris (pH 6.0), 0.5 mM $CaCl_2$, and 10 $\mu$M $^{65}Zn^{2+}$ (45 kBq $L^{-1}$). At each time interval (0, 10, 20, 30, 60, 120, and 240 min), one plant of each ecotype was harvested and the roots were cleaned from any adsorbed Zn for 20 min in 2 L of solution (containing 5 mM MES-Tris (pH 6.0), 5 mM $CaCl_2$, and 100 $\mu$M $ZnCl_2$) before being separated from the shoots. The excised roots were blotted dried, roots, and shoots were oven-dried at 65°C for 48 h and weighed, and $^{65}Zn$ radioactivity was quantified using a gamma detector ($\gamma$-Spectrum Instruments, model GR2519, CANBERRA Co., Australia).

*Experiment IV: concentration-dependent dynamics of Zn uptake by the shoots of the HE ecotype grown in the soil*

The soil used for the pot experiments is an Inceptisol (silty), which was collected at a Suburb of Hanghzou, Zhejiang Province, China. Agrochemical properties of the soil were: pH 6.13, organic matter of 21.4 g $kg^{-1}$, CEC of 6.9 cmol $kg^{-1}$, total N, P, and K of 8.9, 1.02, and 9.8 g $kg^{-1}$, respectively, available N (extracted by 1.0 N NaOH), P (extracted by 0.5 M $NaHCO_3$), and K (extracted by 1.0 N $NH_4Ac$) of 70.2, 9.53, 76.3 mg $kg^{-1}$, respectively, and total and available Zn (extracted with 0.005 M DTPA) of 166 and 1.35 mg $kg^{-1}$. The soil was air-dried, passed through a 1-cm sieve and mixed thoroughly. The Zn treatment levels were: control, 100, 200, 400, 600, 800, 1200, 1600, 2000 mg $kg^{-1}$, applied as $ZnSO_4$. $K_2SO_4$ was used to balance the sulfate level across all treatments. Soils were then incubated at approximately 70% of the maximum field water holding capacity for 12 months to allow aging of the added Zn to more stable forms. Each pot contained 1 kg of soil thoroughly mixed with 0.1 g urea and 0.2 g $KH_2PO_4$ as basal fertilizers. A randomized complete block experimental design was used with each treatment replicated four times. Six plants of uniform seedlings of the HE *S. alfredii* were transplanted to each pot. Plants were grown under greenhouse conditions with natural light, day/night temperature of 30/25 °C and day/night

humidity of 65/80%. Two harvests of the shoots were performed; the first occurred after 3 months growth and the second after an additional 3 months growth. At harvest, shoots were severed approximately 3 cm above the soil surface, washed with distilled water, blotted dry, and fresh weights recorded, then oven dried at 65 °C overnight to obtain dry weights.

*Sample and data analysis*

Dried plant materials were ground with a stainless steel mill and passed through a 0.25-mm sieve prior to element analysis. A known weight of dry sample of each replicate was ashed in a muffle furnace at 550° for 5 h. The ash was dissolved in 1:1 HCl. The solution was transferred to a 50-ml volumetric flask, made to volume and filtered through Whatman No. 40 filter paper. Concentrations of Zn in the filtrates were analyzed by flame Atomic Absorption Spectrophotometry (AA 6800, Shimadzu, Japan).

Analysis of variance (ANOVA) was performed for each measured variable, and mean and standard error (SE) of each treatment was calculated. The statistical analyses were performed using SPSS 10.0.

## Results

*Growth responses of shoots and roots*

At Zn levels ≤1000 $\mu$M, the HE *S. alfredii* grew normally, and there were no visual toxic symptoms. In contrast, growth of the NHE *S. alfredii* was significantly inhibited when Zn levels were ≥250 $\mu$M. At Zn levels between 250 and 1000 $\mu$M, the HE had normal growth, erect stems, and thick dark green leaves, while the NHE had small, thin and light green leaves, and wilting symptoms on leaves and putrescence on the root tip. At the Zn level of 5000 $\mu$M, roots of the HE became black but shoot remained alive. However, the whole plant of the NHE eventually died.

Dry weight yields were similar with increasing Zn levels up to 1000 $\mu$M for the leaves and to 5000 $\mu$M for the stems of the HE (Table 1). The leaf dry weight of the HE significantly decreased only at 5000 $\mu$M Zn, indicating that the HE *S. alfredii* can tolerate high Zn levels. However, dry weight yields significantly decreased at the Zn levels ≥500 $\mu$M for the leaves and ≥250 $\mu$M for the stems of the NHE. Root dry matter yields of the HE increased with increasing Zn levels, and peaked at 500 $\mu$M Zn, but a decrease in root biomass yield occurred at 5000 $\mu$M Zn. For the NHE, however, significant inhibition of root growth was noted at Zn levels ≥250 $\mu$M. Root biomass yields decreased by 46% at 500 $\mu$M Zn, as compared with the control (Table 1). The HE and the NHE *S. alfredii* produced similar leaf, stem, and root dry matter yields when grown at low Zn levels (≤10 $\mu$M). These results indicate that the HE *S. alfredii* has increased its ability to tolerate Zn in nutrient solution and the tolerance capability may have developed from the long-term adaptation of this ecotype in the mined area with high metal soil environments.

*Table 1.* Dry matter yields of leaves, stems, and roots of the hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of *Sedum alfredii* Hance grown at different Zn rates in nutrient solution

| Zn concentration ($\mu$M) | Dry weight (g plant$^{-1}$) | | | | | | Root/Shoot ratio | |
|---|---|---|---|---|---|---|---|---|
| | Leaves | | Stems | | Roots | | | |
| | NHE | HE | NHE | HE | NHE | HE | NHE | HE |
| 1 | 0.38$^{a}$ | 0.41$^{a}$ | 0.20$^{a}$ | 0.22$^{a}$ | 0.071$^{a}$ | 0.068$^{a}$ | 0.122$^{a}$ | 0.108$^{a}$ |
| 10 | 0.36$^{a}$ | 0.40$^{a}$ | 0.20$^{a}$ | 0.19$^{a}$ | 0.071$^{a}$ | 0.074$^{a}$ | 0.127$^{a}$ | 0.125$^{a}$ |
| 50 | 0.33$^{a}$ | 0.42$^{a}$ | 0.18$^{a}$ | 0.19$^{a}$ | 0.066$^{a}$ | 0.075$^{a}$ | 0.129$^{a}$ | 0.123$^{a}$ |
| 250 | 0.29$^{a}$ | 0.39$^{a}$ | 0.16$^{b}$ | 0.20$^{a}$ | 0.051$^{a}$ | 0.081$^{b}$ | 0.113$^{a}$ | 0.137$^{a}$ |
| 500 | 0.21$^{b}$ | 0.44$^{a}$ | 0.14$^{b}$ | 0.21$^{a}$ | 0.038$^{b}$ | 0.088$^{b}$ | 0.109$^{a}$ | 0.135$^{a}$ |
| 1000 | 0.16$^{b}$ | 0.37$^{a}$ | 0.09$^{c}$ | 0.20$^{a}$ | 0.022$^{b}$ | 0.079$^{c}$ | 0.088$^{b}$ | 0.139$^{a}$ |
| 5000 | – | 0.30$^{b}$ | – | 0.21$^{a}$ | – | 0.042$^{d}$ | – | 0.082$^{b}$ |

All the data are the means of three replications, "–" refer to a dead plant.
Values followed by different letters in the same column are significantly different at $P < 0.05$.

The results from the pot experiment with soil culture indicated that shoot dry weight yields of the HE *S. alfredii* progressively increased with increasing Zn rates in the soil, and peaked at 400 and 800 mg Zn $kg^{-1}$, respectively, for the first and the second harvest. The maximum shoot dry matter yield of the second harvest (1.44 g $plant^{-1}$) was twofold higher than that of the first harvest (0.71 g $plant^{-1}$), which may be attributed to the increased adaptation and enhanced root development of *S. alfredii*. There are statistically significant differences ($P < 0.05$) in shoot dry weights between the treatments of 400 vs. control, 100, 1600, or 2000 mg Zn $kg^{-1}$, respectively from the first harvest, and between the treatments of 800 mg Zn $kg^{-1}$vs. each of all the other treatments except for 600 mg Zn $kg^{-1}$ at the second harvest (Table 2). Soil total Zn rate for optimal plant growth of *S. alfredii* was approximately 600 mg $kg^{-1}$.

*Dynamics of Zn uptake and accumulation in leaves, stems, and roots with increasing Zn levels in nutrient solution*

The HE and the NHE of *S. alfredii* (Figure 1) had different patterns of variation in Zn concentrations in leaves, stems, and roots with supply of Zn. Leaf and stem Zn increased rapidly with increasing Zn supply, reaching a maximum at 500 $\mu$M Zn, but significantly decreased at the Zn supply levels >1000 $\mu$M for the HE. For the NHE *S. alfredii*, leaf and stem Zn concentrations increased with increasing Zn levels, reaching a peak at 250 $\mu$M Zn, and then decreased with further increasing Zn levels (Figure 1). The highest Zn concentration in the leaves and stems of the HE were 17,300 and 21,945 mg $kg^{-1}$, respectively, over 16 and 18 times greater than those of the NHE *S. alfredii*.

In contrast, minimal changes in root Zn concentrations of both ecotypes occurred with increasing solution Zn levels up to 50 $\mu$M, followed by a greater increase in root Zn concentration with further increasing solution Zn in the NHE than in the HE. Approximately 1.6-fold higher root Zn concentration in the NHE than in the HE occurred when grown at Zn levels ≥250 $\mu$M (Figure 1c). At Zn levels of 250, 500, and 1000 $\mu$M, significant differences ($P < 0.05$) in root Zn concentration were observed between the HE and the NHE (Figure 1c). These results indicate that the ability of HE *S. alfredii* to translocate Zn from root to shoot has been altered, as compared to the NHE and may play a key role in Zn hyperaccumulation in *S. alfredii*.

Within Zn supply levels from 1 to 1000 $\mu$M, Zn concentrations in the tissues decreased in the order of: stem>leaf>root for the HE *S. alfredii*, and root>stem>leaf for the NHE *S. alfredii* (Figure 1). However, when Zn supply level was ≥5000 $\mu$M, Zn concentration in different plant organs of the HE *S. alfredii* changed in the order of root>stem>leaf, which may be partly associated with root activity (root dehydrogenase activity) severely inhibited (data not shown) and shoot growth ceased, thereby resulting in inhibited Zn transport to shoots. The ion speciation calculation showed that when external Zn levels increased from 1000 to 5000 $\mu$M, the proportion of ZnHPO4 and ZnSO4 in nutrient solution increased by fivefold, therefore, the precipitation of Zn with phosphate and sulfate in the apoplast of roots at higher external Zn supply may also partly account for the shift of root Zn accumulation.

*Table 2*. Shoot dry matter yields of the hyperaccumulating ecotype of *Sedum alfredii* Hance grown at different Zn rates in the soil at the first and second harvests

| Zn rates in soil (mg $kg^{-1}$) | Shoot dry weight (g $plant^{-1}$) | |
|---|---|---|
| | First harvest | Second Harvest |
| Control | $0.584^{cb}$ | $0.401^{f}$ |
| 100 | $0.615^{cb}$ | $0.802^{e}$ |
| 200 | $0.619^{cb}$ | $1.032^{cd}$ |
| 400 | $0.711^{a}$ | $1.152^{cd}$ |
| 600 | $0.601^{cb}$ | $1.379^{ab}$ |
| 800 | $0.600^{cb}$ | $1.436^{a}$ |
| 1200 | $0.650^{cb}$ | $1.208^{cb}$ |
| 1600 | $0.587^{cb}$ | $1.001^{d}$ |
| 2000 | $0.550^{c}$ | $0.301^{f}$ |

Values followed by different letters in the same column are significantly different at $P < 0.05$.

*Dynamics of Zn uptake by the shoots of the HE S. alfredii H with increasing Zn levels in the soil*

At different soil Zn rates, the Zn uptake and accumulation in the shoots of the HE *S. alfredii* exhibited similar patterns to those with nutrient solution Zn. A sharp initial uptake phase

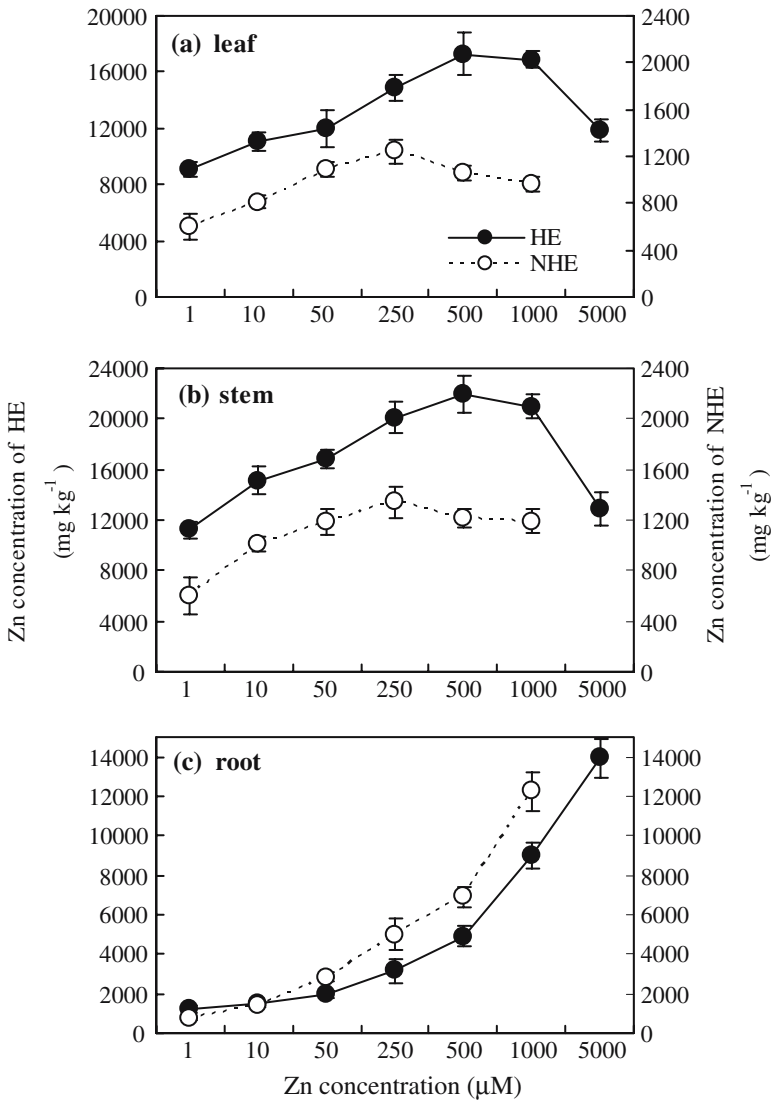


*Figure 1.* Dynamics of Zn uptake and accumulation in leaves, stems, and roots of the two ecotypes *Sedum alfiedii* Hance with increasing external Zn levels in nutrient solution. Data points and error bars represent means ± SE. The right and the left *y*-axes represent data of the NHE and the HE, respectively.

occurred at soil Zn rates ≤100 mg $kg^{-1}$ for the first harvest, and at Zn levels ≤200 mg $kg^{-1}$ for the second harvest (Figure 2). The highest Zn uptake and accumulation were observed at external soil Zn levels around 600 mg $kg^{-1}$, and then the shoot Zn concentration leveled off or slowly decreased with a further increase in soil Zn levels (Figure 2). The maximum shoot Zn concentrations reached 16,000 and 29,000 mg $kg^{-1}$ at the first and the second harvest, respectively, when grown at soil Zn rates optimal for the yield. This is related to better plant growth during the second period after the first cut, probably due to improved establishment of the root system after the first period of adaptation to the cultured soil. These results indicate that the HE *S. alfredii* has an extraordinary ability to hyperaccumulate Zn at relatively low Zn levels in the soil, and the capacity of Zn hyperaccumulation is affected by the vigor of plant growth.

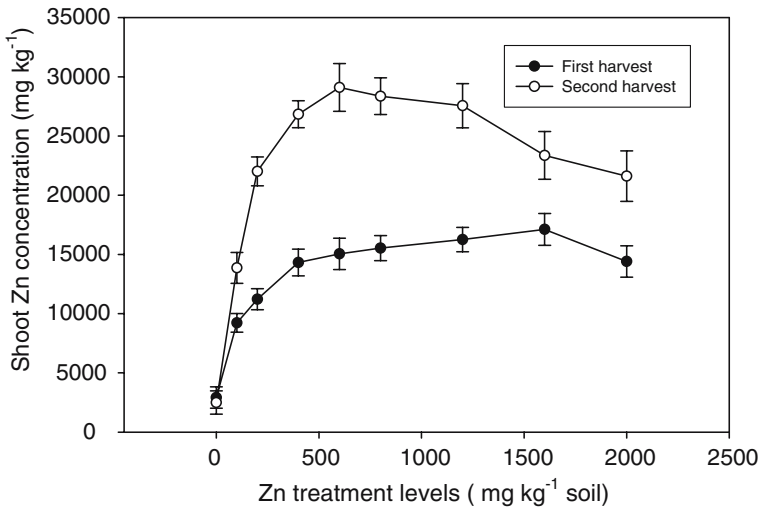


*Figure 2.* Dynamics of Zn uptake and accumulation in the shoots of the HE *Sedum alfiedii* Hance as a function of different Zn rates in the soil at the first and the second harvests. Data points and error bars represent means ± SE.

*Time-course of Zn uptake and accumulation in leaves, stems, and roots in the nutrient solution experiment*

Leaf and stem Zn concentrations of the two *S. alfredii* ecotypes increased with treatment time, reaching a plateau after 24 and 8 days in the 500 $\mu$M Zn treatment for the HE and the NHE, respectively (Figure 3). On day 0 (after plants had been precultured for 2 weeks with 1.0 $\mu$M Zn), leaf and stem Zn concentrations of the HE were approximately 16 and 13 times greater, respectively, than those of the NHE. On day 16, stem Zn concentrations of the HE was 19 times higher than those of the NHE grown at the low Zn level (1 $\mu$M Zn). At the high Zn level (500 $\mu$M), however, leaf and stem Zn concentrations increased with treatment time, and leveled off on days 24 and 16 of treatment for the HE and the NHE, respectively. Moreover, leaf and stem Zn concentrations of the HE were 15 and 21 times higher, respectively, than those of the NHE on day 24 grown with 500 $\mu$M Zn. The maximum stem Zn concentrations in the HE reached over 10,000 mg $kg^{-1}$ even when grown with 1.0 $\mu$M Zn (Figure 3b).

Root Zn concentrations of the two ecotypes increased rapidly with increasing treatment time, and leveled off on day 8 of treatment for the 500 $\mu$M Zn (Figure 3). At the low Zn level (1 $\mu$M), however, root Zn concentrations of both ecotypes increased slightly with exposure time and had a saturated stage between days 4 and day 32 of treatment for both ecotypes (Figure 3c and g). In contrast to leaf and stem, the HE and NHE had a similar pattern of root Zn concentration change with exposure time, but the NHE had approximately 1.2-fold higher root Zn concentration than the HE after 8 days of the treatment with 500 $\mu$M Zn. The differences in root Zn concentrations between the HE and the NHE after day 8 of the treatment were statistically significant ($P < 0.05$). The results indicate that HE *S. alfredii* has greater abilities of Zn transport from root to shoot.

*Short-term uptake and accumulation of $^{65}Zn^{2+}$*

Short-term uptake and accumulation of Zn by the two ecotypes were measured using $^{65}$Zn as a tracer. Similar to the long-term uptake dynamics, $^{65}Zn^{2+}$ uptake into the shoots increased with treatment time, and there were no differences in shoot $^{65}Zn^{2+}$ concentrations between the HE and the NHE *S. alfredii* after the initial 60 min uptake period (Figure 4). At the uptake time of 240 min, $^{65}Zn^{2+}$ uptake to the shoots gradually increased for the HE but levelled off for the NHE. The difference in $^{65}Zn^{2+}$ uptake to the shoots between the two ecotypes became larger with longer treatment time. After 240 min, the amount of $^{65}Zn^{2+}$ translocated to the shoots was

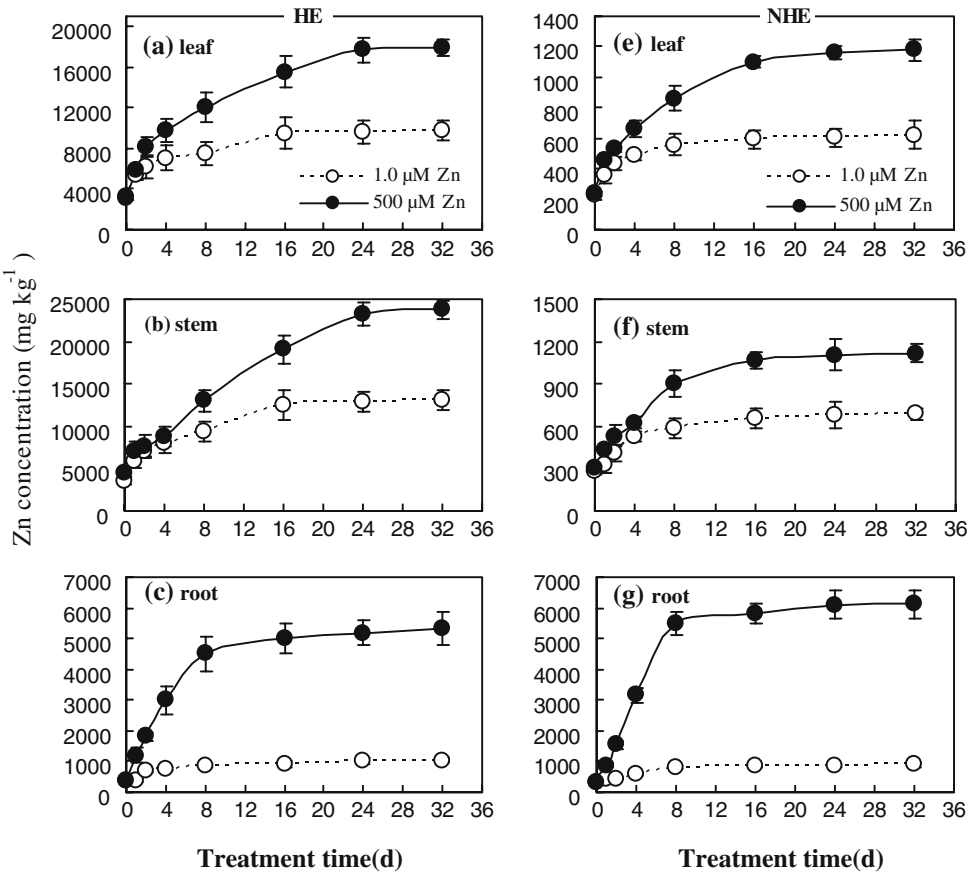


*Figure 3*. Dynamics of long-term Zn uptake and accumulation in leaves, stems, and roots of the two ecotypes of *Sedum alfredii* Hance with exposure time at Zn rates of 1.0 and 500 $\mu$mol $L^{-1}$, respectively. a, b, and c represent Zn concentrations in leaves, stems and roots of the HE *Sedum alfredii* Hance, respectively, and e, f, and g represent Zn concentrations in leaves, stems, and roots of the NHE *Sedum alfredii* Hance, respectively. Data points and error bars represent means ± SE.

2.1-fold higher in the HE than in the NHE. However, much smaller differences in $^{65}Zn^{2+}$ accumulation in the roots occurred between the two ecotypes, although root $^{65}Zn^{2+}$ uptake increased with treatment times. During the first 60 min period, the $^{65}Zn^{2+}$ concentrations in roots of the NHE were significantly higher than those in the HE. Throughout the experiment, only a small proportion of $^{65}Zn$ was translocated from roots to shoots in both ecotypes. The $Zn^{2+}$ concentration ratio of root/shoot was 37 and 61 for the HE and NHE, respectively. This is in agreement with the long-term uptake, in which about 1.2-fold higher root Zn concentrations were measured in the NHE than in the HE during the initial 8-day uptake period (Figure 3). At the uptake time of 240 min, the NHE accumulated a smaller amount of $^{65}Zn^{2+}$ in roots than the HE, presumably due to the high levels of non-labeled $Zn^{2+}$ (100 $\mu$M) and $Ca^{2+}$ (5 mM) in the desorbing solution. After 20-min desorption, 80% of the $^{65}Zn^{2+}$ accumulated during the uptake period was desorbed into the externa1 solution.

## Discussions

*Thlaspi caerulencens* and *Arabidopsis halleri* both belong to the Brassicaceae family, whereas *Sedum alfredii* is a naturally selected Zn hyperaccumulator in an old Pb/Zn mined area belonging to the Crassulaceae family. It is therefore interesting to compare the results obtained in the present study with those reported previously for

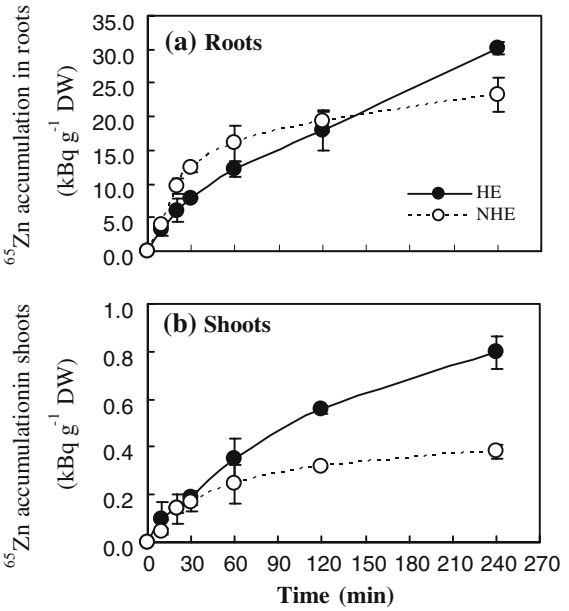


*Figure 4*. Short-term time course of $Zn^{2+}$ accumulation in roots (a) and shoots (b) of two ecotypes *Sedum alfiedii* Hance. Seedlings were immersed with roots in uptake solution containing 2 mM MES-Tris (pH 6.0), 0.5 mM CaCl2, and 10 $\mu$M $^{65}Zn^{2+}$ (45 kBq $L^{-1}$). Following the appropriate incubation period, roots were desorbed for 20 min. Then roots were separated from shoots, blotted, oven-dried at 65 °C and weighed, and their gamma activity was counted. Data points and error bars represent means ± SE.

*T. caerulencens* and *A. halleri*. The Zn hyperaccumulator *S. alfredi* can tolerate Zn levels up to 1000 $\mu$M in nutrient solution and 2000 mg $kg^{-1}$ in the soil without reduction in dry matter yield (Tables 1 and 2), which is close to those of *T. caerulencens* (Brown et al., 1995a, b) and *A. halleri* (Bert et al., 2000; Zhao et al., 2000). The dynamics of shoot Zn accumulation in the HE *S. alfredii* under both solution and soil culture conditions indicated a sharp increase with increasing external Zn levels and peaked at the optimal Zn level of 500 $\mu$M in nutrient solution and 800 mg $kg^{-1}$ in the soil, respectively (Figures 1 and 2), whereas Zn accumulation patterns in shoots of *T. caerulencens* or *A. halleri* increased linearly with increasing Zn levels up to 1000 $\mu$M (Brown et al., 1995a; Zhao et al., 2000). The HE *S. alfredii* accumulated more Zn in the stems than in the leaves (Figures 1 and 3). Brown et al. (1995b) reported that *T. caerulescens* leaves had a larger Zn concentration than stems. These results suggest that differences in the Zn distribution occurred between hyperaccumulators in the Crassulaceae and Brassicacea families.

It has been shown that both non-metallicolous and metallicolous populations of *T. caerulescens* (Assungco et al., 2003; Escarri et al., 2000; Meerts and van Isacker, 1997) and *A. halleri* (Bert et al., 2000, 2002) can hyperaccumulate Zn, with the former often showing a greater accumulation ability than the latter. These studies indicate that Zn hyperaccumulation is a constitutive trait in *T. caerulescens* and *A. halleri*. Similarly, Zhao et al. (2002) reported that arsenic hyperaccumulation is a constitutive property in *Pteris vittata*. In contrast, the present study shows that Zn hyperaccumulation is not a constitutive trait in *S. alfredii*. Only the metallicolous population (HE) of *S. alfredii*, can hyperaccumulate Zn, whereas the non-metallicolous population (NHE) does no hyperaccumulate Zn and is also much less tolerant to Zn than the HE. Plants of the two populations belong to the same species of *S. alfredii* according to their botanic characters. Analysis of phylogenetic tree of the HE and NHE *S. alfredii* and other *Sedum* species using the RAPD method shows the closest genetic linking of these two ecotypes, as compared with other *Sedum* species (X.E. Yang et al., unpublished data). Our results indicate that the HE *S. alfredii* has an extraordinary ability to hyperaccumulate Zn at relatively low Zn levels, and the capacity of Zn hyperaccumulation greatly relies on the plant growth vigor under both nutrient solution and soil culture conditions. The soil culture experiment indicated that shoot Zn accumulation capacity of the HE *S. alfredii* remarkably increased at the second harvest (Figure 2), and this trait is useful for the application of this hyperaccumulator to successive phytoextraction of Zn from the contaminated soils.

Comparative study on short-term (20 min) $^{65}$Zn uptake kinetics revealed that the concentration-dependent Zn influx velocity for Zn in *T. caerulescens* was 4.5 times greater than that in the non-hyperaccumulator *Thlaspi arvense* (Lasat et al., 1996), and this is partly attributed to a constitutively high expression of ZNT1, a high affinity Zn transporter, in both roots and shoots (Pence et al., 2000). Results from

short-term Zn uptake kinetic study in *S. alfredii* indicated that the maximum Zn influx velocity was 3.5 times greater in the HE than in the NHE *S. alfredii* (Li et al., 2005). Furthermore, Zn transport to the shoots was 3.5 times greater in the HE than in the NHE *S. alfredii* after a short-term (240 min) exposure (Figure 4). The root vacuoles of the HE *S. alfredii* sequestered less Zn than the NHE *S. alfredii*, making Zn more readily available for xylem loading in the hyperaccumulator plants (unpublished data), which is in agreement with the reports in *T. caerulescens* (Lasat et al., 1998). The molecular characterization and function of Zn transporters in the hyperaccumulator *S. alfredii* need to be further studied.

The long-term time course of Zn uptake indicated that Zn accumulation in shoots reached a plateau after growing at 500 $\mu$M Zn for 24 and 8 days, respectively for the HE and the NHE (Figure 3). This suggests that the shoot capacity of Zn accumulation may be one of the driving forces in Zn hyperaccumulation, which may be controlled by metal homeostasis in shoot cells. Three zinc transporter cDNAs isolated from the Zn hyperaccumulator *T. caerulescens* show elevated expression in *T. caerulescens* than in the non-accumulator of *T. arvense*, and the increased expression of ZAT gene might contribute to Zn tolerance (Assuncão et al., 2001). Comparative transcript profiling of metal hyperacumulator and non-hyperaccumulator shoots and roots has identified a distinct and tissue specific regulation of different members of the ZRT, IRT-like proteins (ZIP) and nicotianamine synthase (NAS) metal homeostasis gene families in *A. halleri* (Weber et al., 2004). When exposure to low or high Zn levels in nutrient solution, transcript abundance of several genes was found and confirmed to be higher in the hyperaccumulator *A. halleri* than in the non-hyperaccumulator *Arabidopsis thaliana*, and are involved in cellular Zn detoxification (Becher et al., 2004). In *A. halleri*, a NAS, a ZIP transporter, and other genes may be potential Zn hyperaccumulation factors. However, whether the HE *S. alfredii* possesses similar molecular mechanisms of Zn homeostasis as *A. halleri* is unknown. The results from our analysis of subcellular Zn distribution in shoot tissue indicated that *S. alfredii* prevents toxic Zn concentrations in the cytoplasm by sequestering the metal in the vacuoles of epidermal and subepidermal leaf cells (unpublished data). The biochemical and molecular mechanisms of Zn homeostasis in shoot cells of the hyperaccumulator *S. alfredii* need to be further clarified.

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