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Zinc Tolerance and Hyperaccumulation of *Sedum alfredii* Hance: A Greenhouse Experiment with Artificial Polluted Soils

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Heavy metal contamination of soils is a nationwide problem, arising mostly from anthropogenic sources such as mining, smelting, dumping of sewage wastes. Recently, the use of plants to extract heavy metal from soils (phytoextraction) is considered as an effective, in-situ, and highly promising technique for the remediation of polluted soils (Cunningham and Berti 1996; Raskin et al. 1997). Phytoextraction of heavy metals from polluted soils relies on the ability of a small number of higher plant species (termed hyperaccumulator) to grow on polluted soils, and to extract and transport metals from roots to their above ground parts (Salt et al. 1998). Approximately 450 hyperaccumulators have been reported, of which about 14 belong to Zn hyperaccumulator, defined as containing more than 10g/kg Zn in shoots dry matter (DM) (Baker et al. 2000).

Removal of heavy metal from polluted soils by phytoextraction depends on shoot metal concentration and shoot biomass of the hyperaccumulators. Unfortunately, most known hyperaccumulating plants have small biomass and/or lower growth rates. For example, *Thlaspi caerulescens* is a well known Zn hyperaccumulator plant. Hydroponically grown *Thlaspi caerulescens* can accumulate up to 33600 mg/kg Zn in shoots from a solution containing 650mg/L Zn (Brown et al. 1995). Wild populations of *Thlaspi caerulescens* growing on various contaminated sites were shown to have shoot Zn concentration ranging from 13000 to 21000mg/kg DW (Baker et al. 1994). However, *Thlaspi caerulescens* may not be suitable for many larger scale phytoremediation efforts, because these plants are small and slow growing (Ebbs et al. 1997).

One strategy for increasing phytoextraction efficiency is to search for new high biomass metal hyperaccumulators. *Sedum alfredii* Hance is a new Zn-hyperaccumulator found in an old Zn/Pb mining area in China (Yang et al. 2001; Ni et al. 2004). Zinc concentration in its shoots can reach over 20 g/kg grown at 80 mg/L Zn in nutrient solution without showing any toxic symptoms (Long et al. 2002). In addition, it has characteristics of fast growth, large biomass, asexual reproduction, and perennial. The plants of *Sedum alfredii* can grow up to 40 cm heights, propagate 3-4 times in a year if the environmental conditions are favorable (Yang et al. 2002). This species may have significant phytoremediation

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biomass production and fast growth. Before phytoremedition can be effectively exploited on contaminated sites, determination of Zn tolerance and uptake by *Sedum alfredii* is necessary. The objectives of this study were to examine quantitative relationships between Zn concentration in soil and Zn uptake in *Sedum alfredii*, and the tolerance of *Sedum alfredii* to soil Zn.

MATERIALS AND METHODS

Before the pot experiment, the topsoil (0~20 cm) of a upland agricultural soil, collected from Fuyang county, Zhejiang province, P. R. China, as the initial soil was incubated with different doses of Zn (100, 200, 400, 600, 800, 1200, 1600, 2000 mg Zn/kg soil) added as $ZnSO_4 \cdot 7H_2O$ under 70-80% of the maximum field water capacity condition for 12 months. Martinez and Motto (2000) reported that the transformation of heavy metals added as single solutions in mineral soils could get balance after 40-days incubation. After 12 months incubation, the soils were air-dried. Thus, the initial soil and incubated soils with different total Zn contents (165.1 mg/kg soil in the initial soil, 265.1, 365.1, 565.1, 765.1, 965.1, 1365.1, 1765.1, 2165.1 mg/kg soil in the incubated soils) were prepared for the pot experiment. The main properties of the initial soil were as follows: pH (soil : H_2O = 1 : 2.5) 6.13, Organic carbon 12.4 mg/g, Total N 0.89 mg/g, Total P 0.56 mg/g, Hydrolysable N 70.2 mg/kg, Available P 9.53 mg/kg, Total Zn 165.1 mg/kg, Total Cd 0.17 mg/kg, Total Cu 20.1 mg/kg, Total Ni 23.7 mg/kg, Total Pb 54.1 mg/kg.

The initial soil and the eight incubated soils with different Zn level were used as the treatments. 800 g of the prepared soil (based on dry weight), and 0.08 g of urea and 0.16 g of KH_2PO_4 as basal fertilizers were supplied in each plastic pot. The treatments were replicated five times, and randomly arranged, resulting in a total of 45 pots. The pots were placed individually in saucers, the soils were wetted to 70% of the maximum field water capacity by addition of deionized water to the saucer and allowed to equilibrate for 7 days. Water was added to the saucer rather than the surface of the soil in the pot to prevent any soluble Zn being leached from the pot, and the soil being compacted. Subsequently, three pre-prepared seedlings of *Sedum alfredii* Hance were transplanted to each pot. *Sedum alfredii* Hance was collected from an old mining area in Southeast China, and has been proved to be a new Zn-hyperaccumulating plant species (Long et al. 2002; Yang et al. 2002). The details of preparation of seedlings of *Sedum alfredii* Hance were referred to Long et al. (2002) and Ni et al. (2004). During the experiment, the deionized water was added to the saucers to return to the soils to 65%~70% of the maximum field water capacity as required.

The plants (shoots) were excised approximately 40 cm above the soil surface after 35 days growth, when some plant began to flower, and a few of new foliage appeared at the basal of the stem. The re-growth of the plants was re-sampled after further growth for 68 days. Plant shoots were washed with tap water and rinsed with deionized water, dried at 60℃ to constant weight, and the dry matter (DM) was recorded. Samples of plant dry materials were ground with stainless steel for Zn analysis.

The important characteristics of the tested soil were measured with conventional methods (Committee of Agrochem, Soil Sci Soc of China 1983). Soil total metals (Zn, Cd, Pb, Cu, Ni) were measured by the methods from Tessier et al. (1979). Soil water-extractable Zn (WE-Zn), ammonium acetate-extractable (AAE-Zn), and DTPA-extractable (DTPAE-Zn) were measured by shaking 5-g sub-samples of each soil with 25 ml deionized water, 1mol/L NH_4OAC (pH=7.0), or 0.005 mol/L DTPA (pH=7.3) respectively at 20℃ for 2h, all extracts were filtered, zinc concentrations in extracted solutions were determined in these extracts by atomic absorption spectroscopy (AAS). Approximately 0.1 g samples of plant material were weighed accurately into 50-ml borosilicate cup, and mineralized by oven-drying digestion methods at 550℃. Digested sample were dissolved with 10-ml 6.0mol/L HCl, mixed, filtered, and brought to 100 ml with 0.6mol/L HCl. Zinc concentrations in the solutions were determined by AAS.

All results were expressed on a dry weight basis. The significance of differences between the means of the treatments was evaluated by one-way analysis of variance followed by Duncan's multiple range test at 5%, 1% or 1‰ significance.

RESULTS AND DISCUSSION

Shoot dry weights of *Sedum alfredii* Hance were significantly ($p<0.05$) affected by zinc addition, with the greatest growth occurring at 400 Zn mg/kg soil supplied for the first harvest and 800 Zn mg/kg soil supplied for the second harvest and the total of two harvests (Table 1). Total shoot biomass of two harvests was significantly greater for zinc application (except the 2000 Zn mg/kg treatment) than for no Zn addition (the control). It was also noted that no nutrient deficiency nor toxicity symptoms were visible on *Sedum alfredii* plants at all Zn addition treatments (except the 2000 Zn mg/kg treatment), implying that *Sedum alfredii* Hance was tolerant to Zn.

Zinc concentration in shoots of *Sedum alfredii* Hance were also significantly ($p<0.01$) affected by Zn addition, with the highest concentrations occurring at the treatments with 400-1600 mg Zn/kg soil added for the first harvest and at the treatments with 400-1200 mg Zn/kg soil added (Figure 1). Zn concentration in shoots of *Sedum alfredii* with Zn addition treatment was significantly higher than that of the control, and higher than the identified concentration of Zn hyperaccumulation (10000 mg/kg) (Reeves and Baker 2000).

Similar response patterns of Zn accumulation in shoots with Zn addition were noted (Table 2). Total Zn accumulation in shoots of two harvests with Zn addition treatment was significantly higher than that of the control, peaked at the treatment with 800 mg Zn/kg soil added.

Added Zn can react with soil constituent, such as complexation with soil organic matter, sorption on oxides and clay minerals and precipitation as carbonates, hydroxides and phosphates, then its chemical forms and bioavailability to plant are also changed. Metal phytoavailability is a crucial issue for the assessment of

Table 1. Shoot biomass of *Sedum alfredii* Hance grown on the Zn-enriched soils.

Zn added	Shoot biomass (g/pot)		
(mg Zn/kg Soil)	First harvest	Second harvest	Total
0	0.53 ± 0.13 bc	0.36 ± 0.12 d	0.89 ± 0.22 d
100	0.62 ±0.07 abc	0.81 ± 0.19 c	1.43 ± 0.17 c
200	0.64 ± 0.11 ab	1.05 ± 0.17 bc	1.68 ± 0.24 bc
400	0.71 ± 0.14 a	1.15 ± 0.27 ab	1.86 ± 0.30 ab
600	0.59 ± 0.08 abc	1.39 ± 0.43 a	1.98 ± 0.46 a
800	0.60 ± 0.07 abc	1.45 ± 0.33 a	2.06 ± 0.36 a
1200	0.59 ± 0.09 abc	1.19 ± 0.29 ab	1.78 ± 0.33 ab
1600	0.54 ± 0.07 bc	0.99 ± 0.17 bc	1.53 ± 0.20 bc
2000	0.51 ± 0.09 c	0.25 ± 0.12 d	0.76 ± 0.14 d

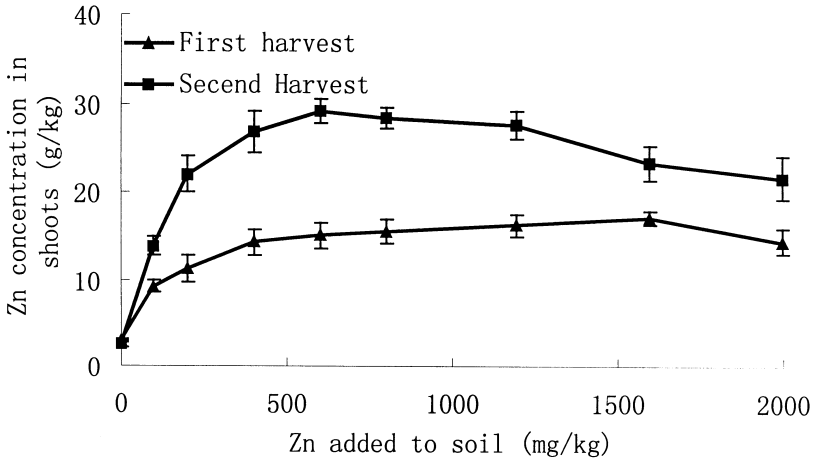

Figure 1. Zn concentration in shoot of *Sedum alfredii* Hance grown on the Zn-enriched soils.

Table 2. Zn accumulation in the shoots of *Sedum alfredii* Hance.

Zn added (mg Zn/kg Soil)	Total soil Zn (A) (mg/pot)	Zn accumulated in shoots (B) (mg/pot)			B/A×100
		First harvest	Second harvest	Total	
0	132	1.55±0.33	0.92±0.40	2.5 e	1.87
100	212	5.73±0.53	11.02±2.05	16.8 d	7.90
200	292	7.10±1.43	23.00±3.99	30.1 c	10.31
400	452	10.0±1.34	30.84±7.50	40.9 b	9.04
600	612	8.91±1.57	40.15±11.53	49.1 a	8.01
800	772	9.43±1.71	41.32±10.24	50.8 a	6.57
1200	1092	9.64±2.12	33.03±8.74	42.7 b	3.91
1600	1412	9.19±1.39	23.37±5.53	32.6 c	2.31
2000	1732	7.27±1.49	5.41±2.67	12.7 d	0.73

Table 3. Concentrations of water-extractable Zn (WE-Zn), ammonium acetate-extractable Zn (AAE-Zn), and DTPA-extractable Zn (DTPAE-Zn) in soils before planting and after two times' harvesting.

Zn added (mg Zn/kg Soil)	WE-Zn (mg/kg)			AAE-Zn (mg/kg)			DTPAE-Zn (mg/kg)		
	Before planting	After planting	Balance	Before planting	After planting	Balance	Before planting	After planting	Balance
0	nd	nd		nd	nd		2.79	2.87	+0.08^{ns}
100	0.50	0.51	+0.01^{ns}	4.14	2.64	-1.50**	38.93	24.71	-14.22***
200	3.14	0.66	-2.48***	11.10	5.93	-5.17***	64.91	42.93	-21.98***
400	10.26	7.47	-2.79**	21.38	36.27	+24.89***	120.8	123.4	+2.60^{ns}
600	32.09	14.97	-17.12***	47.90	57.83	+9.93***	271.4	215.9	-55.5***
800	54.54	23.86	-30.68***	87.71	149.5	+61.79***	443.3	341.1	-102.2***
1200	150.8	94.8	-56.0***	334.4	278.9	-55.5**	741.8	643.9	-97.9*
1600	247.3	111.1	-136.2***	486.6	386.8	-99.8*	1059	750.9	308.1**
2000	385.5	301.9	-83.6***	745.2	442.4	302.8***	1289	842.4	446.6***

nd: not detectable.

*, **, *** Significant between before planting and after harvesting under the same treatment at the 0.05, 0.01 and 0.001 probability levels, respectively, and ns means not significant.

the performance of phytoextraction. The biologically active fraction of Zn in soils mainly consists of its soluble, exchangeable and complexed forms, and many studies used single extractions (e.g. water, NH_4CH_3COO, and DTPA) for evaluating heavy metal phytoavailability in contaminated soil (Arnesen and Singh 1998; Miner et al. 1997). After 12 months incubation (before the pot experiment), phytoavailable Zn of different treatments was assessed through water, NH_4CH_3COO, and DTPA extraction. Concentrations of water-extractable (WE-Zn), ammonium acetate-extractable (AAE-Zn), and DTPA (DTPAE-Zn) extractable Zn increased progressively with Zn additions, and with the as DTPAE-Zn>AAE-Zn>WE-Zn. Under the added Zn≤800mg/kg soil conditions, only about 10% of added Zn can be extracted by water or ammonium acetate. However, a major portion of added Zn was extracted by DTPA, especially at high Zn treatment (≥800mg/kg) (Table 3).

After planting (two harvests), concentrations of water-extractable Zn, ammonium acetate-extractable Zn and DTPA extractable Zn generally decreased, with the comparison of that before planting, except ammonium acetate-extractable Zn concentrations of the treatments with 400, 600 and 800 mg Zn/kg soil added (Table 3). It would be impossible to detect any changes in the total concentration of in the tested soils due to the short duration. Differences due to plant uptake of metals would only be detectable in the more labile fraction of metals. Reduced concentration of water-extractable Zn may be interpreted as an indirect evidence of the effectiveness of uptake Zn from soils by *Sedum alfredii*.

The present study confirmed the exceptionally high tolerance of *Sedum alfredii* to soil Zn pollution as the shoot biomass of Zn added treatments (except the 2000 Zn mg/kg treatment) was significantly higher that of the control. Zn concentration in shoots with Zn addition treatment under pot experiment conditions being higher than 10000 mg/kg DW showed the characteristics of *Sedum alfredii* Hance for Zn hyperaccumulation. About 4% to 10% of total Zn in soil can be removed through harvesting shoots of *Sedum alfredii* under 265 to 1365 mg Zn/kg soil conditions (Table 2), which is considerable for phytoremediating Zn polluted soil by phytoextraction.

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