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Pb uptake, accumulation, subcellular distribution in a Pb-accumulating ecotype of *Sedum alfredii* (Hance)*

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Abstract: Lead concentrations in roots, stems and leaves of accumulating and non-accumulating ecotypes of *Sedum alfredii* (Hance) were studied through a hydroponic experiment with different Pb concentrations supplied as $Pb(NO_3)_2$. Lead concentrations in leaves and stems of the accumulating ecotype were 4-9 times and 3-5 times those of the non-accumulating ecotype, and Pb-accumulated amounts in stems and leaves of the accumulating ecotype were 4-9 times and 8-11 times higher than those of the non-accumulating ecotype, respectively. The results indicated that the accumulating ecotype had better ability to transport Pb from roots to shoots. The subcellular distributions of Pb in the root, stem and leaf tissues were studied using sucrose differential centrifugation. Approximately 50% of Pb contents was found to be associated with the cell wall fraction in stems of the accumulating ecotype and the percentage increased to 80% both in roots and leaves, no matter when plants were grown with different levels of Pb. The results indicated that the distribution of Pb on cell walls of the accumulating ecotype could mainly account for the high tolerance to Pb.

Key words: Pb, Accumulating ecotype, Subcellular distribution
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INTRODUCTION

Lead (Pb) exists in many forms in natural sources throughout the world, and is now one of the most widely and evenly distributed trace metals (Nriagu, 1992). Soil and plants can be contaminated by Pb from paints, gasoline additives, Pb smelting and refining, pesticide production, Pb acid battery breaking (Paff *et al.*, 1995). Considerable importance has been attached to the problem of Pb pollution with the development of modern industry and agriculture. However, most conventional remediation approaches do not provide acceptable solutions to toxic metal pollution. Only recently has the value of metal-accumulating plants for environmental remediation been fully recognized (Baker *et al.*, 1994; Raskin *et al.*, 1994). Phytoextraction, defined as the use of green plants to remove pollutants from the environment (Cunningham *et al.*, 1993), is being considered as a new highly promising technology for the remediation of polluted sites. For phytoextraction to be a feasible remediation tool, plants that are used must be able to take up large concentrations of heavy metals into the roots, to transport these metals to the shoots so that high concentrations are accumulated in the shoots, and to accumulate high biomass (Cunningham *et al.*, 1995). Besides, tolerance to heavy metal is also the key plant characteristic required for phytoextraction.

Our survey of plant population yielded a new Pb-accumulating ecotype, *Sedum alfredii* (Hance) in an old Pb/Zn mining area in Zhejiang Province of China (He *et al.*, 2002). In order to get information on the high Pb-accumulation of *S. alfredii* from soil to shoot, mechanisms that control uptake, translocation and subcellular distribution of Pb by *S. alfredii* must be understood. At present, understanding of these mech-

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anisms is poor (Kumar *et al.*, 1995; Blaylock *et al.*, 1997). Methods to investigate subcellular distribution of Pb were found that represented the basic idea for our study (Weigel *et al.*, 1980; Gabbrielli *et al.*, 1990).

The aim of this study was to investigate the effects of different concentrations of $Pb(NO_3)_2$ on Pb absorption, translocation and subcellular distribution in *S. alfredii*.

MATERIALS AND METHODS

Plant Materials The Pb-accumulating ecotype of *S. alfredii* was obtained from the old Pb/Zn mining area in Zhejiang Province of China. The non-accumulating ecotype of *S. alfredii* was obtained from a suburb of Hangzhou in Zhejiang Province of China.

Hydroponic experiment Healthy and equal-sized plants were chosen and grown for 2 weeks in basic nutrient solution containing 2.00 mmol/L $Ca(NO_3)_2 \cdot 4H_2O$, 0.10 mmol/L KH_2PO_4, 0.50 mmol/L $MgSO_4 \cdot 7H_2O$, 0.10 mmol/L KCl, 0.70 mmol/L K_2SO_4, 10.00 μmol/L H_3BO_3, 0.50 μmol/L $MnSO_4 \cdot H_2O$, 1.0 μmol/L $ZnSO_4 \cdot 7H_2O$, 0.20 μmol/L $CuSO_4 \cdot 5H_2O$, 0.01 μmol/L $(NH_4)6Mo_7O_{24}$, 100 μmol/L Fe-EDTA (Yang *et al.*, 2001). The nutrient solution was continuously aerated and renewed every 4 days. After growth for 14 days, the plants were transferred to modified nutrient solution, in which KH_2PO_4 concentration was adjusted to 0.005 mmol/L in order to prevent precipitation of Pb. The Pb^{2+} concentrations in nutrient solutions were 0, 20, 40, 80, 160, 320 mg Pb /L supplied as $Pb(NO_3)_2$. Each Pb treatment was replicated three times. Each replication consisted of 18 plants. Plants were harvested 30 days after treatment. The nutrient solution was continuously aerated and renewed every 4 days.

At harvest, roots were immersed in 20 mmol/L Na-EDTA for 15 min, and then the whole plants were rinsed with deionized water. The fresh weight of roots, stems and leaves was measured. The samples were divided into two groups: one for experimentally investigating Pb subcellular distribution (Weigel and Jager, 1980), while the other was dried and ground to pass through 60 mesh for determining the dry weight and the total element concentration.

Fractionation of roots, stems and leaves. The samples for determining Pb subcellular distribution were immediatedly frozen in liquid nitrogen, dried in a freeze dryer (ALPHA 1 – 4) and weighed again. Samples (0.500g) were immersed in 20 mL extractant (0.25 mol/L sucrose + 50 mmol/L Tris-HCL (pH 7.5) + 1.0 mmol/L dithioerythritol), shaken at low speed for 2 h, and then centrifuged at 300 × g for 5 min. The pellet was extracted twice again with 20 ml extractant, and centrifuged as before. The resulting pellet was designated as crude cell wall fraction (I). The supernatant from the three extractions was then centrifuged at 20000 × g for 45 min to separate cell organelles and membrane. The pellet was taken as membrane fraction (II). The resultant supernatant solution was referred to soluble fraction (III). The three fractions were dried at 60℃ in forced-air oven for determination of the element concentration.

Determination of Pb The samples for determining the Pb concentration were digested with concentrated HNO_3 at 180℃ – 200℃ for 7 h, then diluted to 25 ml, and analyzed by flame atomic absorption spectrometry(A6800).

RESULTS

Lead concentrations in roots, stems and leaves of both ecotypes with different Pb treatments are shown in Fig.1. Lead supply gave a rise to Pb concentrations in all plant tissues of the accumulating ecotype but only in roots and stems of the non-accumulating ecotype. The maximum Pb concentration in the stems of the accumulating ecotype was 915 mg/kg, 5 times that of non-accumulating ecotype, and the maximum concentration of leaves was 162 mg/kg, 4 times that of non-accumulating ecotype. From Fig.1, it can also be seen that the accumulating ecotype's ability of transporting Pb from roots to stems was stronger than that of the non-accumulating ecotype. For example, Pb concentrations in the stems and leaves of accumulating ecotype with supply of 320 mg Pb /L were 540% and 447% those in the non-accumulating ecotype, respectively. At the same time, the Pb concentration in the roots of the accumulating ecotype was only 125% that in the non-accumulating ecotype.

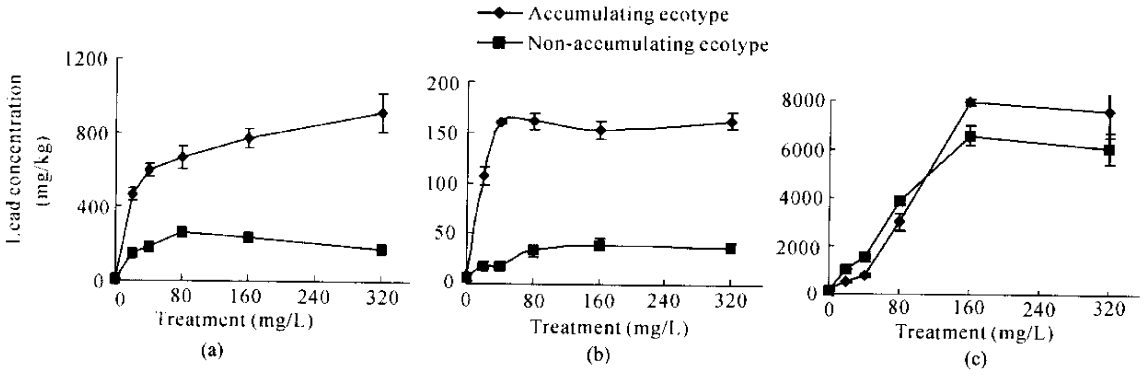

Fig.1 Pb concentrations in roots, stems and leaves of the accumulating ecotype and the non-accumulating ecotype with different levels of Pb treatments

(a)Pb concentrations in stems; (b)Pb concentrations in leaves; (c)Pb concentrations in roots

Table 1 Pb accumulation in roots, stems and leaves of the accumulating ecotype and the non-accumulating ecotype under different levels of Pb treatments (μg/plant)

Treatment (mg/L)	Accumulating ecotype						Non-accumulating ecotype					
	0	20	40	80	160	320	0	20	40	80	160	320
Root	–	22 d	36 d	146 c	453 a	374 b	–	33 d	48 c	102 b	122 a	118 a
Stem	1 f	48 e	57 d	66 c	77 ab	88 a	1 d	12 b	14 b	18 a	18 a	10 c
Leaf	2 c	19 b	27 a	30 a	28 a	30 a	1 c	2 b	3 b	3 a	4 a	3 a
ratio of stem/root	–	2.2	1.6	0.4	0.2	0.2	–	0.4	0.3	0.2	0.1	0.1
ratio of leaf/stem	2.1	0.4	0.5	0.5	0.4	0.3	1.4	0.2	0.2	0.2	0.2	0.3

For each treatment, amounts of tissue biomass were multiplied by Pb concentrations to determine element accumulation (μg/plant) for individual tissue types. Pb accumulations in roots were highest, followed by those in stems and the lowest were in leaves (except the accumulating ecotype at 20 – 40 mg Pb /L treatments). Stem/root ratios of accumulated Pb averaged 0.9 in the accumulating ecotype under different Pb treatments, 4.5 times that of the non-accumulating ecotype. Leaf/stem ratios of accumulated Pb averaged 0.41 in the accumulating ecotype, as compared to 0.22 in the non-accumulating ecotype. Stem/root ratios of accumulated Pb in the accumulating ecotype exceeded 1.0 for the 20 – 40 mg/L treatments, suggesting that there was a positive mechanism for transporting Pb from root to stem at low Pb concentrations.

In the tissues of root, stem and leaf, considerable amounts of Pb were accumulated in both soluble fraction and cell wall fraction, and only rather small quantities were detected in the membrane fraction.

As for the Pb content in the roots, the presence of Pb had some effects on both ecotypes. Lead stimulated the Pb accumulation in cell wall and the soluble fractions. However, the stimulation of Pb accumulation in the cell wall fraction was much stronger than that in the soluble fraction. For the accumulating ecotype, about 80% of Pb was distributed in the cell wall fraction even under the 320 mg Pb /L treatment, while less than 50% of Pb was found in the cell wall fraction of roots of the non-accumulating ecotype at Pb ≥ 160 mg/L (Table 2). On the other hand, the amount of Pb absorbed by roots of the accumulating ecotype averaged 1.23 times that in the non-accumulating ecotype at Pb ≥ 160 mg/L (Fig. 1). At the same time, however, the Pb concentration in the soluble fraction of the accumulating ecotype was only 43 % that of the non-accumulating ecotype (Table 2). This meant that the restriction of Pb penetrating into the symplasm was relatively higher in the accumulating ecotype.

Table 2 Pb distribution within root cell of the accumulating ecotype and the non-accumulating ecotype under different levels of Pb treatments (mg/kg DW)

Pb treatment (mg/L)	Soluble fraction		Membrane fraction		Cell wall fraction	
	Accumulating ecotype	Non-accumulating ecotype	Accumulating ecotype	Non-accumulating ecotype	Accumulating ecotype	Non-accumulating ecotype
0	–	–	–	–	–	–
	–	–	–	–	–	–
20	11.42d	211.6d	4.09c	17.70cd	426.5d	722.9cd
	(2)	(23)	(1)	(1)	(97)	(76)
40	29.67d	304.0d	7.32c	30.54bcd	700.6d	1055c
	(4)	(22)	(1)	(1)	(95)	(76)
80	229.0c	1546c	31.27b	65.62abc	2602.5c	1901b
	(8)	(44)	(1)	(1)	(91)	(54)
160	1530.4a	3466a	75.92a	71.74ab	6045a	2769a
	(20)	(55)	(1)	(1)	(79)	(44)
320	1254b	2941b	62.12a	86.55a	5653b	2528a
	(18)	(53)	(1)	(1)	(81)	(46)

* The number in parentheses means the percentages of Pb in different fractions.

Lead supply resulted in higher Pb concentration in roots of the accumulating ecotype at 160 mg Pb/L – 320 mg Pb/L treatments. However, only approximately 20% of Pb uptaken by roots of the accumulating ecotype could be found in the soluble fraction under 160 mg/L – 320 mg/L treatments, and its Pb concentrations averaged 43% that in the case of the non-accumulating ecotype (Table 2). Verkleij and Schat (1990) showed that plants commonly store toxic elements in the vacuoles of cortical tissue of roots outside the endodermis or in cell walls, thereby preventing the metals from being uptaken into rhizomes and aboveground tissues. It was suggested that the accumulating ecotype accumulated Pb mostly in cell walls of roots, while the non-accumulating ecotype accumulated Pb in vacuoles of cortical tissue of roots outside the endodermis, which also restricted Pb translocation up to shoots.

In the case of stems, Pb supply also increased Pb concentrations in both cell wall fraction and soluble fraction for both ecotypes. As for the accumulating ecotype, the stimulation of Pb accumulation was stronger in cell wall fraction than in soluble fraction. Over 50% of Pb was accumulated in the cell walls fraction even under 320 mg Pb /L treatment, while less 50% of Pb was accumulated in cell wall fraction for the non-accumulating ecotype at Pb $\geqslant$ 160 mg/L (Table 3). This also meant that the restriction of Pb penetrating into the symplasm was relatively higher in the stems of the accumulating ecotype. However, the Pb concentrations in the soluble fraction and cell walls fraction of the accumulating ecotype were 2 – 5 times and 2 – 8 times those of the non-accumulating ecotype, respectively (Table 3). Therefore, it was obvious that the Pb transport from root and stem was greater in the accumulating ecotype, as compared with the non-accumulating ecotype.

In leaves, the Pb concentrations of the cell wall fraction increased with increasing Pb concentrations in solution; while the Pb concentrations of the soluble fraction and membrane fraction had not significant differences. As Pb concentrations in solutions increased from 20 mg/L to 320 mg/L, the Pb content of cell wall fraction increased from 79.2% to 88.9% in leaf cells of accumulating ecotype, from 67.9% to 84.1% in the non-accumulating ecotype (Table 4). It was suggested that the Pb in leaves was mostly accumulated on the cell walls. However, the Pb concentrations of the cell wall fraction of the accumulating ecotype were 4 – 8 times that of the non-accumulating ecotype under different Pb treatments (Table 4).

Table 3 Pb distribution within stem cell of the accumulating ecotype and the non-accumulating ecotype under different levels of Pb treatments(mg/kg DW)

Pb treatment (mg/L)	Soluble fraction		Membrane fraction		Cell wall fraction	
	Accumulating ecotype	Non-accumulating ecotype	Accumulating ecotype	Non-accumulating ecotype	Accumulating ecotype	Non-accumulating ecotype
0	–	–	–	–	–	–
	–	–	–	–	–	–
20	144.8c	37.64c	8.23ab	3.43a	294.0d	64.4bc
	(32)	(29)	(2)	(3)	(66)	(69)
40	173.2c	57.35bc	9.96ab	1.71a	360.3c	103.7b
	(32)	(35)	(2)	(1)	(66)	(64)
80	227.1b	98.22a	16.61ab	3.73a	402.8b	188.0a
	(35)	(40)	(3)	(1)	(62)	(77)
160	303.5a	101.3a	15.61ab	3.61a	425.9b	76.91b
	(41)	(55)	(2)	(2)	(57)	(44)
320	414.8a	81.05ab	30.42a	2.56a	509.8a	64.39bc
	(44)	(55)	(3)	(2)	(53)	(43)

* The number in parentheses means the percentages of Pb in different fractions

Table 4 Pb distribution within leaf cell of the accumulating ecotype and the non-accumulating ecotype under different levels of Pb treatments(mg/kg DW)

Pb treatment (mg/L)	Soluble fraction		Membrane fraction		Cell wall fraction	
	Accumulating ecotype	Non-accumulating ecotype	Accumulating ecotype	Non-accumulating ecotype	Accumulating ecotype	Non-accumulating ecotype
0	–	–	–	–	–	–
	–	–	–	–	–	–
20	13.79ab	4.54a	1.48a	0.32a	82.73c	10.36c
	(14)	(30)	(2)	(2)	(85)	(68)
40	36.22a	4.24a	2.57a	2.98a	107.21b	17.51b
	(19)	(17)	(2)	(12)	(80)	(71)
80	13.30ab	3.77a	2.23a	0.47a	118.44ab	22.60ab
	(10)	(14)	(2)	(2)	(88)	(84)
160	15.35ab	4.90a	3.06a	0.61a	120.58ab	28.33a
	(11)	(12)	(2)	(4)	(87)	(84)
320	13.68ab	3.96a	2.68a	1.14a	130.13a	27.08a
	(9)	(12)	(2)	(8)	(89)	(81)

* The number in parentheses means the percentages of Pb in different fractions

DISCUSSION

The accumulating ecotype of *S. alfredii* from the old Pb / Zn mining area had the ability to accumulate Pb primarily in its roots, and transport and concentrate it in stems and leaves at much higher concentrations than in the non-accumulating ecotype. This is supported by the observation that the Pb concentrations in the stems and leaves of the accumulating ecotype were much higher than those of the non-accumulating ecotype. Also supporting this is the observation that the stem/root ratios of accumulated Pb in the accumulating ecotype exceeded 1.6 under the 40 mg Pb /L treatment. Under the 40 mg Pb /L treatment, the Pb concentration in stems was 65.5%, while that in leaves increased to 98.67% of the maximum Pb concentration. This indicated a better ability of the accumulating ecotype in transporting Pb from root to shoot at

low-Pb concentrations.

Yang *et al*.(2001) found that the accumulating ecotype of *S. alfredii* from the old Pb/Zn mining area also had the ability to uptake Zn in roots and hyperaccumulate it in shoots. In the presence of Zn, Zn concentration in stems of the accumulating ecotype was highest, followed by that in leaves, the lowest was in roots. Under Pb treatments, the Pb concentration in roots of the accumulating ecotype was highest, followed by that in stems and leaves. However, Pb is not generally considered as an essential element for the growth of plants. Lead uptake and accumulation by most plant species is very limited (Macnair, 1993). If the major goal of the phytoremediation process is to remove the maximum amount of element in the shortest time possible, then the selection should be based on the rate of element accumulation in harvestable tissues as well as on plant biomass accumulation. Under this standard, the accumulating ecotype would be perfect for the extraction of Pb from the contaminated soils.

From our results it can be concluded that Pb accumulated mainly on the cell walls of the roots, stems and leaves. This phenomenon was observed in both ecotypes, although the contents of Pb in the cell wall fraction were lower in the case of the non-accumulating ecotype. For the accumulating ecotype, the Pb concentrations detected on the cell walls of the roots, stems and leaves under different Pb treatments averaged 89%, 61%, 86% respectively, while in the non-accumulating ecotype, the Pb distributions in the cell wall fraction averaged 59%, 59%, 77%, respectively. The Pb concentrations in the cell wall fraction of the stems and leaves of the accumulating ecotype averaged 472% and 568% those of the non-accumulating ecotype under different levels of Pb treatments. Brown *et al*. (1972) showed that the high tolerance of Pb in plants results from Pb accumulation only in the cell wall without Pb penetrating into the symplasm. It is likely that this mechanism is more efficient in the accumulating ecotype, which appears to be more tolerant to toxic effects. As for the non-accumulating ecotype, the decrease in biomass may result from the higher contents of Pb distributed in the soluble fraction of roots, stems and leaves, when compared with the accumulating ecotype (He *et al*., 2002).

References

Baker, A. J., McGrath, S. P., Sidoli, C. M. D. and Reeves, R.D., 1994. The possibility of in situ heavy metal decontamination of polluted soil using crops of metal-accumulating plants. *Resour Conserv. Recycl*, **11**:41 – 49.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D. and Raskin, I., 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ Sci Technol*, **37**:860 – 865.

Brown, D.H. and Slingsby, D.R., 1972. The cellular location of lead and potassium in the lichen Cladonia rangiformis(L). *New Phytol*, **71**:297 – 305.

Cunningham, S.D. and Berti, W.R., 1993. Remediation of contaminated soils with green plants: an overview. *In Vitro Cell Dev. Biol*, **29**:207 – 212.

Cunningham, S.D., Berti, W.R. and Huang, J.W., 1995. Remediation of Contaminated Soils and Sludges by Green Plants. *In*: Hinchee R.E., Means J.L., Burris D.R., Eds. Bioremediation of inorganics. Batelle Press, Columbus, p.33 – 54.

Gabbrielli, R., Panddfini, T., Vergnano, O. and Palandri, N.R., 1990. Comparison of two serpentine species with different nickel tolerance strategies. *Plant and Soil*, **122**:271 – 277.

He, B., Yang, X.E., Ni, W.Z., Wei, Y.Z., Long, X.X. and Ye, Z.Q., 2002. *Sedum alfredii* Hance: a new lead-accumulating ecotype. *Acta Botanica Sinca*, **44**(11):1365 – 1370.

Kumar, P.B.A.N., Dushenkov, V., Motto, H. and Raskin, I., 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ Sci Technol*, **29**:1232 – 1238.

Macnair, M.R., 1993. The genetics of metal tolerance on vascular plants. *New Phytologist*, **124**:541 – 559.

Nriagu, J.O., 1992. Toxic metal pollution in Africa. *Sci Total Environ*, **121**:1 – 37.

Paff, S.W. and Bosilovich, B.E., 1995. Use of Pb reclamation in secondary lead smelters for the remediation of lead contaminated sites. *Journal of Hazardous Materials*, **40**:139 – 164.

Raskin, I., Kumar, P.B.A., Dushenkov, S. and Salt, D.E., 1994. Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol*, **5**:285 – 290.

Verkleij, J.A.C. and Schat, H., 1990. Mechanisms of Metal Tolerance in Plants. *In*: Heavy Metal Tolerance in Plants-Evolutionary Aspects, CRC Press, Boca Raton, FL., p.179 – 193.

Weigel, H.J. and Jager, H.J., 1980. Subcellular distribution and chemical form of cadmium in bean. *Plant physiol*, **65**:480 – 482.

Yang, X.E., Long, X.X., Ni, W.Z. and Ni, S.F., 2001. Zinc tolerance and hyperaccumulation in a new ecotype of *Sedum alfredii* Hance. *Acta Phytoecolgica Sinica*, **25**(6): 670 – 677.