## Cadmium and Other Metal Uptake by *Lobelia chinensis* and *Solanum nigrum* from Contaminated Soils

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**Abstract** Cadmium concentrations in two plant species and their corresponding soils were evaluated in a metal contaminated area. The average Cd concentrations reached 36.9 and 141 mg kg<sup>-1</sup> in *Solanum nigrum* leaves and *Lobelia chinensis* shoots, respectively. There is a significant relationship between the Cd concentration in the aerial tissues and the corresponding soils on a logarithmic scale. Under the hydroponic culture conditions, the maximum Cd concentration in the *S. nigrum* leaves and *L. chinensis* shoots were 1,110 and 414 mg kg<sup>-1</sup>, respectively. Cd concentration was higher in the roots than in the aerial parts. The two plants may be used in suitable phytoremediation process.

**Keywords** Cadmium · Accumulation · Plant · Phytoextraction · Contaminated soil

Phytoextraction, which refers to the use of metal-accumulating plants that can extract and translocate contaminants to the harvestable above ground parts, is of growing interest because of its low environmental impact and cost-effectiveness, even if a longer time is usually

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required for this kind of clean-up process (Salt et al. 1998). It has been well recognized that the success of any phytoextraction technique depends upon suitable plant species that can accumulate heavy metals and produce large amounts of biomass. The metal concentrations in plant shoots are generally more important than plant biomass yields (McGrath 1998; Shen et al. 2002), and the use of hyperaccumulators is very important for phytoextraction of heavy metals in polluted soils. Hyperaccumulators are defined as high plant species whose shoots contain >100 mg Cd kg<sup>-1</sup>, >1,000 mg Ni, Pb and Cu kg<sup>-1</sup>, or >10,000 mg Zn and Mn kg<sup>-1</sup> (dry wt) when grown in metal rich soils (Baker and Brooks 1989).

Cadmium is a highly toxic and nonessential element. No suitable method is yet available to selectively remove Cd in a short time without harmful effect on the properties of soil. As an alternative, phytoextraction of Cd from contaminated soils may be needed in some cases (McGrath 1998). Some plant species, such as *Thlaspi caerulescens* and *Arabidopsis halleri*, have been shown to accumulate Cd above 100  $\mu$ g g<sup>-1</sup> in shoot DM (Baker and Brooks 1989; Küpper et al. 2000; Lombi et al. 2000; Bert et al. 2002). Recently, *Viola baoshanensis* (Liu et al. 2003) and *Salsola kali* (de la Rosa et al. 2004) have been reported to be capable of hyperaccumulating Cd from soils.

Solanum nigrum L. is an annual herb with 0.3–1 m in height, which is often found in contaminated areas. It has been previously identified as a Cd hyperaccumulator with a maximum concentration of 125 mg kg<sup>-1</sup> from the pot experiment (Wei et al. 2005). Lobelia chinensis Lour. is a perennial herb with stolons. It normally grows 0.06–0.2 m in height with low biomass. Preliminary result in our field investigation showed that S. nigrum and L. chinensis collected in the contaminated sites of the Xiangxi area were able to take up large amounts of cadmium (Peng et al.

2006). The present study was undertaken using field study combined with solution culture experiments to investigate the Cd accumulation capacities by the two plant species.

## **Materials and Methods**

Plant and soil sampling was carried out in the Xiangxi autonomous prefecture (109°10′00″E–109°22′30″E, 27°44′30″N–29°28′00″N) located in the northwest of Hunan Province, Southern China. Xiangxi has a mean annual temperature of 13.5–16.7°C with an average annual rainfall of 1,419 mm. The region has an average altitude of 800–1,200 m with a subtropical mountainous moist climate.

The samples of the plants and soil were collected from four contaminated sites: two wastelands around the Xiangxi Zn and Mn smelters in Jishou City; one mine tailing ruin of the Tuanjie Pb–Zn mine in Huayuan County; one wasteland site around the Zhengxin Mn smelter in Huayuan County. Plant samples included the leaves, stems and roots of *S. nigrum* and the shoots of *L. chinensis* (lack of root samples due to low biomass). At least ten sections of leaves, stems and roots from each plant were collected at each sampling location, and then they were mixed to give a representative sample. The soils in which the sampled plants were growing were also collected for chemical analysis.

In hydroponic experiments, the seeds of S. nigrum were germinated in a mixture of perlite and vermiculite in plastic dishes moistened with tap water for 15 days under the following condition: 16 h per day length with a light intensity of 350 µmol photons m<sup>-2</sup> s<sup>-1</sup> supplied by fluorescent tubes, 25/20°C day/night temperature, and 60%-70% relative humanity (RH). After germination, the seedlings were transplanted to a vessel containing 2.5 L Hoagland nutrient solution at pH 5.6 (1 mM KH<sub>2</sub>PO<sub>4</sub>; 1 mM KNO<sub>3</sub>; 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>; 1 mM MgSO<sub>4</sub>; 20 μM Fe-EDTA; 46 μM H<sub>3</sub>BO<sub>3</sub>; 9 μM MnCl<sub>2</sub>; 0.77 μM ZnSO<sub>4</sub>; 0.32 μM CuSO<sub>4</sub>; 0.11 μM H<sub>2</sub>MoO<sub>4</sub>). Thirty-five days later, the seedlings were treated with 0 (control), 5, 10, 20, 50, 100, 200 and 500 µM CdCl<sub>2</sub>. Each treatment was replicated in three vessels with each containing ten plants. The nutrition solutions were renewed every 2 days. After 15 days, plants were harvested.

Stolons of *L. chinensis* were collected from the sampling site around a zinc smelter wasteland in Jishou City. The stolons were inserted into the soil from a vegetable field in order to obtain the breeding young plants for use in the hydroponic experiments in a glasshouse. When enough stolons grew up, the new branches were cut and transferred to 500 mL containers filled with 500 mL tap water for 10 days. The tap water was replaced with 500 mL Hoagland nutrient solution after the new root developed and

grew to about 30 mm. About 40 days later, the asexual young plants were treated with 0 (control), 5, 10, 50, 100, 200 and 500  $\mu$ M CdCl<sub>2</sub>. Fifteen days after the treatment, the plants were harvested.

All the plants samples were dried at 80°C in an oven and ground into fine powder in an agate mortar. Soil samples were air-dried at room temperature and ground in an agate mortar, and sieved to 80 mesh (<180 µm). Plant samples were digested with concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (87:13, v/v) and soil samples with concentrated HF:HNO<sub>3</sub>:HClO<sub>4</sub> (4:1:1, v/v). Metal concentrations were determined using inductively coupled plasma atomic emission spectrometry (Perkin Elmer Optima 3000 DV) and atomic absorption spectrometer with graphite furnace (TAS-986). The accuracy of chemical analysis was checked using a standard reference plant material (NIST SRM 1573). There was good recovery of Cd in the reference material, and the precision of analytical results was <10%.

## **Results and Discussion**

Table 1 shows that the concentrations of Cd in the soils varied between 3.13 and 1,100 mg kg<sup>-1</sup> DW, and were rather high compared with the safety threshold concentration in soils (0.2 mg kg<sup>-1</sup>) for agricultural use developed by the Chinese Government (GB 15618-1995). The concentrations of Pb, Zn and Cu in soils varied from 42 to 11,700 mg kg<sup>-1</sup>, 250 to 5,930 mg kg<sup>-1</sup> and 8.91 to 3,960 mg kg<sup>-1</sup>, respectively (Table 1). In most soil samples, the concentrations of Pb, Zn and Cu exceeded the maximum values permitted for agricultural soils set by Chinese Government (GB 15618-1995).

Tables 2 and 3 show the Cd concentrations in the leaves (or shoots) and roots of S. nigrum and L. chinensis, respectively. The maximum Cd concentration was 205 mg kg $^{-1}$  in the shoots of L. chinensis collected at the Datianwan I site (Table 3). Cadmium concentrations in the shoots of L. chinensis were generally high. The ability of L. chinensis to accumulate Cd in their aerial parts was extraordinary in comparison with other plant species grown at the same contaminated sites (Peng et al. 2006). The S. nigrum species had been previously identified as a Cd hyperaccumulator with a maximum concentration of 125 mg kg<sup>-1</sup> from the pot experiment with the soil containing 25 mg Cd kg<sup>-1</sup> (Wei et al. 2005). In the present study, all the leaf samples of S. nigrum at the contaminated sites had lower than 100 mg kg<sup>-1</sup> Cd in dry matter. The normal concentration of Cd in plants ranged from 0.05 to 0.2 mg kg<sup>-1</sup> (Kabata-Pendias and Pendias 1992). Wenzel and Jockwer (1999) suggested that the plant with the concentration of Cd in shoots greater than 50 mg kg<sup>-1</sup> should be regarded as Cd hyperaccumulator species.



**Table 1** The ranges and mean concentrations of Cd, Pb, Zn and Cu in the soils and the safety thresholds for agricultural production developed by the Chinese Government (STAPRCG)

Site	No.	Cd (mg kg <sup>-1</sup> ) range (a.m.)	Pb (mg kg <sup>-1</sup> ) range (a.m.)	Zn (mg kg <sup>-1</sup> ) range (a.m.)	Cu (mg kg <sup>-1</sup> ) range (a.m.)
Datianwan I	22	7.87–1,100	72.8–3,180	806–5,930	32.0–560
		(194)	(1,200)	(2,290)	(131)
Datianwan II	10	7.23-282	72.8-2,160	537-4,240	36-3,960
		(124)	(968)	(2,470)	(840)
Tuanjie	10	3.13-146	174–11,700	1,109-3,970	8.91-93.6
		(47.6)	(3,800)	(2,600)	(40.1)
Huayuan	9	3.14-7.12	42.1–215	250-584	26.2-38.8
		(4.88)	(92.6)	(393)	(31.7)
STAPRCG		≤0.20	≤35	≤100	≤35

STAPRCG, safety thresholds for agricultural production developed by the Chinese Government (GB 15618-1995); a.m, arithmetic mean

**Table 2** The ranges and mean concentrations of Cd, and bioaccumulation factors in *S. nigrum* collected at four contaminated sites

Site	No.	Cd Range (a.m.) (mg kg <sup>-1</sup> DW)		BF
		Leaf	Root	
Datianwan I	5	32.6-61.1 (49.4)	16.9–33.4 (27.4)	0.64
Datianwan II	4	41.7-63.4 (65.8)	_	3.15
Tuanjie	7	16.6-67.7 (27.8)	4.88-18.8 (8.64)	2.54
Huayuan	6	18.5–33.2 (22.4)	0.44-1.86 (1.12)	5.24

a.m, arithmetic mean; BF, bioaccumulation factor = metal concentration in leaf/metal concentration in soil

Table 3 The ranges and mean concentrations of Cd, and bioaccumulation factors in *L. chinensis* collected at Datianwan I site in summer and autumn

	No.	Cd Range (a.m.) (mg kg <sup>-1</sup> DW)	BF
Summer	5	81.1–205 (144)	1.21
Autumn	5	116–182 (137)	2.05

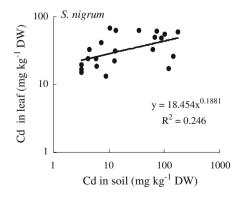
a.m, arithmetic mean; BF, bioaccumulation factor = metal concentration in leaf/metal concentration in soil

Regarding the translocation of Cd from root to shoot, the Cd concentration in leaves of S. nigrum was all significantly higher than that in the roots. This pattern was not clear for the species L. chinensis, due to the lack of root Cd concentrations (root samples were not enough for chemical analysis in the present study). The ratio of the Cd concentration in plants to the Cd concentration in soil represents the bioaccumulation factor (BF). In the shoots of L. chinensis, the average BF was 1.63, and 60% of the plants had a BF > 1.0. As for S. nigrum, the average BF was 2.96. Zhao et al. (2003) reported that the BF of Cd hyperaccumulator T. caerulescens ranged from 0.2 to 73.

Figure 1 shows the relationships between the concentration of Cd in *S. nigrum* leaves or *L. chinensis* shoots and the concentration of total Cd in soil. On a logarithmic scale, the concentration of Cd in plant tissues correlated significantly with the soil Cd concentration. Similar relationship of the shoot-soil Cd was also reported for *T. caerulescens* (Zhao et al. 2003).

The concentrations of Cu in normal plants ranged of 5–30 mg kg $^{-1}$  (Kabata-Pendias and Pendias 1992). In the present study, the range of Cu concentrations in aboveground plant tissue was from 14 to 44 mg kg $^{-1}$  in S.

Fig. 1 Relationship between Cd concentrations in plants and soils



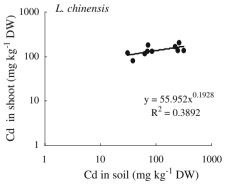




Table 4 The ranges and mean concentrations of Pb,Zn and Cu in leaf or shoot of the two plant species collected at contaminated sites

Plant species No.		Pb (mg kg <sup>-1</sup> ) range (a.m.)	Zn (mg kg <sup>-1</sup> ) range (a.m.)	Cu (mg kg <sup>-1</sup> ) range (a.m.)
S. nigrum	22	18.1–382 (77.9)	250–1,570 (896)	14.1–43.5 (24.5)
L. chinensis	10	60.9–1,150 (297)	1,243–2,270 (1781)	10.5-87.2 (31.4)

*nigrum* and from 10 to 87 mg kg $^{-1}$  in L. chinensis (Table 4). Most of samples analyzed showed the Cu concentrations were well below 30 mg kg<sup>-1</sup>. The average Pb concentrations were 297 and 77.9 mg kg<sup>-1</sup> in L. chinensis and S. nigrum, respectively (Table 4). The highest Pb concentration of 1,150 mg kg<sup>-1</sup> was found in the shoots of L. chinensis, which was greater than the criterion of 1,000 mg kg<sup>-1</sup> for Pb hyperaccumulators (Baker and Brooks 1989). This specimen also contained higher Cd, Zn and Cu than other specimens of L. chinensis. The concentration of Zn in the plants ranged from 250 to 1,570 and 1,243 to 2,270 mg kg<sup>-1</sup> with the mean values of 896 and  $1,780 \text{ mg kg}^{-1}$  in species S. nigrum and L. chinensis, respectively. Although the limit of hyperaccumulation  $(10,000 \text{ mg kg}^{-1})$  was never exceeded, considerably higher Zn concentrations were found in all specimens than the values of 27-150 mg kg<sup>-1</sup> in normal plants from uncontaminated soils. S. nigrum has been shown to accumulate up to 1,430 and 3,690 mg kg<sup>-1</sup> Zn in the leaves and stems, respectively (Marques et al. 2008).

Under hydroponic culture conditions, the 5  $\mu$ M Cd treatment had no significant effect on the shoot dry weight of *S. nigrum* (Table 5). At 10  $\mu$ M Cd and more, the negative effect on the shoot dry weights was significant. Compared with the control, 5–100  $\mu$ M Cd increased the root dry weight of *S. nigrum*. The root dry weights of *S. nigrum* and *L. chinensis* decreased significantly with Cd concentration >200 and >100  $\mu$ M, respectively.

The concentrations of Cd in the leaves and stems of *S. nigrum* increased as the concentration in the solution medium increased from 0 to 200  $\mu$ M. At 5  $\mu$ M, the plants accumulated up to 167 and 182 mg kg<sup>-1</sup> of Cd in the leaves and stems of *S. nigrum* (Table 5). The highest concentration of Cd in leaves was found in 200  $\mu$ M Cd treatment, which represented almost 7 times the amount found at 5  $\mu$ M Cd. For *L. chinensis*, the maximum Cd concentration in the shoots was 414 mg kg<sup>-1</sup> found at the 500  $\mu$ M Cd treatment.

As has been observed in shoot tissues, the concentration of Cd in roots drastically increased as the Cd concentration increased in solution. The roots of the two plant species had higher concentrations of Cd than the aerial parts in all Cd treatments. This result was different from our observation of the field survey. Sun et al. (2006) observed that the concentration of Cd in the shoots of S. nigrum was always higher than that in the root using pot culture experiments. A probable explanation is that high amount of Cd was likely to precipitate with phosphate deposit on the root surface or in the apoplast due to high concentration of phosphate in Hoagland solution. When the Cd concentration in medium is high under hydroponic culture conditions, the plant intracellular accumulation rate is lower than the metal sorption by the root apoplastic pathway (Citterio et al. 2003). Nedelkoska and Doran (2000) suggested that Cd uptake by fine roots was due to sorption rather than intracellular mechanisms. In comparison with

Table 5 The dry weights (DW) and Cd concentration of S. nigrum and L. chinensis plants under hydroponics conditions

Cd (μM)	S. nigrum					L. chinensis			
	DW (mg/plant)		Cd (mg kg <sup>-1</sup> )		DW (mg/plant)		Cd (mg kg <sup>-1</sup> )		
	Shoot	Root	Leaf	Stem	Root	Shoot	Root	Shoot	Root
0	390 a	54.1 b	4.97 f	1.78 h	21.4 g	31.5 a	29.5 a	0.31 f	1.51 g
5	389 a	73.7 a	167 e	182 fg	1,510 f	30.4 a	26.4 ab	62.1 e	338 f
10	336 b	75.9 a	262 d	261 f	2,100 ef	21.9 b	25.0 ab	68.5 e	508 e
20	299 bc	71.9 a	261d	558 e	2,840 de	_	_	-	_
50	260 bc	76.3 a	586 c	783 d	3,440 cd	32.3 a	29.4 a	142 d	3,030 d
100	268 bc	72.2 a	752 b	1,510 c	5,060 bc	22.6 b	23.9 b	245 с	4,540 c
200	160 d	43.0 c	1,110 a	2,230 b	6,340 b	23.4 b	19.7 c	348 b	7,900 a
500	93.3 e	15.2 d	714 b	3,440 a	13,600 a	26.7 b	20.4 c	414 a	6,320 b

Same letters stand for no significant difference at p < 0.05



the results obtained in the field survey, the lower shoot/root ratio of Cd concentrations in *S. nigrum* was probably due to the much denser fibrous root systems under hydroponic culture conditions. In the field survey, the root samples of *S. nigrum*, were mainly composed of taproots because of difficulty in collecting the fine fibrous roots from soil.

In conclusion, the results of hydroponic culture experiment showed that *S. nigrum* and *L. chinensis* can accumulate Cd up to 262 and 142 mg kg<sup>-1</sup>, respectively, in *S. nigrum* leaves and *L. chinensis* shoots without significant decrease in plant growth. It is suggested that both *S. nigrum* and *L. chinensis* may be Cd tolerant plants since the roots of the two species had higher concentrations of Cd than the aerial parts.

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