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Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator—*Lonicera japonica* Thunb

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ABSTRACT

Phytoremediation using hyperaccumulators is a promising technique of removing soil pollutants. In the study, growth responses, cadmium (Cd) accumulation capability and physiological mechanisms of *Lonicera japonica* Thunb. under Cd stress were investigated. Exposed to 5 and 10 mg L^{-1} Cd, the plants did not show any visual symptoms, furthermore, the height, dry biomass of leaves, roots and total and the chlorophyll (CHL) content were obtained different grade increase. When the concentration of Cd was up to 50 mg L^{-1}, the height, dry biomass of leaves and roots had not significant differences compared with the control. The indexes of tolerance (IT) were all above 0.8. The maintenance of high superoxide dismutase (SOD) and catalase (CAT) activities was observed along with the increased Cd concentration, suggesting strong internal detoxification mechanisms inside plant cells. After 21 days exposure to 25 mg L^{-1} Cd, stem and shoot Cd concentrations reached 344.49 ± 0.71 and 286.12 ± 9.38 μg g^{-1} DW, respectively and the plant had higher bioaccumulation coefficient (BC) and translocation factor (TF). According to these results, it was shown *L. japonica* had strong tolerance and accumulation capability to Cd, therefore it is a potential Cd-hyperaccumulator.

1. Introduction

Over the past five decades, the worldwide release of cadmium (Cd) has reached 22,000 t (metric ton) [1]. Cadmium contamination in soils has become a global concern as Cd is not only absorbed by plants or other life forms, but it is easily transferred to human food chain. Therefore, it is important and urgent to develop methods to cleanup Cd-contaminated soils.

Phytoremediation has become a promising soil remediation technique where hyperaccumulators or accumulators are used to take up large quantities of pollutant metals [2,3]. Phytoremediation takes advantage of the natural processes of plants. These processes include water and chemical uptake, metabolism within the plant, and the physical and biochemical impacts of plant roots [2]. More than 400 species of hyperaccumulators have been documented in the world, however, few of them have been considered as Cd-hyperaccumulators [4,5], and phytoremediation technology has not so far been widely used in practice for such problems as low biomass, slow growth rate and long growing seasons of hyperaccumulators or accumulators, which are generally confined to endemic species growing in mineral and rocky soil [4]. Thus, it is very important to identify new universal and feasible hyperaccumulators or accumulators of Cd as the groundwork for the successful phytoremediation of Cd-contaminated soils [6].

Cd is not essential to plant growth, and it can cause various phytotoxic symptoms including leaf chlorosis, root putrescence, growth inhibition, etc. The normal range of Cd concentration in leaf tissue (dry weight) of some species is 0.05–0.2 μg g^{-1} [7], however, hyperaccumulators have been known to accumulate Cd above 0.01% dry tissue (100 μg g^{-1}) [4]. Cd was also known to induce the burst of active oxygen species (AOS) in plant tissues, leading to oxidative stress [7–12]. To control the level of AOS, plants have evolved enzymatic and non-enzymatic defense systems [13,14]. Among these defense systems antioxidative enzymes, especially superoxide dismutases (SOD) and catalases (CAT) play an important role in scavenging ROS through a series of complex reactions [15–19]. Accordingly, hyperaccumulators or accumulators should take an effective Cd tolerance strategy by the cooperation of antioxidative enzymes under Cd stress [20].

Lonicera japonica Thunb. (Japanese honeysuckle), as a popular ornamental, has become established in temperate and tropical regions worldwide in the past 150 years, and it is also widely used in Asian medicine [21]. It has deep root and shoots could reach as long as 150 cm. It possesses characteristics of high biomass, easy cultivation, extensive competitive ability, wide geographic distribution, strong resistance to environmental stress like bacterial, viral and oxidative stress [22]. However, little information is available on the accumulation potential and tolerance to Cd on *L. japonica*.

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In the study, we investigated responses of the plant to varied Cd supply levels in hydroponic system, including Cd concentration in plant tissues, antioxidative enzymes (SOD and CAT), chlorophyll (CHL) content and lipid peroxidation. The aims were to identify the capability of accumulation and tolerance of *L. japonica* to Cd and assess Cd tolerance strategies taken by *L. japonica* in contaminated environment. Furthermore it can provide a reference for screening hyperaccumulators for phytoremediation of soils contaminated by Cd.

2. Materials and methods

2.1. Plant culture and Cd exposure

Cuttings of *L. japonica* Thunb. were collected from a non-contaminated field in the Shenyang Botanical Garden of Chinese Academy of Sciences and propagated in sterilized sand. After 6 weeks, plants were transformed to 500 ml adumbral containers for hydroponics in a greenhouse, four plants for each. The nutrient medium was a modified Hoagland solution [23] containing the following ingredients (mmol L^{-1}): $Ca(NO_3)_2{\cdot}4H_2O$ 5.00, $MgSO_4{\cdot}7H_2O$ 2.00, KNO_3 5.00, KH_2PO_4 1.00, H_3BO_3 0.05, $ZnSO_4{\cdot}7H_2O$ 0.80×10^{-3}, $MnCl_2{\cdot}4H_2O$ 9.00×10^{-3}, $CuSO_4{\cdot}5H_2O$ 0.30×10^{-3}, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.02×10^{-3}, Fe–EDTA 0.10.

The nutrient medium was continuously aerated with an aquarium air pump, renewed once every 3 days, and the pH was daily adjusted to 5.8 ± 0.1 with 0.1 M HCl or 0.1 M NaOH. After the plants were cultivated for 1 week in 50% Hoagland solution, the nutrient medium was changed into 100% Hoagland solution for next 2 weeks. Then $CdCl_2{\cdot}2.5H_2O$ was added into the solution to get: 0 (CK), 5, 10, 25 and 50 (mg L^{-1}), equal as 0 (CK), 44.48, 88.96, 222.40 and 444.80 (μM), respectively. The experiment was repeated three times. The plants were grown in a greenhouse at a temperature of 23 ± 2 °C in October 2007 and harvested 1, 2 and 3 weeks later for analysis.

2.2. Assays of CHL, lipid peroxidation and enzyme activity

Fresh tissue (0.1 g) was homogenized in a pre-chilled mortar under ice-cold conditions in 5.0 ml 50 mM cold Na-phosphate buffer (pH 7.8), with 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP). After centrifuging at $13{,}000 \times g$ for 30 min at 4 °C the supernatant was used for further analyses.

After 14 days Cd exposure, the chlorophyll (CHL) content was measured in 80% acetone extract of 0.1 g leaf tissue [24]. Lipid peroxidation was estimated by the concentration of malondialdehyde (MDA), the major thiobarbituric acid (TCA) reactive material, as described by [25]. All enzyme activities were calculated on the base of fresh weight (FW). Superoxide dismutase (SOD) was measured by the inhibition of nitroblue tetrazolium (NBT) reduction [26], taking the enzyme extract that inhibits 50% of the reduction as one unit. Catalase (CAT) was measured by the decrease in absorbance at 240 nm (extinction coefficient, 39.4 mM^{-1} cm^{-1}) [27].

2.3. Measurements of plant biomass and Cd content in plant tissues

The plants were harvested after they were exposed to Cd for 21 days. The harvested plants were rinsed with tap water, and the roots were immersed in 20 mM Na_2–EDTA for 15 min to remove Cd adhered to the root surface [28]. Then the plants were separated into leaves, stems and roots. They were then separately rinsed with running tap water and distilled water, wiped with tissues and weighed. They were then dried at 105 °C for 30 min, then at 70 °C until weight was constant for Cd content measurement.

Dried plant materials were weighed and ground. The powders were digested with a concentrated acid mixture of $HNO_3/HClO_4$ (3:1, v/v). The Cd concentration in plant tissues was determined with an Optima3000 ICP-AES instrument (Perkin-Elmer, USA).

2.4. Data analysis

The translocation factor (TF) indicated the ability of plants to translocate heavy metals from the roots to the shoots [29]. It was calculated as:

$$\mathrm{TF} = \frac{\text{the metal concentration in shoots}}{\text{the metal concentration in roots}} \quad (1)$$

The bioaccumulation coefficient (BC), or enrichment factor, was described as [30]:

$$\mathrm{BC} = \frac{\text{the heavy metal concentration in the plant}}{\text{the heavy metal concentration in the solution}} \quad (2)$$

The index of tolerance (IT) was calculated as maximum root length in cadmium solution divided by maximum root length in control solution [31].

2.5. Statistical analyses

All measurements were replicated three times. Average values and standard deviations (S.D.) were calculated by the Microsoft Office Excel 2003 for all the data in this paper. One-way analysis of variance was carried out with SPSS11.0. The significant difference was set between treatments at $p < 0.05$ or $p < 0.01$. Multiple comparison was also made by the least significant difference (LSD) test.

3. Results and discussion

3.1. Effect of Cd on plant growth

Cd is a non-essential and toxic heavy metal element for plant growth inhibition is known as a result of Cd toxicity. After 21 days exposure to 5 and 10 mg L^{-1} Cd, *L. japonica* did not show any visual symptoms. When the concentration of Cd was up to 50 mg L^{-1}, the chlorosis on the leaves and dark brown spots on the roots were observed, however, the height, and the dry biomass of leaves and roots had not significant differences compared with the control with the prolongation of exposure time (Table 1). Generally, the biomass and the index of tolerance (IT) of plants may give an important index to identify it as a hyperaccumulator or accumulator. In other words, the biomass and the IT value should not decrease significantly at the threshold concentration of inhibiting plant growth [32]. In the present study, exposed to 25 mg L^{-1} Cd, the plant did not induce significant damage and the leaf, root and total biomass had no significant differences compared with the control. With the increase of Cd concentration in the medium, the root lengths of the plant decreased gradually, however, no statistical differences were observed compared with the control. Furthermore, the IT values in the plants under Cd stress were all above 0.8 and especially exposed to the higher Cd concentration (25 and 50 mg L^{-1}), and they were not obviously different from that of the control after 21 days Cd exposure. It seemed that *L. japonica* could maintain normal growth at 25 mg L^{-1} Cd and have high tolerance to not less than 50 mg L^{-1} Cd. Furthermore, plant growth was improved at the low Cd concentrations and the height and the leaf, root and total biomass increased along with the increase in Cd concentration in the medium. Based on these growth traits, it is suggested that *L. japonica* has a potential in phytoremediation of Cd-contaminated soils, since the tolerance to metal toxicity is a crucial characteristic for hyperaccumulators or accumulators [28].

Table 1
Growth responses of *L. japonica* after 21 days Cd exposure.

Cd concentration in the medium (mg L^{-1})	Root length (cm)	Height (cm)	Leaf biomass dry weight (g)	Root biomass dry weight (g)	Total biomass dry weight (g)	IT
0	13.83 ± 2.88a	13.20 ± 2.79ab	1.39 ± 0.61ab	1.72 ± 0.12b	4.01 ± 0.93ab	
5	12.13 ± 3.35a	13.23 ± 2.92ab	1.43 ± 0.78a	1.76 ± 0.11a	4.06 ± 0.65a	0.88
10	11.93 ± 1.55a	14.17 ± 3.15a	1.42 ± 0.15ab	1.72 ± 0.46b	3.97 ± 0.61b	0.86
25	11.77 ± 2.82a	13.97 ± 1.68ab	1.38 ± 0.46ab	1.70 ± 0.25b	3.95 ± 0.77bc	0.85
50	11.26 ± 3.18a	12.53 ± 2.38b	1.35 ± 0.55b	1.68 ± 0.51b	3.89 ± 0.81c	0.81

Data are means ± S.D. (n = 3). Different letters within the columns indicate significant differences at the 5% level according to the LSD test.

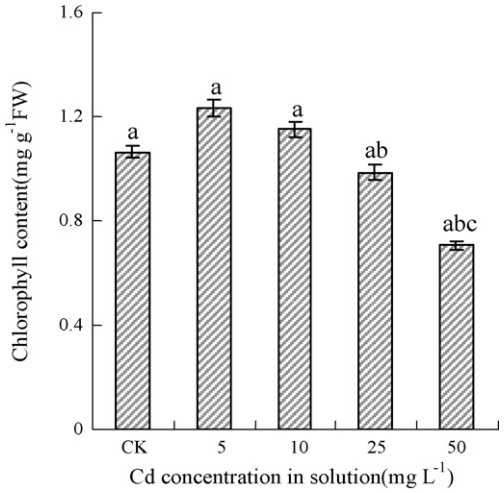

Fig. 1. Effect of Cd concentrations in medium on chlorophyll content in leaves of *L. japonica* after 14 days exposure. Values represent mean ± S.D. Different letters indicate significant differences at the 5% level according to the LSD test.

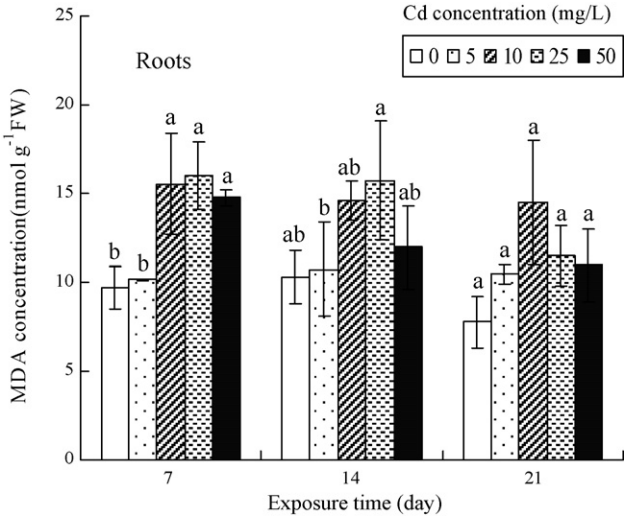

Fig. 2. Effect of Cd concentrations in medium on malondialdehyde (MDA) content in roots of *L. japonica* after 7, 14 and 21 days exposure. Values represent mean ± S.D. Different letters indicate significant differences at the 5% level according to the LSD test.

3.2. Effect of Cd on physiological characteristics

In our study, Cd accumulation in *L. japonica* was accompanied by an induction of many physiological changes, some of which is directly attributed to metal tolerant ability of plants. The chlorophyll content in leaves did not show statistical differences, especially when plants were exposed to 25 and 50 mg L^{-1} Cd (Fig. 1). The increase in the CHL content by exposure to 5 mg L^{-1} Cd may indicate an improved growth. The same phenomenon has been found for studying the enhancement of aluminum to plant growth as [33], the explanation of which contained increased Fe solubility, promotion of P uptake, and protection against Cu/Mn toxicity. However, the decreased chlorophyll content related to higher Cd concentration could be used to monitor Cd-induced damage [24,34], the phenomenon is also proposed as hormesis by de la Rosa et al. [35].

It is known that the most widely accepted indicator of oxidative damage is the accumulation of malondialdehyde (MDA), which is a breakdown product of lipid peroxidation [36]. In our study, the elevation in MDA content in roots compared with the control, indicating the plants were subjected to Cd-induced oxidative stress (Fig. 2). This is in accordance with other study [37]. Cd is shown to induce lipid peroxidation, resulting in AOS formation. As a defensive mechanism, antioxidative enzymes, especially SOD

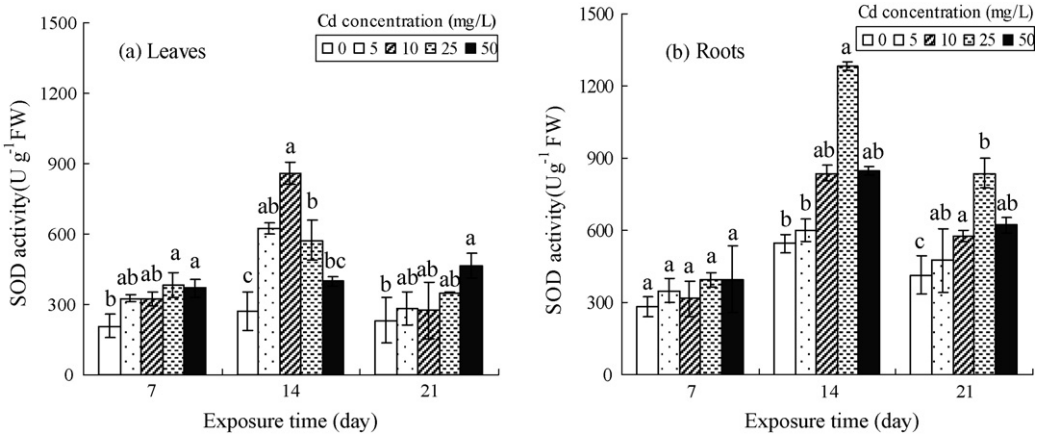

Fig. 3. Effect of Cd concentrations in medium on the activity of superoxide dismutases (SOD) in leaves (a) and roots (b) after 7, 14 and 21 days exposure. Values represent mean ± S.D. Different letters indicate significant differences at the 5% level according to the LSD test.

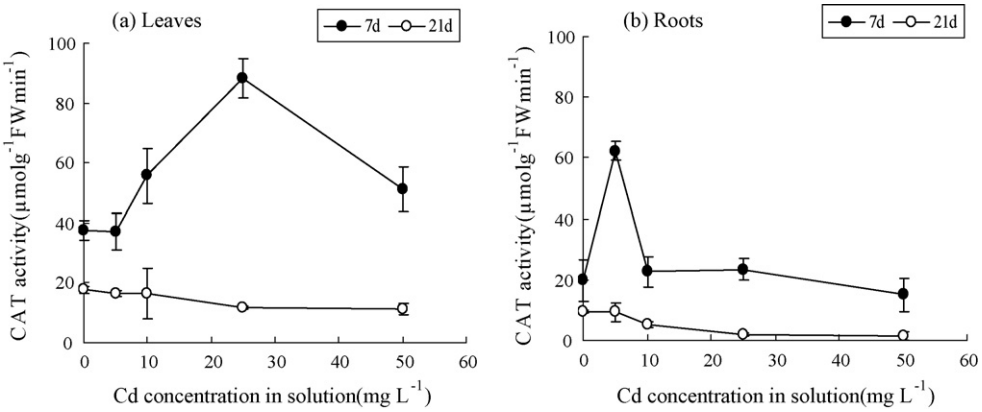

Fig. 4. Effect of Cd concentrations in medium on the activity of catalases (CAT) in leaves (a) and roots (b) after 7 and 21 days exposure. Values represent mean ± S.D.

and CAT play an important role in scavenging AOS. The enzyme SOD is an essential component of antioxidation system in plants as it can dismutate O_2^- to H_2O_2 and O_2. The enzyme CAT is one of the key enzymes for the detoxification of H_2O_2 via two electron transfer [15,16]. In our study, increased SOD and CAT activity in leaves and roots were observed along with the increased Cd concentration in the medium (Figs. 3 and 4). This is in agreement with the results in metal-accumulators (*Thlaspi caerulescens* and *Brassica juncea*) [38] and *Solanum nigrum* L. [39]. After 1, 2 and 3 weeks exposure to 25 mg L^{-1} Cd, *L. japonica* can maintain high SOD activity, raising by 84.7%, 112.4% and 51.3% in leaves and 39.5%, 135.2% and 102.5% in roots compared with the control, indicating a high level of SOD activity might protect plants from oxidative damage induced by Cd toxicity. Similar results were reported in a potential Cd-hyperaccumulator *Bidens pilosa* L. [40]. Moreover, CAT activity increased significantly by 134.1% in leaves and 18.5% in roots relative to the control after 7 days Cd exposure. The maintenance of high SOD and CAT activities in *L. japonica* under Cd stress represents an important feature of metal-accumulator tolerating Cd toxicity. The results showed that *L. japonica* had an effective tolerance strategy to Cd in the medium and inside plant cells, suggesting its strong internal detoxification mechanisms, which is a necessary feature of Cd-hyperaccumulators [20,41].

3.3. Cd accumulation by L. japonica

After 21 days exposure, Cd concentrations in leaves, stems and roots of *L. japonica* all increased significantly with increasing Cd concentrations in the medium ($p < 0.01$; Table 2). There was a positive linear correlation between Cd uptake in the tissues and Cd concentrations in the medium. Cadmium accumulation in roots, stems and leaves is represented by the following Eqs. (3)–(5):

$$Y = 29.80X + 73.99 \, (R^2 = 0.9885, \; p < 0.01) \quad (3)$$

$$Y = 9.63X + 26.44 \, (R^2 = 0.9462, \; p < 0.01) \quad (4)$$

$$Y = 0.93X + 4.77 \, (R^2 = 0.9884, \; p < 0.01) \quad (5)$$

where Y is Cd accumulation in roots, stems and leaves, respectively, and X is the concentration of Cd in the medium. In the present study, stem and shoot Cd concentrations exposed to 25 mg L^{-1} Cd were 344.49 ± 0.71 and 286.12 ± 9.38 μg g^{-1} DW, respectively, more than 100 μg g^{-1} dry tissue, which is the threshold value of Cd-hyperaccumulator [4]. When plants were exposed to 50 mg L^{-1} Cd, the concentration of accumulated Cd reached the maximum of 1555.71 ± 13.12 μg g^{-1} DW in roots and 470.25 ± 7.23 μg g^{-1} DW in stems. At the same level of Cd concentration, there was a significant gradient of Cd concentrations from roots to stems and leaves. Cd accumulation in roots was higher than that in stems or leaves, which was observed for some found hyperaccumulators and accumulators (Table 3). Compared with these found plants, *L. japonica* could accumulate a larger amount of Cd more rapidly. The above results indicated *L. japonica* has the basic characteristics of Cd-hyperaccumulators.

The bioaccumulation coefficient (BC) was used to evaluate the metal accumulation efficiency in plants, and as shown in Table 2, the BCs in *L. japonica* under different Cd treatments were always higher than 1. Moreover, the elevated translocation factor (TF) demonstrated that the plant had the ability to tolerate and translocate Cd to shoots, which suggested *L. japonica* had stronger tolerance to Cd and had a good potential in accumulating Cd more efficiently. Based on higher BC and TF values, the high Cd accumulation in the stems and shoots indicated that *L. japonica* has the potential of hyperaccumulation in phytoremediation application for Cd-contaminated soil.

Tong [42] suggested plants used for phytoremediation of soil polluted by heavy metals should grow fast, develop a large amount of biomass quickly and be easy to cultivate and harvest, preferably multiple times/year. *L. japonica*, is a twining semi-evergreen vine native to Japan, Korea and eastern of China [21] and it possesses the

Table 2
Effect of Cd stress on Cd accumulation characteristics of *L. japonica* after 21 days exposure.

Cd concentration in the medium (mg L^{-1})	Cd concentration in plant tissues (μg g^{-1} DW)				BC	TF
	Root	Stem	Leaf	Shoots		
0	4.12 ± 0.35	4.04 ± 0.34	5.31 ± 0.51	4.26 ± 0.12		
5	220.71 ± 8.83d	49.33 ± 1.03d	7.97 ± 0.20d	42.01 ± 6.55cd	21.92	0.19
10	477.76 ± 10.36c	130.88 ± 6.23c	16.65 ± 1.34c	113.00 ± 14.21c	25.41	0.24
25	793.60 ± 22.35b	344.49 ± 0.71b	25.46 ± 2.44b	286.12 ± 9.38b	19.08	0.36
50	1555.71 ± 13.12a	470.25 ± 7.23a	52.22 ± 2.93a	402.96 ± 11.26a	16.93	0.26
	sig. (*p*)	<0.01	<0.01	<0.01	<0.01	

Data are means ± S.D. ($n = 3$). Different letters within the columns indicate significant differences at the 1% level according to the LSD test; BC, the bioaccumulation coefficient; TF, the translocation factor.

Table 3
Comparison of Cd accumulation in some found hyperaccumulators and accumulators.

Species	Cd concentration in roots (mg kg^{-1})	Cd concentration and plant parts (mg kg^{-1})	Treatment time (*d*)	Culture medium	References
Thlaspi caerulescens[a]	609	319 (shoots)	63	Soil	[43]
Lonicera japonica Thunb.	793.6	344.5 (stems) 286.1 (shoots)	21	Nutrient	The study
Solanum nigrum L.[a]	–	310 (leaves)	35	Soil	[39]
Echinochloa polystachya[a]	299	233 (leaves)	58	Nutrient	[7]
Iris tectorum	330	171 (shoots)	42	Nutrient	[44]
Arabidopsis halleri[a]	660	157 (shoots)	30	Nutrient	[45]
S. melongena	–	121 (leaves)	35	Soil	[39]
I. lactea var. chinensis[a]	402	120.7 (shoots)	42	Nutrient	[44]

–: Not reported.
[a] Hyperaccumulators.

advantages of high biomass, deep roots, easy cultivation, extensive competitive ability, and wide geographic distribution, which are the disadvantages of most found hyperaccumulators. In the present study, it is more important to find firstly that *L. japonica* has a good capability in accumulation and tolerance to Cd.

4. Conclusions

The present study suggested that *L. japonica* has a high tolerant ability to Cd based on its good growth and an effective tolerance strategy. Exposed to low concentrations Cd, *L. japonica* did not show any visual symptoms, even plant growth was improved. When the plants exposed to the highest Cd concentration (50 mg L^{-1}), there were no significant differences in the height and leaf and root biomass in the plants compared with the control. The IT values were all above 0.8. The elevated MDA content in roots indicated the plants were subjected to Cd-induced oxidative stress. As a defensive mechanism, antioxidative enzymes, especially SOD and CAT play an important role in scavenging AOS. The maintenance of high superoxide dismutase (SOD) and catalase (CAT) activities were observed along with the increased Cd concentration, suggesting strong internal detoxification mechanisms inside plant cells. Furthermore, the plant can accumulate Cd in stems and shoots above 100 μg g^{-1} dry tissue, which is the threshold value of Cd-hyperaccumulator, and have higher BC and TF values. Besides these above, *L. japonica* possesses the advantages of high biomass, deep roots, easy cultivation, extensive competitive ability, and wide geographic distribution, which will supply a gap of most found Cd-hyperaccumulators. According to these traits, it is shown *L. japonica* is a potential Cd-hyperaccumulator and the new found plant would provide an important material for understanding the tolerance strategies of Cd hyperaccumulation in plant cells. Therefore, *L. japonica* may have the ability of hyperaccumulation in phytoremediation application for Cd-contaminated soil. On the one hand, as a popular ornamental, *L. japonica* had dual merits of beautification and phytoremediation. On the other hand, our results would give people a caution that how to balance the advantages of the plant as a common medicine plant in Asian and the hidden trouble with the capability of Cd hyperaccumulation.

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