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Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator

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ABSTRACT

Recently, researchers are becoming interested in using hyperaccumulators for decontamination of heavy metal polluted soils, whereas few species that hyperaccumulate cadmium (Cd) has been identified in the plant kingdom. In this study, the physiological mechanisms at the seedling stage and growth responses and Cd uptake and accumulation at flowering and mature stages of *Bidens pilosa* L. under Cd treatments were investigated. At the seedling stage, when soil Cd was lower than 16 mg kg^{-1}, the plant did not show obvious symptom of phytoxicity, and the alterations of chlorophyll (CHL), superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA), and soluble protein (SP) did not have significant differences when compared with the control. At the flowering and mature stages, under low Cd treatments ($\leq$16 mg kg^{-1}), the application of Cd could facilitate plant growth, resulting in 3.9–11.0% and 5.9–13.8%, respectively, increase in shoots dry biomass compared with the control. The Cd concentrations in stems, leaves and shoots exceeded 100 mg kg^{-1} when soil Cd was at 8 mg kg^{-1}, and they were positively correlated with Cd concentration in soils, the bioaccumulation factor (BF) and translocation factor (TF) values were all greater than 1.0. Thus, it is clear that *B. pilosa* has the basic characteristics of a Cd-hyperaccumulator. All the results elementarily indicated that *B. pilosa* is a potential Cd-hyperaccumulating plant.

1. Introduction

Cadmium (Cd) is one of the most hazardous and ubiquitous contaminants in soil and water generated from industrial and agricultural activities such as mining and smelting of metalliferous ores, electroplating, wastewater irrigation, and abuse of chemical fertilizers and pesticides [1,2]. It can reduce the yield of crops and may pose a potential hazard to human health by way of food chain, in particular, induce some fatal diseases such as the "itai-itai disease". Therefore, cleanup of Cd-contaminated soils is emergent and imperative [3,4]. However, current technologies resort to soil excavation and either land filling or soil washing followed by physical or chemical way to separate some contaminants. Moreover, the cost of soil remediation is highly variable and depends on the contaminants of concern, soil properties, and site conditions [5–7]. So scavenging metal-contaminated soils via conventional engineering methods is prohibitively expensive [8]. As an emerging and promising technology, phytoremediation is a cost-effective, environment-friendly and sustainable method. Many studies have demonstrated that the cost of metal phytoextraction is only a fraction of that associated with conventional engineering technologies [9–11].

It is found that hyperaccumulators can accumulate exceptionally high concentration of heavy metals in their tissues. By definition, hyperaccumulators are herbaceous and woody plants that accumulate and tolerate without visible symptoms a 100 times or greater metal concentrations in shoots than those usually found in common non-hyperaccumulators growing in the same environment [12]. At present, four main standards of a hyperaccumulator can be summarized, including (1) accumulating capability: the threshold values of metal concentrations in plants have been used to define metal hyperaccumulators including 10,000 mg kg^{-1} dry weight of shoots for Zn and Mn, 1000 mg kg^{-1} for Co, Cu, Ni, As and Se, and 100 mg kg^{-1} for Cd [13–15]; (2) bioaccumulation factor (BF) index: the ratio of metal concentration in plants to that in soil is greater than 1.0, sometimes reaching 50–100 [16]; (3) translocation factor (TF) index: the ratio of metal concentration in shoots to that in roots is greater than 1.0, which is used to measure the effec-

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Fig. 1. *Bidens pilosa* L.

tiveness of a plant in translocating a metal from roots to shoots; and (4) tolerance capability: as a metal-hyperaccumulator, the plant species should have high tolerance capability to heavy metals, particularly, under a certain concentration hyperaccumulating plants do not show visibly toxic symptoms such as necrosis, whitish-brown chlorosis and the significant decrease in shoot biomass [17–20]. Superoxide dismutases (SOD) and peroxidases (POD) play an important role in scavenging reactive oxygen species (ROS) produced under oxidative stress, malondialdehyde (MDA) and soluble protein contents have been used to assess oxidative stress of chemical pollutants.

To date, more than 400 species have been identified as hyperaccumulators. However, most of hyperaccumulators can only bioconcentrate Ni, about 30 either Cu, Co, Mn and/or Zn, even few species accumulate Cd, As and Pb [3,21,22]. And the majority of hyperaccumulators have been screened out from the temperate zone as well as tropical regions. These plants are generally restricted to endemic species growing on mineralized soil and related rock types [23–25], such as *Thlaspi caerulescens*, *Thlaspi rotundiolium* ssp., *Pteris vittata* L., and *Alyssum wulfenianum* growing on mine tailings and metalliferous sites, *Thlaspi goesingense* found in serpentinitic sites, and so on. Moreover, they are small, slow growing and do not produce a high biomass. So screening out new tolerant, naturalized and higher yield Cd-hyperaccumulating cultivars may be highly useful for the remediation of contaminated soils.

B. pilosa (Railway Beggarticks) is an annual weed in various fields, widely growing from the tropical zone to the subtropical zone in Asia, America and other continents. In contrast to other documented Cd-hyperaccumulators, *B. pilosa* has the characteristics of stronger tolerance to adverse environment, faster growing and higher biomass (Fig. 1). The objectives in present study were: (1) to assess the metal-tolerance strategies adopted by *B. pilosa* in Cd polluted soils; and (2) to evaluate the capability of Cd uptake and accumulation of *B. pilosa*.

2. Materials and methods

2.1. Soil preparation and plant culture

The tested soil was collected from agricultural fields in the Shenyang Ecological Experiment Station, Chinese Academy of Sciences (123°41′N and 41°31′E). It belongs to the temperate zone with a semi-moist continental climate, 5–9 °C average annual temperature, 520–544 kJ cm^{-2} total annual radiation, 650–700 mm average annual precipitation, and 127–164 days frostless duration per year. The coldest month (average −14 °C) is in January and the warmest month (average 24 °C) is in July. This soil is meadow burozem with 22.1% clay, 43.4% silt and 34.5% sand, and organic matter, total *N*, pH and Cd concentration were 1.52%, 0.11%, 6.50 and 0.20 mg kg^{-1}, respectively. 2.5 kg of surface (0–20 cm) soil samples which were ground to pass through a 4 mm mesh were placed in each plastic pot (20 cm in diameter, 15 cm in height), after having mixed with seven levels of Cd ($CdCl_2 \cdot 2.5H_2O$): 0, 8, 16, 24, 32, 50, and 100 mg kg^{-1}, and then incubated for 4 weeks. Three uniform seedlings of *B. pilosa* (5–6 cm height and 4–5 fronds) were transplanted into each pot. Each treatment was replicated three times and arranged in a completely randomized design. To simulate field conditions, the plants were grown under open field conditions and no fertilizers were added. Loss of water was made up using tap water (no Cd detected) to reach 75% of the field water-holding capacity and maintained this humidity by daily watering throughout the cultivation, and a petri dish was placed under each pot to collect potential leachate during the experiment. After growing for 40, 120 and 130 days (June 24, September 14 and September 24 in 2007), namely, the seedling phase was used for analyzing physiological characteristics, the flowering and mature phases were used for evaluating characteristics of plant growth and Cd uptake and accumulation.

2.2. Experiment 1: determination of CHL, lipid peroxidation, SP and enzyme activity

The third to sixth pair of leaves from the top of main stems and branches were picked up and weighted for fresh weight (FW). The chlorophyll (CHL) content in seedlings of *B. pilosa* was determined in 80% acetone extract of leaves as described by Hegedüs et al. and expressed as mg g^{-1} fresh weight [26]. Malondialdehyde content was determined as described by Wang and Zhou, and expressed as mg g^{-1} FW [27].

About 1.0 g of the leaf was ground in an ice-cooled mortar with 5 mL of ice-cooled 50 mM Na-phosphate buffer (pH 7.8, containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone). The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was used for enzyme activity determination.

Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich [28]. The activity of peroxidase was determined using guaiacol as substrate according to the method described by Wu and von Tiedemann [29]. The soluble protein (SP) concentration in the supernatant was determined by the method of Bradford and expressed as mg g^{-1} FW [30].

2.3. Experiment 2: measurement of plant biomass and determination of heavy metals

At the flowering and mature stages, plants were immersed in a 0.01 M HCl solution to remove any external Cd and rinsed with deionized water [31]. Then the plants were separated into roots, stems, leaves and flowers (or seeds), and dried at 100 °C for 10 min, then at 70 °C in an oven until completely dry, then weighed for dry weight (DW).

The plant and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v) [32]. The concentrations of heavy metals were determined by a flame atomic absorption spectrophotometer with a 1.3 nm spectral band width (WFX-120, made in China). The wavelength for Cd determination was 228.8 nm.

2.4. Statistical analysis

All treatments were replicated three times in the experiments. The means and standard deviations (S.D.) were calculated by the Microsoft Office Excel 2003. One-way analysis of variance was carried out with SPSS10.0. When a significant ($P < 0.05$ or $P < 0.01$) difference was observed between treatments, multiple comparisons were made by the LSD test.

Table 1
Effects of Cd pollution on *B. pilosa* physiological characteristics

Concentration of Cd (mg kg^{-1})	CHL (mg g^{-1} FW)	SOD (Ku/g FW)	POD (Ku/g FW)	MDA (mol/g FW)	SP (mg g^{-1} FW)
CK	1.80 ± 0.40a	1.15 ± 0.42a	0.03 ± 0.01b	4.05 ± 0.06b	1.16 ± 0.39a
8	1.65 ± 0.09a	1.09 ± 0.42a	0.04 ± 0.01b	4.31 ± 0.51b	0.84 ± 0.18ab
16	1.55 ± 0.33a	0.92 ± 0.13a	0.04 ± 0.02b	4.23 ± 0.10b	0.84 ± 0.16ab
32	1.75 ± 0.44a	0.75 ± 0.41a	0.07 ± 0.06b	4.93 ± 0.47b	0.70 ± 0.04b
50	1.40 ± 0.08a	1.21 ± 0.41a	0.13 ± 0.1ab	6.11 ± 0.01a	0.66 ± 0.03b
100	1.38 ± 0.20a	1.19 ± 0.30a	0.22 ± 0.11a	6.12 ± 1.00a	0.68 ± 0.03b

Data are means ± S.D. (n = 3). One-way ANOVA (1 factor: different Cd treatments) were performed for each parameter. Means with different letters are significantly different from each other (P < 0.05) according to the LSD test.

3. Results

3.1. Physiological responses of B. pilosa under Cd stress

The physiological characteristics of *B. pilosa* under different Cd treatments are shown in Table 1. The chlorophyll content in leaves was gradually inhibited with increasing soil Cd concentrations, however, the CHL content in leaves did not show statistical differences. Especially, when plants were grown at the Cd levels of 50 and 100 mg kg^{-1}, the content of CHL decreased by 22.2% and 23.3%, respectively, compared with the control.

Changes of SOD activity were negligible with increasing Cd concentrations in soils. The SOD activities reached the bottom at the level of 32 mg kg^{-1} Cd, and then increased slightly, but compared with the control, all the changes were not significant (P > 0.05). The POD activity increased gradually with the various treatments of Cd, although the POD activity did not rise significantly until soil Cd concentration reached 100 mg kg^{-1}. It resulted in 1.33-, 3.33- and 6.33-fold increase at the levels of 32, 50 and 100 mg kg^{-1} Cd when compared with the control.

MDA formation is considered as the general indicator of lipid peroxidation [33]. MDA levels tended to increase with increasing soil Cd concentrations. However, comparing with the control, the MDA levels increased markedly only when the Cd levels in soil were 50 and 100 mg kg^{-1}, respectively. The Cd treatments tended to reduce the SP content in leaves, and decreased significantly at soil Cd concentrations ≥32 mg kg^{-1}.

3.2. Effects of Cd stress on plant growth

Generally speaking, a plant with high tolerant capability can normally grow in contaminated soil, and thus its biomass cannot significantly decrease due to stress of environmental pollution. Fig. 2 shows the dry biomass of *B. pilosa* roots and shoots at two growing stages under Cd treatments. At the flowering stage, there were no significant (P > 0.05) differences in the shoot dry biomass between treatments of Cd at 0–32 mg kg^{-1}. However, the application of Cd could enhance plant growth under low Cd levels (soil Cd concentration ≤16 mg kg^{-1}), the shoot dry biomass at the levels of 8 and 16 mg kg^{-1} Cd resulted in 3.7%, 9.8% increase, respectively, when compared with the control. However, the shoot biomass observed was affected with significant (P < 0.05) decrease when the Cd concentration in soils was up to 50 mg kg^{-1}. Similarly, low concentration of Cd (≤16 mg kg^{-1}) increased the dry biomass of shoots at the mature stage, resulted in 5.9–13.8% increase relative to the control plants. However, it showed significant reduction (P < 0.05) in shoot biomass at the level of Cd ≥ 24 mg kg^{-1}.

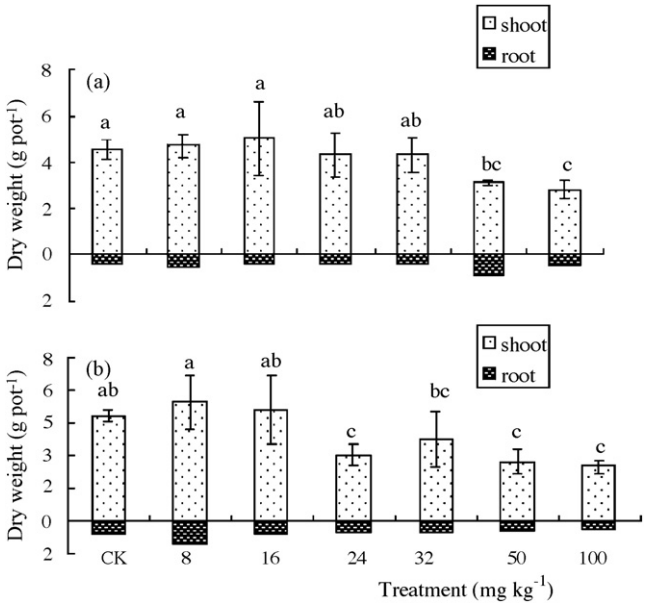

Fig. 2. Effects of Cd on the growth of *B. pilosa*: (a) flowering stage, and (b) mature stage. Data are means ± S.D. (n = 3). One-way ANOVA (1 factor: different Cd treatments) were performed for the dry biomass of shoots and roots. Means with different letters are significantly different from each other (P < 0.05) according to the LSD test.

Table 2
Concentration of Cd in *B. pilosa* at the flowering stage under Cd treatments (mg kg^{-1})

Concentration of Cd (mg kg^{-1})	Root	Stem	Leaf	Flower
CK	0.5 ± 0.1	3.2 ± 0.9	4.5 ± 1.1	Not detected
8	31.3 ± 6.0b	110.5 ± 12.7c	147.3 ± 29.6d	32.3 ± 4.9c
16	64.9 ± 38.2b	189.1 ± 29.4b	196.2 ± 51.5bcd	55.0 ± 5.8b
24	80.0 ± 10.3b	222.2 ± 15.5ab	189.1 ± 38.3cd	71.5 ± 7.1a
32	159.3 ± 58.9a	214.7 ± 28.4b	230.9 ± 30.5bc	67.6 ± 8.1a
50	156 ± 19.3a	208.4 ± 6.6b	252.7 ± 13.4ab	34.9 ± 3.2c
100	201.1 ± 60.1a	256 ± 31.2a	303.2 ± 22.9a	54.5 ± 4.5b
Sig. (P)	0.001	<0001	0.002	<0.001
F	8.7	14.1	8.1	23.3

Data are means ± S.D. (n = 3). One-way ANOVA (1 factor: different Cd treatments) were performed for each parameter on Cd concentration. Data in the same column followed by different letters are significantly different from each other (P < 0.05) according to the LSD test.

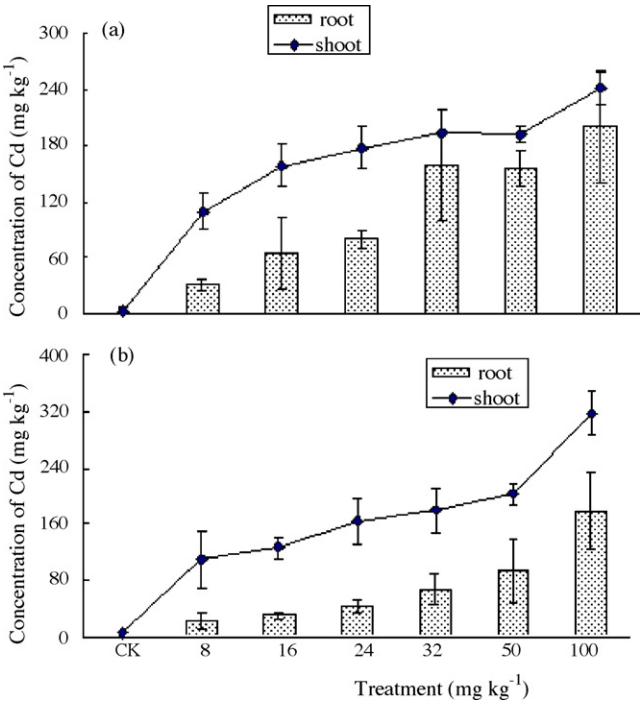

Fig. 3. Concentration of Cd in *B. pilosa* at different growing stages under Cd treatments: (a) flowering stage, and (b) mature stage. Note: shoot Cd concentration is calculated as: $(C_{root} \times M_{root} + C_{stem} \times M_{stem} + C_{leaf} \times M_{leaf})/(M_{root} + M_{stem} + M_{leaf})$, where C is the concentration of Cd and M is the mass of plant, and three plants were in each treatment.

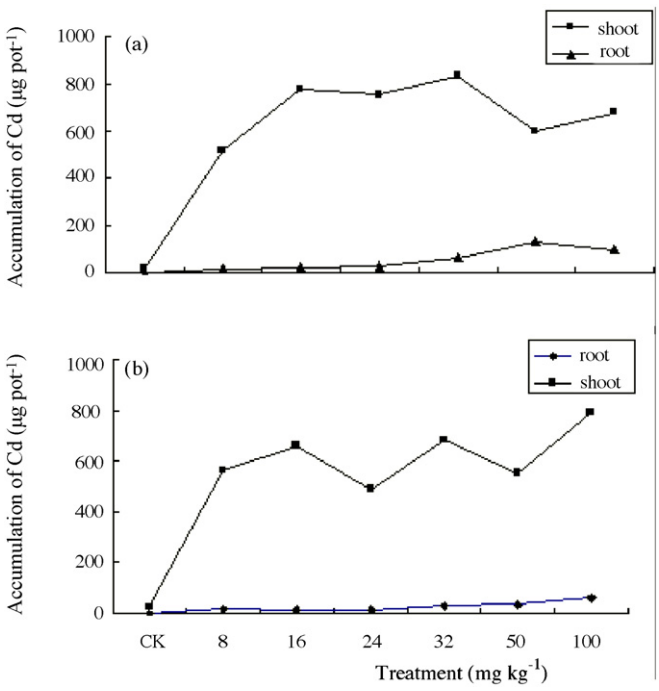

Fig. 4. Accumulation of Cd in *B. pilosa*: (a) flowering stage, and (b) mature stage. Note: shoot Cd accumulation is $(C_{root} \times M_{root} + C_{stem} \times M_{stem} + C_{leaf} \times M_{leaf})$, where C is the concentration of Cd and M is the mass of plant, and three plants were in each treatment.

3.3. Cd concentration in plant tissues

The amount and distribution of Cd accumulated in *B. pilosa* under various Cd treatments are shown in Tables 2 and 3 and Fig. 3. At the flowering and mature stages, the Cd accumulation in all parts of plant increased with increasing concentrations of Cd spiked in the soils, and there was a significant positive linear correlation between shoot Cd uptake and Cd concentrations in soils. The corresponding regression equations can be expressed as:

$$Y_{FS} = 32.68X + 23.4 \quad (R^2 = 0.83, P < 0.001) \tag{1}$$

$$Y_{MS} = 42.1X - 10.3 \quad (R^2 = 0.91, P < 0.001) \tag{2}$$

where Y_{FS} and Y_{MS} is Cd concentration in shoots at the flowering stage and the mature stage, respectively, X is the concentration of Cd in soils. Meanwhile, the Cd concentration in plant was in the sequence: leaf > stem > root > flower and shoot > root. At the flowering stage, when the soil Cd concentration reached 8 mg kg^{-1}, Cd contents in the stems, leaves and shoots were up to 110.5, 147.3 and 110.0 mg kg^{-1}, respectively. And at the same level of Cd, the concentration of Cd in stems, leaves and shoots were up to 107.7, 144.1 and 109.4 mg kg^{-1} at the mature stage (Table 3). Obviously, there were negligible differences between the two growing stages, and all of them exceeded the threshold value as a Cd-hyperaccumulator [14].

At the flowering and mature stages, the dominating Cd uptake by *B. pilosa* was in the shoots, up to 82.1–97.1% and 92.4–97.6%, respectively, in the whole plant (Fig. 4), and the accumulation of Cd in shoots reached 831.6 and 682.5 μg pot^{-1}. The large amounts of heavy metals accumulated in the aboveground parts of a hyperacccumulator were favorable to shift out metals from soils by harvesting aboveground parts, and then through appropriate treatments, it was to reach the aim of ecological remediation of contaminated soils by heavy metals [3,17].

4. Discussion

It is necessary to define and use a scientific standard for hyperaccumulator identification and phytoremediation of contaminated soils by heavy metals [34]. A Cd-hyperaccumulator should contain 100 mg kg^{-1} Cd in its tissues, whereas the normal level of Cd in most of plants is only 0.1 mg kg^{-1} [3,16]. Except the arbitrary value, the following traits are known to be shared by all hyperaccumulators: the BF is greater than 1.0, and higher Cd concentrations are found in shoots than roots. Moreover, Cd-hyperaccumulators should possess hypertolerance to Cd in the medium and inside plant cells, which indicates that they hold strong internal detoxification mechanisms [3,35,36].

4.1. Probable Cd tolerant mechanisms of B. pilosa

There has been growing interest in plants that hyperaccumulate heavy metals due to their unique physiology and potential use in phytoremediation for decontaminating polluted soil [37,38]. As a hyperaccumulator, the basic characteristics are those plants have high tolerant capability and exhibit a variety of responses to mechanical stresses that enable them to tolerate and evolve resistance to adverse conditions that are toxic to most other plants [39], and hypertolerance is a pivotal property that makes hyperaccumulation possible [40].

As concerning visible Cd toxic symptoms, plants show symptoms such as necrosis and whitish-brown chlorosis under Cd stress [41]. Therefore, the reduction of CHL content might be used to monitor the Cd induced damage to the growth of *B. pilosa* [26,42]. In the present study, with increasing soil Cd concentrations, CHL accumulation was inhibited. However, the change in CHL content under Cd treatments was insignificant (Table 1).

Cd has been reported to enhance lipid peroxidation in many plant species, e.g., *Triticum aestivum* [27], *Oryza sativa* [43] and *Allium. sativum* [44]. However, in this study, even when soil Cd

Table 3
Concentration of Cd in *B. pilosa* at the mature stage under Cd treatments (mg kg^{-1})

Concentration of Cd (mg kg^{-1})	Root	Stem	Leaf	Seed
CK	0.7 ± 0.1	4.5 ± 2.2	6.5 ± 2.0	2.1 ± 0.6c
8	21.7 ± 11.3c	107.7 ± 58.3d	144.1 ± 45.9d	27.9 ± 17.bc5
16	31.1 ± 4.9c	135.0 ± 21.7cd	159.3 ± 6.1cd	43.6 ± 12.5b
24	43.1 ± 9.9bc	186.2 ± 18.0bcd	220.1 ± 42.9bc	61.4 ± 7.6bc
32	67.2 ± 32.0c	193.5 ± 53.7bc	216.0 ± 48.9bcd	48.2 ± 23.8bc
50	94.2 ± 45.1b	234.1 ± 69.2b	242.1 ± 44.2b	48.3 ± 15.9a
100	178.9 ± 54.8a	376.0 ± 26.6a	400.7 ± 49.3a	101.2 ± 19.9
Sig. (*P*)	<0.001	<0.001	<0.001	0.004
F	10.5	12.9	14.0	6.5

Data are means ± S.D. ($n = 3$). One-way ANOVA (1 factor: different Cd treatments) were performed for each parameter on Cd concentration. Means in the same column with different letters are significantly different from each other ($P < 0.05$) according to the LSD test.

concentration was up to 32 mg kg^{-1}, MDA concentration did not show any significant increase. The results indicated an indirect or undetectable Cd effect on the plasma membrane of plant cells [45].

SOD catalyses the dismutation of O_2^- to H_2O_2 and O_2 and plays a key role in quenching active oxygen [33,46]. SOD activity did not show any significant changes under all Cd treatments, even though it failed to the lowest level at Cd level of 32 mg kg^{-1}, decreasing by 34.8% compared with the control, and at a soil Cd concentration of 100 mg kg^{-1}, SOD activity increased by 58.7% (Table 1).

POD can catalyze $H_2O_2^-$ dependent oxidation of substrates, is involved in removing oxygen radicals formed in plant tissues due to exposure to chemicals or heavy metals in soils and can thus take part in improving mechanical protection in plant tissues [46]. *B. pilosa* was able to maintain high POD activity for detoxifying active oxygen under higher Cd treatments, raising by 1.3, 3.3 and 6.3 times, respectively, at the Cd levels of 32, 50 and 100 mg kg^{-1} relative to plants under the control (Table 1). The enhancement of POD activity could be resulted from either ionic microenvironment or tissue specific gene expression in plants. Moreover, POD participating in lignin biosynthesis could build up a physical barrier against toxic heavy metals [26,46].

Under the stress of heavy metals, the SP content in plants decreased [29,33]. Table 1 showed that the SP content decreased markedly at the concentration of Cd ≥32 mg kg^{-1} and reduced progressively with increasing of Cd concentration.

For a hyperaccumulator, usually plant biomass at time of seed maturity may be an end-point of a bioassay [41,47]. The insignificant decrease in aboveground biomass of a hyperaccumulator growing in a soil contaminated with heavy metals seriously is one of characteristics of a hyperaccumulator distinguished from a normal plant [18]. No reduction in plant height and shoot dry biomass of Cd-hyperaccumulator (*Solanum nigrum* L.) was noted when the plants were grown at Cd concentration of ≤25 mg kg^{-1} [17]. Similarly, the shoot and root dry matter yields of Cd/Zn hyperaccumulator (*Sedum alfredii* H.) did not reduce at the external Cd level of ≤200 μmol L^{-1}. Moreover, slight stimulation on shoot growth was noted at relatively low Cd levels (25–100 μmol L^{-1}) [41]. In this study, as shown in Fig. 2, the dry biomass of *B. pilosa* shoots at the mature stage did not decrease significantly until the concentration of Cd spiked was up to 24 mg kg^{-1}. Moreover, at the level of Cd ≤ 16 mg kg^{-1}, the aboveground biomass was observed slightly increase compared with the control, which suggested that a certain concentrations of Cd could facilitate the plant growth, and also indicated that the plant has strong tolerance to Cd stress. Therefore, it could be suggested that *B. pilosa* must be considered to be a species of great potential for phytoextraction purposes in polluted soils [48].

However, tolerant species are not necessarily hyperaccumulators. Tolerant non-accumulators can exclude metals from entering their root tissues [49], but hyperaccumulators can take up particularly high amounts of a toxic substance, usually a metal or metalloid, in their shoots during normal growth and reproduction [50,51].

4.2. Hyperaccumulating characteristics of *B. pilosa*

The tolerant mechanisms of Cd tolerant plants have been reported previously [17,52,53], including two strategies: exclusion and accumulation [3]. As for the accumulation strategy, plants accumulated high amounts of Cd in their tissues, which only a small amount of Cd was stored in the roots and the rest was all translocated to the shoots. In this study, when the level of Cd in soils reached 8 mg kg^{-1} at the flowering and matures stages, the concentration of Cd in stems and leaves was more than 100 mg kg^{-1} (Tables 2 and 3). Meanwhile, Fig. 3 shows that the accumulation of Cd in shoots at the two growing stages reached 110.0 and 109.4 mg kg^{-1}, respectively. They all exceeded the critical level for a Cd-hyperaccumulator [3,54,55]. Furthermore, the content of Cd in aboveground parts of plants increased with increasing concentration of Cd at the gradual experiments. The ability of *B. pilosa* to take up high concentration of Cd implies that this plant species might have highly effective Cd-scavenging mechanisms.

To evaluate Cd accumulation efficiency in plants, the bioaccumulation factor, which is defined as the ratio of the metal concentration in the plant tissue to that in the soil [32], was calculated. As depicted in Table 4, the BF values of *B. pilosa* at the flowering stage were 2.4–13.7, but at the mature stage, there were higher BF values (3.2–13.7) for the plant under stress of Cd with the same concentration. However, the BF values at the flowering and mature stages decreased with increasing soil Cd concentrations. In other words, the relationship between the BF values and soil Cd concentrations is negative and logarithmic linear, which indicated a diminishing efficiency of Cd accumulation with increasing soil Cd concentrations. Nevertheless, the BF values were all higher than 1.0 under different Cd treatments. The BF values are more important than shoot concentration *per se* when one considers the potential of phytoextraction for a given species [38]. This may suggest that *B. pilosa* has a steady feature of Cd accumulation.

Table 4
Bioaccumulation and transfer factors of Cd in *B. pilosa*

Cd concentration (mg kg^{-1})	Flowering stage		Mature stage	
	TF	BF	TF	BF
CK		7.4		14.4
8	13.7	3.6	13.7	5.6
16	9.9	2.9	7.9	4.2
24	7.4	2.3	6.8	3.9
32	6.1	1.3	5.7	3.0
50	3.8	1.2	4.1	2.7
100	2.4	1.3	3.2	1.9

Table 5
Newly found Cd-hyperaccumulating plants

Species	Cd contents (mg kg^{-1})	Parts of plant	Growing conditions	TF	BF	References
Thlaspi caerulescens	2120	Rosetter	Zn smelter			[59]
Viola baoshanensis	1168	Shoot	Pb/Zn mine	1.3	2.4	[60]
Thlaspi rotundifolium ssp. *cepaeifolium*	108	Shoot	Mineralized soils			[24]
Cardaminopsis halleri	281	Leaf	Metal smelter	1	3.5	[61]
Thlaspi praecox	1351	Seed	Lead mine and smelter			[62]
Thlaspi praecox	2700	Shoot	50 mmol L^{-1} hydroponic culture			[63]
Sedum alfredii Hance	9000	Leaf	400 mmol L^{-1} hydroponic culture	>1.5		[41]
Solanum nigrum L.	124.6	Leaf	25 mg kg^{-1} pot culture	2.1	5.0	[64,65]
Rorppa globosa	150.1	Leaf	25 mg kg^{-1} pot culture	2.6	6.0	[20]

The transfer factor is defined as the ratio of the metal concentration in shoots to that in roots, which is used to evaluate the effectiveness of a plant in translocating metal from roots to shoots [32]. The TF values of *B. pilosa* at the flowering and mature stages were 1.3–7.4 and 1.9–14.4, respectively (Table 4). Higher TF values may suggest that the plant can uptake Cd from soil and store them in the shoots with great efficiency [3]. In a word, *B. pilosa* has the basic characteristics of a Cd-hyperaccumulator [3,23,36,55,56]. Combined with higher BF and TF values, the strong Cd accumulation in the stems and leaves indicated that *B. pilosa* is potentially useful for remedying Cd-contaminated sites.

4.3. Cd-hyperaccumulators

Last decade there was an exponentially increasing interest in the phenomenon of metal hyperaccumulation since the property has been proposed as an emerging technology of phytoextraction, both in terms of cleanup of contaminated land and phytomining noneconomic mineral deposits [51]. *T. caerulescens* is one of the best-known Cd-hyperaccumulators, this species has been shown to hyperaccumulate Cd up to 1000 mg kg^{-1} [57–59], and has been studied extensively as the only acknowledged Cd-hyperaccumulator until now. Other hyperaccumulating plants have been found in naturally occurring metal-rich sites [7], they are *Thlaspi rotundifolium* ssp. *Cepaeifolium*, *Cardaminopsis haller*, *Thlaspi praecox*, and so on (Table 5).

In China, Tang et al. took the lead in finding the Cu-hyperaccumulator *Commelina communis* L. [60]. In the 21st century, much work on hyperaccumulators has been conducted, and there was the discovery of many new metal hyperaccumulators. Yang et al. reported that the Zn-hyperaccumulator *Sedum alfredii* Hance absorbed Zn in shoots at 4134–5000 mg kg^{-1} [61]. Afterwards, they found that *Sedum alfredii* Hance also hyperaccumulated Cd in aboveground, and the concentration of Cd in leaves and stems could accumulate a maximum of approximately 9000 and 6500 mg kg^{-1} at 400 μmol L^{-1} Cd solution [41]. According to Liu et al., *Viola baoshanensi*, a Cd-hyperaccumulator, was screened out from a Pb/Zn mining area, and the average Cd concentration in shoots and roots of this species was up to 1168 and 981 mg kg^{-1}, respectively, varying from 456 to 2310 mg kg^{-1} in the shoots, and from 233 to 1846 mg kg^{-1} in the roots. In greenhouse experiments, Cd concentration in its shoots reached 4825 mg kg^{-1} at 50 mg L^{-1} Cd solution. The TF values in this plant were greater than 1.0 at all Cd treatments, with an average of 1.67 (1.14–2.22) [62].

However, there are two new Cd-hyperaccumulators have been notarized from clean soils, which are *S. nigrum* L. [17,63,64] and *Rorippa globosa* (Turcz.) [20,32]. The Cd accumulation in stems and leaves of *S. nigrum* was 103.8 and 124.6 mg kg^{-1}, respectively, when soil Cd was 25 mg kg^{-1} [63,64]. And at the same concentration of Cd added, *R. globosa* accumulated 107.0 and 150.1 mg kg^{-1} Cd in their stems and leaves, respectively [20]. Much literature have shown the metallicolous ecotype species is more tolerant to heavy metals and higher capacity of heavy metal uptake and accumulation than those non-metallicolous ecotype one [65,66], but the characteristics of hyperaccumulation may be the function of certain intrinsic gene in plants that could hyperaccumulate heavy metals [18]. In any case, the finding of hyperaccumulators in uncontaminated sites would offer us other new methods for screening out hyperaccumulators.

5. Conclusions

In the present study, *B. pilosa* showed the high tolerant ability at the physiological and biochemical levels and based on good plant growth. Furthermore, at the flowering and mature stages, the concentration of Cd in stems, leaves and shoots was more than 100 mg kg^{-1}, the threshold value of a Cd-hyperaccumulator, when soil Cd concertino was higher than 8 mg kg^{-1}. Meanwhile, the BF and TF values were all greater than 1.0. According to these standards, *B. pilosa* can be validated as a Cd-hyperaccumulator. Because of insurmountable shortcoming in most found Cd-hyperaccumulators such as slow growing, low biomass and regional types, the finding of Cd hyperaccumulation in *B. pilosa* will fill a gap of known hyperaccumulator plants and it has the potential for phytoremediation of heavy metals contaminated soils. However, *B. pilosa* was primarily notarized as a Cd-hyperaccumulator, and it is necessary to be further confirmed and optimized in the future research.

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