Journal of Hazardous Materials 165 (2009) 1023–1028

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Joint effects of arsenic and cadmium on plant growth and metal bioaccumulation: A potential Cd-hyperaccumulator and As-excluder *Bidens pilosa* L

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ARTICLE INFO

Article history:
Received 25 June 2008
Received in revised form 3 October 2008
Accepted 20 October 2008
Available online 5 November 2008

Keywords:
Bidens pilosa L.
Cd-hyperaccumulator
Phytoremediation
Co-contamination of Cd and As
Joint effect

ABSTRACT

Joint effects of arsenic (As) and cadmium (Cd) on the growth of *Bidens pilosa* L. and its uptake and accumulation of As and Cd were investigated using the field pot-culture experiment. The results showed that single Cd ($\leq$25 mg kg^{-1}) and As ($\leq$50 mg kg^{-1}) treatments could promote the growth of *B. pilosa*, resulting in 34.5–104.4% and 21.0–43.0%, respectively, increase in the dry biomass of shoots while compared with that under the control conditions. However, under the co-contamination of As and Cd, there was an antagonistic effect on the growth of the plant. The concentrations of As and Cd accumulated in tissues of the plant increased with an increase of As and Cd in soils. In particular, the levels of Cd in stems and leaves reached 103.0 and 110.0 mg kg^{-1}, respectively, when soil Cd was 10 mg kg^{-1}. Furthermore, the BF and TF values of Cd were greater than 1.0. However, the highest content of As in roots of the plant was only 13.5 mg kg^{-1} when soil As was at a high level, i.e. 125 mg kg^{-1}, and the TF values of As were less than 0.1, indicating that *B. pilosa* can be considered as a potential Cd hyperaccumulator and As excluder. The presence of As had inhibitory effects on Cd absorption by the plant, in particular, the accumulation of Cd in stems, leaves and shoots decreased significantly, with 42.8–53.1, 49.3–66.4 and 37.6–59.5%, respectively, reduction when the level of soil As was up to 125 mg kg^{-1} compared with that under no addition of As. Whereas, when Cd was added to soils, it could facilitate As accumulation in tissues of the plants and the As concentrations in shoots increased with increasing Cd spiked in soils. The interactive effects of Cd and As may be potential for phytoremediation of Cd and/or As contamination soils.

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1. Introduction

Owing to agricultural and industrial practices such as application of pesticides and chemical fertilizers, wastewater irrigation, precipitation from heavy coal combustion, and smelter wastes and residues from metalliferous mining, large areas of cultivated land in the world have been contaminated by heavy metals [1–3]. In China, there are probably areas of 14,000,000 ha sites contaminated by arsenic (As), cadmium (Cd) and other heavy metals, involving 25 areas in 11 provinces [4]. The concentration of As typically varies from below 5 mg kg^{-1} in non-contaminated soils [5] to as high as 3800 mg kg^{-1} in contaminated soils [6]. According to Liao et al., (2005), the concentration of As in soils near the industrial districts of Chenzhou City, South China was 11–1217 mg kg^{-1} [7], and the As level in the Shimeng area was higher, up to 129–3831 mg kg^{-1} [6]. Meanwhile, metal enriched soils might contain about 600 mg kg^{-1} of Cd [8]. The concentration of Cd in farmland soils in the Zhangshi sewage irrigation areas in the western suburb of Shenyang was 2–25 mg kg^{-1} [9,10]. The high levels of As and Cd could adversely affect biological function of living organisms as a teratogen, carcinogen or mutagen as well as having detrimental effects on the digestive system, respiratory system and immune system [11–14].

Thus, the cleanup of Cd and As contaminated soils is emergent and imperative. Fortunately, there has been ongoing development of a variety of technologies to remedy contaminated soils [15–19]. However, the technologies currently available for the remediation of metal/metalloid contaminated soils are notoriously hard to be applied, because they are prohibitively expensive and time consuming [18,20–22]. As a result, it is highly desirable to develop more cost-effective remediation methods. Recently, phytoremediation, the use of plants, including trees and grasses, to extract, sequester, and/or detoxify hazardous contaminants from media, such as soil,

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doi:10.1016/j.jhazmat.2008.10.097

water, and air [18,23,24], is regarded as a tangible alternative with great potential for affordable remediation of polluted sites. As a green technology, phytoremediation helps to prevent landscape destruction and enhances activity and diversity of soil microorganisms to maintain healthy ecosystems [25]. It has been found that certain wild and ornamental plant species have the ability to accumulate vast amounts of toxic heavy metals and nonmetals [26–30]. For example, *Pteris vittata* L. and *Pityrogramma calomelanos* hyperaccumulate As [27,31], *Sedum alfredii* H. has been considered as a Zn and Cd hyperaccumulator [32,33], and several wild species accumulate Ni, Au, TI and Mn [34–36].

The concomitance of As and Cd in the environment is a ubiquitous phenomenon and the interaction between As and Cd may have synergic, antagonistic or additive effects on Cd uptake and accumulation by plants. The presence of As in soils could promote the absorption of Cd by alfalfa at co-contamination soils by As and Cd [37]. Similarly, As in soils could also facilitate Cd uptake in rice plants, the content of Cd in rice roots, stems, leaves and seeds increased with increasing concentration of As in the soils [38]. Appropriate concentration of As in soils could promote Cd uptake and accumulation by *Solanum nigrum* and *Rorippa globosa* when the plants grew under the co-contamination of As and Cd. However, high concentrations of As produced greater inhibitory effect on the growth of plants and their uptake of Cd [39,40]. Interaction between metals may occur at the root surface, and affect metal uptake and translocation [41]. So the interference mechanisms of As and Cd may help for phytoremediation of contaminated soils by As and Cd [40].

Bidentis pilosa L. (Railway Beggarticks) is an annual weed in various fields, widely distributing from the tropical zone to the subtropical zone in Asia, America and other continents. In contrast to other documented remediation plants, *B. pilosa* has the characteristics of stronger tolerance to adverse environment, faster growing and higher biomass. In this sense, it is an ideal natural resource for the remediation of contaminated soils. The main aims of this work were to examine the growing response of *B. pilosa* and its uptake, distribution, and accumulation of As and Cd in response to the co-contamination of As and Cd as well as the interaction mechanisms between As and Cd in soils.

2. Materials and methods

2.1. Field site and soil characterization

The field pot-culture experiment was located at the Shenyang Ecological Experimental Station (41°31′N and 123°41′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) in July. Soil samples were collected from the surface (0–20 cm) of a field in the station, this soil is meadow burozem with 22.1% clay, 43.4% silt and 34.5% sand, and organic matter, total N and pH were 1.52%, 0.11% and 6.50, respectively, the concentration of Cd, Cu, Zn, Pb and As was 0.2, 32.9, 28.1, 11.1 and 2.1 mg kg^{-1}, respectively.

2.2. Experimental procedure

Plastic pots with 15 cm in height and 20 cm in diameter were used. Air-dried soil of 2.5 kg was sieved through a 4 mm sieve, and then placed into each pot after mixed with Cd and As. Four levels of Cd (0, 5, 10, and 25 mg kg^{-1}) and As (0, 25, 50, and 125 mg kg^{-1}) were used in this experiment, resulting in a total of 16 treatments. The Cd and As treatments were referred to as CK, Cd5, Cd10, Cd25, As25, As50, As125, Cd5As25, Cd5As50, Cd5As125, Cd10As25, Cd10As50, Cd10As125, Cd25As25, Cd25As50 and Cd25As125. Cd and As were applied as $CdCl_2{\cdot}2.5H_2O$ and $Na_2HAsO_4{\cdot}7H_2O$, and then incubated for 4 weeks. A petri dish was placed under each pot to collect potential leachate during the experiment.

Three seedlings of *B. pilosa* with the similar size, which were about 3 weeks old, 4–5 cm height with 3–4 leaves were transplanted into each pot. To simulate field conditions, the plants were grown under open field conditions and no fertilizer was added. Loss of water was made up using tap water (no Cd and As detected) to sustain 75% of water holding capacity. The plants were harvested after 70 days when they reached their physiological maturity. They were washed thoroughly first with running tap water followed by distilled water, and dried at 100 °C for 10 min, then at 70 °C in an oven until completely dry.

2.3. Plant and soil analysis

The plant and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). The concentrations of the heavy metals including Cd were determined using the atomic absorption spectrophotometry (WFX-120) [39,40]. The As concentration was determined using the hydrogen generation atomic fluorescence spectroscopy [7,30,42].

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.

3. Results

3.1. Response of plant growth to different treatments of Cd and As

Fig. 1a shows effects of the single Cd or As on the dry biomass of *B. pilosa*. It revealed that low concentrations of Cd (≤25 mg kg^{-1}) and As (≤50 mg kg^{-1}) spiked in soils could facilitate the growth and development of the plant, resulting in 34.5–104.4 and 21.0–43.0% increase, respectively, relative to those under the control conditions. Especially at the level of 10 mg kg^{-1} Cd, the statistical analysis performed to the data showed that shoot dry weight was significantly ($P < 0.05$) increased and obtained the maximum value of 5.4 g pot^{-1}. However, it was observed that the high level of As (125 mg kg^{-1}) impacted plant growth, with 9.1% reduction in shoot biomass compared with the control group.

As shown in Fig. 1b, there was an antagonistic effect on the growth of the plant under co-contamination of As and Cd in soils. In other words, joint effects of As and Cd enhanced the growth of the plant to a certain extent (except for Cd5As25 and Cd25As125). When the concentration of Cd in soils was 5 mg kg^{-1}, the shoot dry weight increased with increasing of As spiked in soils. At the same Cd level of 10 mg kg^{-1}, shoot biomass increased with an increase in the concentration of As and decreased with further increasing As level. When the application of Cd was up to 25 mg kg^{-1}, the inhibition of plant growth was becoming evident with an increasing concentration of As in soils, and the dry weight of shoots reached the lowest at the treatment of Cd25As125. Similarly, at the same concentration of As (25 mg kg^{-1}), shoot biomass increased with increasing Cd concentration. However, with further increasing concentrations of As (50 and 125 mg kg^{-1}), co-contamination of As and

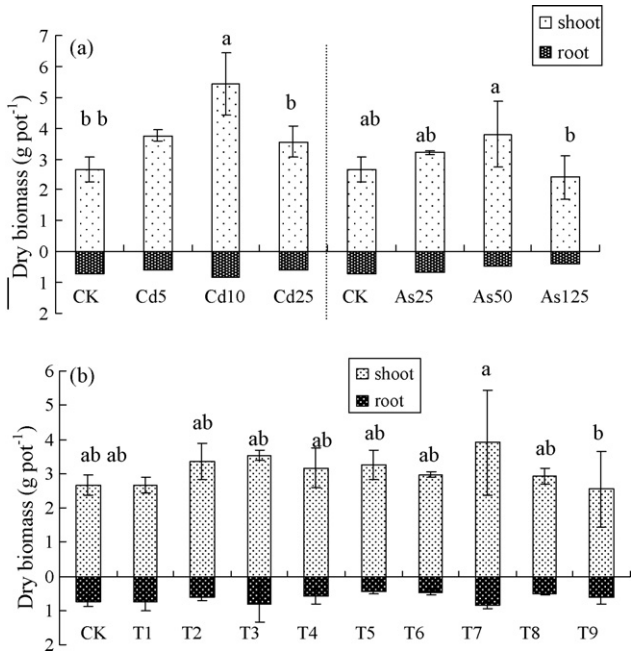

Fig. 1. Dry biomass of *B. pilosa*. (a): single treatments of Cd and As, and (b): joint Cd and As treatments. *Note*: key to metal applications (mg/kg): T1 = Cd5As25; T2 = Cd5As50; T3 = Cd5As125; T4 = Cd10As25; T5 = Cd10As50; T6 = Cd10As125; T7 = Cd25As25; T8 = Cd25As50; T9 = Cd25As125. Means with different letters are significantly different from each other ($P < 0.05$) according to the LSD test.

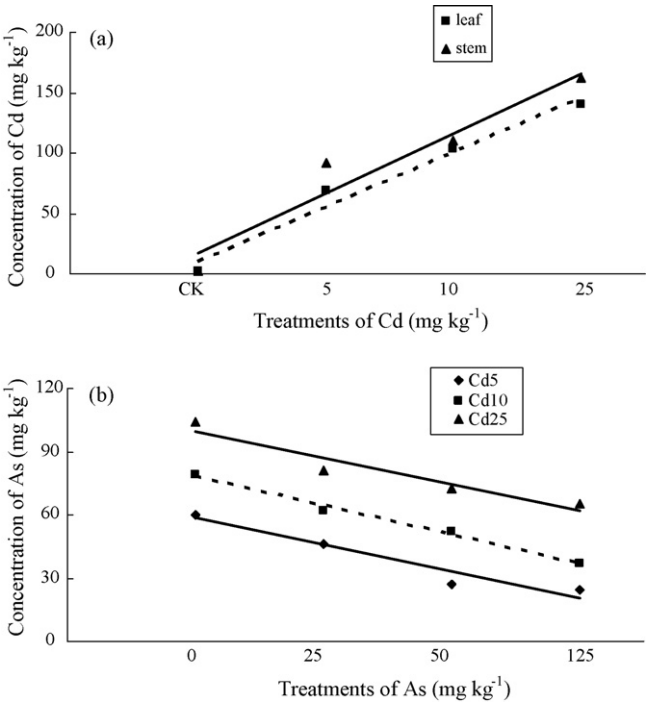

Fig. 2. Relationships between metal absorption by *B. pilosa* and dose of metals exposed in soils. (a): single Cd treatments, and (b): co-contamination of Cd and As.

Cd had a synergic prohibitive effect on plant growth, but there was insignificantly ($P > 0.05$) different in aerial parts of the plant at all treatments compared with that under the control. The results above suggests that *B. pilosa* had a strong capability to endure the joint toxicity of As and Cd at appropriated levels.

3.2. Cd concentration and accumulation in B. pilosa

The amount and distribution of Cd uptake and accumulation in *B. pilosa* are shown in Table 1. The concentration of Cd in all parts of the plant was positively proportional to the concentration of Cd in soils under single treatments of Cd. The corresponding relationship can be expressed using the following regression equations (Fig. 2a):

$$Y_{\text{stem}} = 45.04X - 34.2 \quad (R^2 = 0.97,\ P < 0.01) \tag{1}$$

and

$$Y_{\text{leaf}} = 49.36X - 31.7 \quad (R^2 = 0.93,\ P < 0.01) \tag{2}$$

where Y_{stem} and Y_{leaf} are Cd concentrations in stems and leaves, respectively; X is the concentrations of Cd in soils. In all the treatments, the concentrations of Cd in stems and leaves of *B. pilosa* were higher than those in roots and soils, and the BF and TF values varied from 3.2 to 1.7 and 12.1 to 2.6, respectively (Table 2). Furthermore, the Cd concentrations reached 103.0 and 140.4 mg kg^{-1} in stems and 110.0 and 161.5 mg kg^{-1} in leaves, respectively, when soil Cd was 10 mg kg^{-1} and 25 mg kg^{-1}. Especially under the co-contamination of Cd10As25, Cd25As25 and Cd25As50, the accumulation of Cd in the leaves was up to 100.1, 122.8 and 113.7 mg kg^{-1}, respectively. All exceed the threshold value of

Table 1
Concentration of Cd in *B. pilosa* growing in soils spiked with As and Cd.

Treatment (mg kg^{-1})	Cd concentration (mg kg^{-1}) (mean ± S.D.)			
	Root	Stem	Leaf	Seed
CK	0.4 ± 0.01	1.8 ± 0.9	2.8 ± 1.0	0.6 ± 0.02
Cd5	18.4 ± 2.1a	68.4 ± 9.8a	92.5 ± 9.4a	18.4 ± 3.2a
Cd5As25	14.5 ± 2.7ab	58.6 ± 18.8ab	64.2 ± 26.1a	12.9 ± 5.8ab
Cd5As50	13.1 ± 2.6b	38.3 ± 1.6bc	33.2 ± 10.3b	10.3 ± 2.0b
Cd5As125	14.1 ± 3.7ab	32.9 ± 8.6c	31.1 ± 6.9b	9.6 ± 0.1b
Cd10	26.3 ± 4.9a	103.0 ± 11.7a	110.0 ± 16.1a	19.0 ± 10.8a
Cd10As25	26.4 ± 3.4a	100.1 ± 19.5a	69.6 ± 16.1b	21.7 ± 2.0a
Cd10As50	21.3 ± 1.5ab	65.3 ± 16.6b	71.9 ± 15.9b	18.7 ± 3.1a
Cd10As125	16.0 ± 4.8b	48.3 ± 11.5b	48.6 ± 18.8b	14.0 ± 2.8a
Cd25	45.6 ± 5.2a	140.4 ± 21.4a	161.5 ± 15.6a	32.7 ± 3.0a
Cd25As25	42.3 ± 5.5ab	122.8 ± 30.5ab	95.8 ± 40.5b	27.3 ± 3.9ab
Cd25As50	31.2 ± 1.0b	113.7 ± 24.7ab	81.9 ± 22.0b	24.5 ± 3.6b
Cd25As125	32.1 ± 9.2b	80.3 ± 28.1b	87.5 ± 9.2b	26.0 ± 3.1b

Data are means ± S.D. ($n = 3$). One-way ANOVA were performed for each parameter. Different letters following data within the same column mean concentrations of the Cd form are significantly different ($P < 0.05$) according to the LSD test.

Table 2
Bioaccumulation and translocation factors of Cd and As in *B. pilosa* under the co-contamination of Cd and As.

Treatment (mg kg^{-1})	Cd		Treatment (mg kg^{-1})	As	
	TF	BF		TF	BF
Cd5	12.1	3.2	As25	0.04	0.37
Cd5 + As25	9.2	3.2	As25 + Cd5	0.05	0.29
Cd5 + As50	6.2	2.4	As25 + Cd10	0.05	0.54
Cd5 + As125	3.2	1.9	As25 + Cd25	0.05	0.50
Cd10	7.9	3.0	As50	0.04	1.07
Cd10 + As25	5.4	2.1	As50 + Cd5	0.05	0.76
Cd10 + As50	5.2	2.4	As50 + Cd10	0.06	0.43
Cd10 + As125	2.9	2.3	As50 + Cd25	0.06	0.79
Cd25	4.2	2.3	As125	0.03	0.27
Cd25 + As25	4.9	1.7	As125 + Cd5	0.05	0.64
Cd25 + As50	3.7	2.3	As125 + Cd10	0.05	0.91
Cd25 + As125	2.6	2.0	As125 + Cd25	0.08	0.44

Note: bioaccumulation factor (BF) is defined as the ratio of the metal concentration in shoots to that in the soil; and transfer factor (TF) is defined as the ratio of the metal concentration in shoots to that in roots.

Table 3
Interactive effects of As and Cd on Cd accumulation (mg kg^{-1}) (mean ± S.D.) in the shoots of *B. pilosa*.

Cd treatment (mg kg^{-1})	As treatment (mg kg^{-1})			
	0	25	50	125
5	60.3 ± 7.8a c	45.9 ± 17.6ab a	27.0 ± 4.8bc b	24.4 ± 4.5c c
10	79.2 ± 7.4a b	62.3 ± 7.9ab a	51.9 ± 10.6bc b	36.7 ± 10.4c b
25	104.1 ± 12.1a a	81.0 ± 26.6ab a	72.8 ± 14.8ab a	65.0 ± 12.6b a

Data are means ± S.D. (n = 3). Means followed by different letters differ at P < 0.05 (LSD test). Letters beside means refer to the difference at the same concentration of Cd and under means refer to the difference at the same concentration of As.

100 mg kg^{-1} Cd, which is used to define a Cd-hyperaccumulator [11,43,44].

As shown in Fig. 2b, Table 1 and Table 3, the presence of As had inhibitory effects on Cd uptake in the plant under co-contamination of As and Cd. The concentrations of Cd accumulated in all parts of the plants decreased with increasing of As spiked in soils, and there was a significantly negative linear correlation between shoot Cd uptake and As concentrations in soils. The corresponding regression equations can be expressed as:

$$Y_{Cd5} = -12.66X_{As} + 71.1 \quad (R^2 = 0.93,\ P < 0.01) \tag{3}$$

and

$$Y_{Cd10} = -13.79X_{As} + 92.1 \quad (R^2 = 0.99,\ P < 0.01) \tag{4}$$

and

$$Y_{Cd25} = -12.55X_{As} + 112.1 \quad (R^2 = 0.92,\ P < 0.05) \tag{5}$$

where Y_{Cd5}, Y_{Cd10} and Y_{Cd25} are Cd concentrations (mg kg^{-1}) in shoots when soil Cd was 5, 10 and 25 mg kg^{-1}, respectively; X_{As} is the concentration (mg kg^{-1}) of As in soils. At the low soil As level (treated with 25 mg kg^{-1}), it was found by the variance analysis that there were no significant differences in Cd accumulation in stems compared with that under no addition of As. However, when soil As level was up to 125 mg kg^{-1}, it markedly inhibited Cd accumulation and uptake, as a result, the Cd contents in tissues of the plants reduced significantly (except for the Cd concentrations in roots and seeds when treated with Cd5As125 and Cd10As125), resulting in 51.9, 53.1 and 42.8%, respectively, reduction of Cd concentration in the stems and 66.4, 55.8 and 45.8%, respectively, reduction of Cd concentration in the leaves. Meanwhile, the Cd contents in the leaves were also dramatically decreased when soil Cd was 10 and 25 mg kg^{-1}, respectively.

3.3. As uptake and partitioning in B. pilosa

Fig. 3 shows the amount of As accumulated in the tissues of *B. pilosa*. As shown in Fig. 3, the concentrations of As in the plants strikingly increased with increasing soil As. However, the ability of As uptake and translocation by *B. pilosa* was weak, the highest As uptake by leaves of the plant was only 11.6 mg kg^{-1} when treated with 10 mg kg^{-1} Cd and 125 mg kg^{-1} As. Furthermore, most of As taken up was retained in the roots, and 48.2–78.6% of As absorption by the plant was concentrated in its roots, the BFs and TFs values were 0.27–1.07 and 0.03–0.06, respectively (Table 2).

It is revealed in Table 4 that the coexistence of Cd in the growing media had positive effects on the absorption of As in tissues of *B. pilosa*. As shown in Table 4, applying Cd to soils could facilitate As uptake by shoots, with 1.1–1.3, 1.2–1.6 and 1.1–1.4 times increase, respectively, compared with those under single As treatments (25, 50 and 125 mg kg^{-1} As). This result was similar to that report by Sun et al. (2008), who found that the concentration of Cd in stems of *S. nigrum* increased from 6.43 mg kg^{-1} at the treatment of As50Cd10 to 15.85 mg kg^{-1} under the application of As50Cd50. However, there were insignificant (P > 0.05) differences among the accumulation of As in shoots of the plants when the co-contamination of As (except for 50 mg kg^{-1} As) and Cd took place.

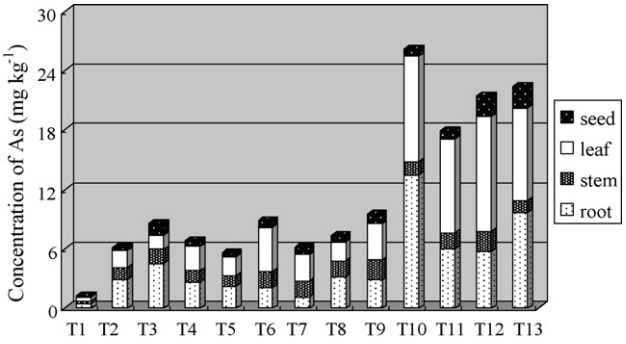

Fig. 3. Effects of As and Cd-As joint stress on As accumulation in *B. pilosa*. *Note*: key to metal applications (mg kg^{-1}): T1 = CK; T2 = As25; T3 = Cd5As25; T4 = Cd10As25; T5 = Cd25As25; T6 = As50; T7 = Cd5As50; T8 = Cd10As50; T9 = Cd25As50; T10 = As125; T11 = Cd5As125; T12 = Cd10As125; T13 = Cd25As125.

Table 4
Interactive effects of As and Cd on As accumulation (mg kg^{-1}) (mean ± S.D.) in the shoots of *B. pilosa*.

As treatment (mg kg^{-1})	Cd treatment (mg kg^{-1})			
	0	5	10	25
25	1.07 ± 0.10a c	1.30 ± 0.54a b	1.40 ± 0.63a b	1.13 ± 0.51a c
50	1.39 ± 0.07b b	1.71 ± 0.41ab b	2.20 ± 0.25a b	2.23 ± 0.63a b
125	3.68 ± 0.94a a	3.91 ± 2.58a a	5.17 ± 0.43a a	4.28 ± 0.31a a

Data are means ± S.D. (n = 3). Means followed by different letters differ at P < 0.05 (LSD test). Letters beside means refer to the difference at the same concentration of As and under means refer to the difference at the same concentration of Cd.

4. Discussion

There are four indicators to define a Cd hyperaccumulator: (1) the threshold value of Cd accumulated in a plant is up to 100 mg kg^{-1} [11,43,44]; (2) bioaccumulation factor (BF) index, the ratio of metal concentration in a plant to soil is greater than 1.0 [11,44]; (3) translocation factor (TF) index, the quotient of metal concentration in shoots to roots is greater than 1.0, which is used to measure the effectiveness of a plant in transferring a metal from roots to shoots; and (4) tolerance capability, the aboveground biomass of a hyperaccumulator should not decrease significantly at the concentration of the critical value that is an important endpoint index for judging it as a hyperaccumulator. In the present study, the low concentrations of single Cd or As and the co-contamination of Cd and As could enhance plant growth (Fig. 1), indicating that *B. pilosa* had a strong capability to endure the toxicity of Cd and As. There was a significantly (P < 0.01) positive linear relationship found between the Cd uptake by all parts of *B. pilosa* and the concentrations of Cd in soils (Table 1). The contents of Cd accumulated in stems and leaves reached 103.0 and 110.0 mg kg^{-1}, respectively, when treated with 10 mg kg^{-1} Cd. Even though the co-contamination of Cd and As (Cd10As25, Cd25As25 and Cd25As50), Cd accumulation in the stems was 100.1, 122.8 and 113.7 mg kg^{-1}, respectively. What is more, it also showed the BF and TF values were greater than 1.0 (Table 2). All exceed the Cd-hyperaccumulator threshold value [11,43,45]. According to these

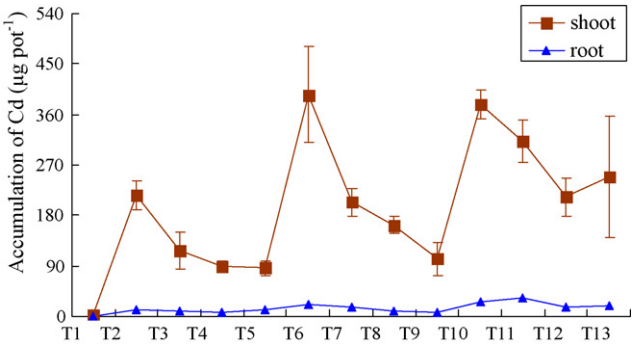

Fig. 4. Joint stress of As and Cd on Cd accumulation in *B. pilosa*. *Note*: key to metal applications (mg kg^{-1}): T1 = CK; T2 = Cd5; T3 = Cd5As25; T4 = Cd5As50; T5 = Cd5As125; T6 = Cd10; T7 = Cd10As25; T8 = Cd10As50; T9 = Cd10As150; T10 = Cd25; T11 = Cd25As25; T12 = Cd25As50; T13 = Cd25As125.

standards, *B. pilosa* could be considered as a Cd hyperaccumulator.

Metal-excluding plants can normally survive in high levels of contaminated soils and the contents of heavy metals accumulated in aboveground tissues and roots of such plants are all very low or else only low heavy metal concentrations occur in aboveground parts even though the concentrations in roots may be very high [46]. In our study, the concentrations of As in roots and shoots of *B. pilosa* were only 2.1–13.5 and 1.1–5.2 mg kg^{-1}, respectively, and relatively large percent of As was concentrated in the roots. Moreover, the BF and TF values of the plants were lower than 1.0 (except for 50 mg kg^{-1} As), indicating As was limited when transporting into the shoots. And when compared with other plants such as *Pteris vittata*, *Pityrogramma calomelanos*, and *Lemna gibba* [12,27,47], all the parameters (As concentrations, BFs and TFs) were very low (Fig. 3, and Table 2). Therefore, *B. pilosa* can also be considered as a potential As-excluder. If metal-excluding mechanisms could be discovered and metal-excluding genes could be transplanted to crops, the finding of metal-excluding plants would be highly useful to the safety of agricultural products and decontamination of heavy metal polluted soils [18,46].

There is a trend that antagonistic and synergistic responses of the plant reflected bioaccumulation patterns in the binary mixture of Cd + As, Cd + Pb, As + P, and Cd + Cu [17,29,37,48,49]. The presence of Pb could facilitate Cd uptake and accumulation in the shoots of *Impatiens Balsamina*, while adding Cd could restrain its Pb absorption [30]. Ye et al. (2003) [50] demonstrated that the accumulation of Cd and Zn in the shoots and roots of *S. alfredii* increased with an increase in Zn/Cd supply levels, when Zn supply at levels ≤500 μmol L^{-1} increased the Cd concentration in the plant, whereas high Zn supply decreased root Cd but did not affect leaf Cd concentrations in *S. alfredii*. According to An et al. (2004), under the co-contamination of Cd and Cu, the accumulation of Cu in shoots was adversely influenced by the presence of Cd in soil, and Cd uptake in the shoots decreased by Cu addition, but Pb addition increased the uptake of Cu by the roots [51].

In this study, the interaction between Cd and As in soil had antagonistic effects on Cd uptake in *B. pilosa*, and the content of Cd in all parts of the plant was evidently reduced (Tables 1 and 3). Under the given Cd levels, the low As concentration (50 mg kg^{-1}) did not markedly prohibit Cd accumulation in the shoots compared with that under no addition of As. However, when the concentration of As in soil was up to 125 mg kg^{-1}, Cd concentration accumulated in the shoots decreased significantly, resulting in 59.5, 53.7 and 37.6% reduction, respectively, relative to the plants growing under single treatment of Cd. Furthermore, Cd accumulation in the shoots decreased significantly ($P < 0.05$) with increasing concentration of As in soil, resulting in 97.8–129.5, 190.6–292.2 and 65.5–165.5 μg pot^{-1} reduction, respectively, compared with that under the treatments of Cd alone (Fig. 4).

However, when Cd was added to soils, it showed an active effect on As uptake by *B. pilosa* (Table 4). The highest As absorption by shoots was 1.40 and 5.17 mg kg^{-1} under the co-contamination of As25Cd10 and As125Cd10, especially at the level of As50Cd10, the concentration of As in the shoots was significantly increased compared with that under no addition of Cd. The results of this work were consistent with that by Xiao et al. (2008), As uptake and transportation by *P. vittata* was not inhibited at the level of ≤20 mg kg^{-1} Cd. Moreover, the frond As concentration was increased by 103.8% when soil Cd was 20 mg kg^{-1} compared with that under the treatment without addition of Cd [52]. Fayiga and Ma (2006) reported the presence of heavy metals (Pb, Cd and Zn) reduced As concentration in the fronds of *P. vittata* from 1630 to 608 mg kg^{-1}, whereas, As uptake in the plants was increased using phosphate rock to immobilize metals in soils, with As concentrations in the fronds increased from 608 to 1046 mg kg^{-1} [53].

In soil, heavy metals compete for sorption sites, resulting in one metal displacing the weaker competing metal from soil particles into the soil solution, the outcome of the competition depends largely on the concentration ratio of the competing metals and their respective sorption characteristics. The ratio of metal concentrations in the soil solution is thus different from the ratio of total metal concentrations in soil [54,55]. This means that the ability of metal uptake by the plants not only rests with the concentration of heavy metals but also relates to their combinations [37]. These interactions at the substrate level, bioavailability and mixture toxicity of metals closely affect the uptake of heavy metals by a plant. The coexistence of Cd and As decreased the ion activity on the root surfaces, meanwhile, the As and Cd equilibrium reaction [$3Cd^{2+} + 2AsO_4^{3-} \rightleftharpoons Cd_3(AsO_4)_2$] in solution may also have limited the ion activity of Cd, reduced ion activity may play an important role in the depression of Cd uptake by plants in the coexistence of As [56,57].

5. Conclusions

Co-contamination of Cd and As had interactive effects on the growth of *B. pilosa*, and antagonistic effects on Cd uptake and additive effects on As absorption in the plant. The coexistence of Cd and As decreased Cd uptake in the plants, but the concentration of As in shoots increased with increasing Cd in soil. Meanwhile, *B. pilosa* had basic characteristics of Cd hyperaccumulation and As exclusion. The joint mechanisms in *B. pilosa* make it useful for phytoremediation of soils co-contaminated by As and Cd.

Acknowledgements

This work was supported by the Ministry of Science and Technology, People's Republic of China as an 863 project (2006AA06Z386) and by the Joint Sino-Russian Centre of Natural Resources and Eco-Environmental Science, Chinese Academy of Sciences.

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