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Short communication

Bidens tripartite L.: A Cd-accumulator confirmed by pot culture and site sampling experiment

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ABSTRACT

Characteristics of accumulation and tolerance of cadmium (Cd) in *Bidens tripartite* L. were investigated to identify Cd-accumulating properties. In this study, pot culture experiment and site sampling experiments were conducted to assess whether this plant is a heavy metal hyperaccumulator or accumulator. The results indicated that the Cd enrichment factor (concentration in plant/soil) and Cd translocation factor (concentration in shoot/root) of *B. tripartite* was principally >1 in pot culture and concentration gradient experiments. Shoot biomass was not reduced significantly ($p < 0.05$) compared to the controls. However, the Cd concentration in *B. tripartite* shoots was not higher than 100 mg kg^{-1}, the threshold concentration for a Cd-hyperaccumulator. In the site sampling experiment, *B. tripartite* also showed Cd-accumulator properties. Based on these results, *B. tripartite* could be identified as a Cd-accumulator. Thus, *B. tripartite* should only be considered as a Cd-accumulator.

1. Introduction

Phytoremediation is the name given to technologies that use plants to clean up contaminated sites. Many techniques and applications are presented under phytoremediation. Remediation of heavy metal contaminated soil is challenging due to its unique properties. The use of physical or chemical methods for this purpose is often not cost effective [1–3]. The success of phytoextraction, as an environmental cleanup technology, depends on several factors including the extent of soil contamination, metal availability for uptake into roots (bioavailability), and plant ability to intercept, absorb, and accumulate metals in shoots [4]. Phytoextraction is widely regarded as a promising technology that mainly uses hyperaccumulator and accumulator to remove contaminants from the soil; much more attention has been paid to the phytoremediation of heavy metal contaminated soils in many countries [5]. So far many hyperaccumulators and accumulators have been reported, but the technology of phytoremediation is not effectively used in practice [6]. Some of the main reasons include many inherent shortcomings of these plants; such as shallow root systems, low biomass, slow growth, weak disease-resistance and insect-resistance, thus resulting in low remediation efficiency [7]. In addition, very little advances in application of transgenic technology to the construction of ideal hyperaccumulative plants have been made [8]. An exhaustive screening of potential hyperaccumulator and accumulators is warranted for the effective application of phytoremediation technology.

Usually, hyperaccumulators should have four basic properties: (1) accumulation capacity, refer to exceed the specified critical values in a hyperaccumulator's shoots (stems or leaves), and the suggested critical values are 1000 mg kg^{-1} for As, Pb, Cu, Ni, and Co, 10,000 mg kg^{-1} for Zn and Mn, 100 mg kg^{-1} for Cd, and 1 mg kg^{-1} for Au, on a dry mass basis [9,10]; (2) translocation factor (TF), i.e. the ratio of the metal concentration in the shoot to that in root should be greater than 1 [9,10]; (3) enrichment factor (EF), which is the ratio of the metal concentration in the plant to that in soil, should be greater than 1 [11]; and (4) tolerance capacity, i.e. the biomass should be statistically similar when grown on contaminated and uncontaminated control soils under experimental conditions [11]. For an accumulator, a plant should meet the last three criteria as mentioned above for a hyperaccumulator. However, the main difference between an accumulator and an hyperaccumulator lies in the first criterion, the accumulation capacity. While the metal concentrations in the shoot of the hyperaccumulators need to exceed the critical values as specified above for different metals, the same is

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not a prerequisite for accumulators. However, an accumulator does need to show an increasing metal accumulation in the shoot when grown on soils with progressively increased metal contents, e.g., in concentration gradient experiments [5,11].

Previously, hyperaccumulative characteristics of 18 weed species from nine families, with respect to Cd, Pb, Cu, and Zn, have been systematically examined using a block experiment (Cd: 0.5, Pb: 59.7, Cu: 29.1 and Zn: 64.1 mg kg^{-1} in soil) [12]. The results showed that the EFs of Cd in shoots of *Bidens tripartite* was higher than 1, and the concentration of Cd in shoots was higher than that in roots, denoting basic characteristics of Cd-hyperaccumulators. The present work describes the experiments designed and conducted to explore the Cd-accumulating potential of *B. tripartite* and further identify it as a hyperaccumulator.

2. Materials and methods

2.1. Identification of accumulative characteristics

The pot culture experiment was conducted in the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (41°31′N and 123°41′E). It is a temperate zone with semi-moist continental climate. The surface (0–20 cm) meadow burozem soil collected from a field with organic matter of 1.52%, cation exchange content of 23.7 cmol kg^{-1} with the following background concentrations of heavy metals (Cd: 0.2, Pb: 14.2, Cu: 12.4 and Zn: 40 mg/kg) was used as the culture medium [13]. As per the National Soil-Environmental Quality Standard of China (NSEQSC), the tested soil is very clean [14].

The single Cd treatment (T_1) and the joint Cd, Pb, Cu and Zn treatment (T_2) were designed based on the NSEQSC guidelines (Table 1). In the treatment T_1, the spiked concentration of Cd was 10 mg kg^{-1}. In the treatment T_2, the concentrations of Cd, Pb, Cu and Zn spiked to the tested soil were 10, 1000, 400, and 1000 mg kg^{-1}, respectively. The concentrations of spiked Cd, Pb, Cu and Zn are 10, 2, 1 and 2 fold(s) as many as their corresponding criterion values in the third level of the NSEQSC, respectively, which are in accordance with the current situations and levels of soils contaminated by heavy metals in northeast China [1,5]. The forms of the heavy metals used to spike the soils are $CdCl_2{\cdot}2.5H_2O$, $Pb(CH_3COO)_2{\cdot}3H_2O$, $CuSO_4{\cdot}5H_2O$, and $ZnSO_4{\cdot}7H_2O$, respectively. The treatment without external heavy metal addition was regarded as the control one (CK_1).

Weed seedlings of *B. tripartite* with the identical growth phase selected from the fields in the station were transplanted into the pots with treatments CK_1, T_1 and T_2 (2 plants per pot with ¢ = 20 cm, *H* = 15 cm and 2.5 kg of soil). Plants in pots were growing in the natural field. Loss of water by evaporation from pots was made up by using tap water (no Cd, Pb, Cu and Zn detected). No fertilizer was added. All the treatments were replicated three times and were harvested after their seed maturity (about 120 d).

2.2. Concentration gradient experiment

The concentration gradient experiment was also arranged in the station. There were six treatments including the control (CK_2, without external Cd added); R_1, R_2, R_3 and R_4. The soils of R_1 to R_4 were spiked with 10, 25, 50 and 100 mg kg^{-1} of Cd respectively. The form of Cd added to the soil was $CdCl_2{\cdot}2.5H_2O$. Two seedlings of *B. tripartite* with identical growth phase were transplanted into the treated pots. The soil properties, pots and management methods were same as the screening experiment. All tested plants were harvested after their seed maturity.

2.3. Site sampling experiment in a sewage irrigation area

The Zhangshi irrigation region was selected as the site for sampling experiment, which is located about 35 km from the research station in the west suburb of Shenyang city. Since 1962, most farmland (about 2800 hm^2) in the area has been contaminated by irrigating rice paddies with Cd containing industrial wastewater drained from Shenyang Weigong hypaethral trench [15]. Although the use of this water for irrigation has been discontinued since it was observed that there is Cd contamination in it, yet the Cd concentration in contaminated soils has remained constant [1]. We randomly collected four plants in autumn to evaluate accumulation of Cd by *B. tripartite*.

2.4. Sample analysis and data processing

The plant samples were separated into roots, stems, leaves, and inflorescences and rinsed with tap water to remove dirt and later on carefully washed with deionized water and dried at 105 °C for 5 min, followed by drying at 70 °C in an oven until completely dry. Nearly half of stems, leaves and inflorescences were mixed together to be used to examine Cd concentration in shoots. The dried plant samples were ground to a powder and passed through a sieve. Collected soil samples were air-dried and ground using a mortar and pestle and passed through a 0.25 mm sieve. The plant and soil samples were digested with a solution containing 87% of concentrated HNO_3 and 13% concentrated $HClO_4$. The concentrations of extractable heavy metals in soils were extracted by using 0.1 mol L^{-1} HCl. Heavy metal concentration was determined by using an atomic absorption spectrophotometer (AAS, Hitachi 180-80 with a 1.3 nm spectral band width). The wavelengths for the determination of Cd, Pb, Cu and Zn are 228.8, 283.3, 324.8 and 213.8 nm, respectively [11–13]. Measured values of heavy metals were checked by using a certified standard reference material (SRM 1547, Peach Leaves) obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The content of soil organic matters was determined by using the general methods. The pH was determined with the pH meter (PHS-3B), and the ratio of soil and water was 1:2.5. All the val-

Table 1
Accumulative characteristics of *B. tripartite* in pot culture experiment.

Treatment	Heavy metal	Root (mg kg^{-1})	Stem (mg kg^{-1})	Leaf (mg kg^{-1})	Inflorescence (mg kg^{-1})	Shoot (mg kg^{-1})	EF	TF
CK_1	Cd	0.3 ± 0.04	0.4 ± 0.03	0.8 ± 0.07	0.3 ± 0.04	0.4 ± 0.05		
	Pb	0.4 ± 0.05	1.6 ± 0.17	0.8 ± 0.09	0.7 ± 0.06	1.4 ± 0.12		
	Cu	11.5 ± 0.26	8.6 ± 0.87	6.4 ± 0.54	10.3 ± 1.25	8.7 ± 0.91		
	Zn	12.9 ± 1.24	27.2 ± 2.89	90.6 ± 9.63	47.6 ± 4.31	35.3 ± 3.67		
T_1	Cd	12.9 ± 1.32c	31.1 ± 3.27a	36.2 ± 4.02a	9.9 ± 1.01c	26.7 ± 2.98ab	2.68	2.08
T_2	Cd	12.7 ± 1.34d	29.6 ± 3.13b	39.6 ± 4.03a	10.1 ± 1.28d	24.4 ± 2.65c	2.43	1.93
	Pb	106.9 ± 11.2a	35.7 ± 4.37b	11.4 ± 1.21d	0.9 ± 0.08e	26.9 ± 2.54c	0.03	0.27
	Cu	46.9 ± 4.52a	12.4 ± 1.31c	18.9 ± 1.86b	10.2 ± 1.11c	12.7 ± 1.43c	0.03	0.27
	Zn	111.4 ± 12.2c	106.9 ± 10.8c	471.3 ± 30.2a	99.6 ± 10.9d	144.9 ± 12.8b	0.10	0.91

Note: Different letters in each line denote a significant difference among of treatment means ($p < 0.05$); EF, enrichment factor. TF, translocation factor.

Table 2
Accumulative characteristics of *B. tripartite* in concentration gradient experiment.

Treatment	Root (mg kg^{-1})	Stem (mg kg^{-1})	Leaf (mg kg^{-1})	Inflorescence (mg kg^{-1})	Shoot (mg kg^{-1})	EF	TF
R_1	12.9 ± 1.68d	30.3 ± 1.03b	37.9 ± 2.41a	10.0 ± 1.09d	25.6 ± 1.62c	2.59	1.99
R_2	16.5 ± 2.45b	40.5 ± 4.66a	41.1 ± 3.24a	16.9 ± 2.21b	35.4 ± 4.70a	1.42	2.15
R_3	33.0 ± 5.67b	62.7 ± 5.60a	59.3 ± 6.87a	25.9 ± 1.52b	53.3 ± 6.67a	1.07	1.61
R_4	50.9 ± 3.64c	81.6 ± 3.60a	82.8 ± 4.41a	36.2 ± 4.82	71.7 ± 3.51b	0.72	1.41

Note: Different letters in each line denote a significant difference among of treatment means ($p < 0.05$); EF, enrichment factor; TF, translocation factor.

ues expressed are mean ± SD (standard deviation) of the three replicates. Data were analyzed by one-way ANOVAs with the Duncan's multiple range test to separate means. Paired *t*-tests were used with significance levels set at $p < 0.05$ [16]. Differences were considered significant at $p < 0.05$. Data were processed with the softwares of Excel and SPSS 11.5. All results were expressed on a dry weight basis.

3. Results and discussion

3.1. Accumulative characteristics of B. tripartite to Cd in pot culture experiment

Plant biomass to indicate plant growth is an important factor for successful application of phytoextraction since its effectiveness depends on both plant biomass and elemental concentrations in a plant. When evaluating the bioaccumulation potential of a hyperaccumulator for phytoremediation purposes, its aboveground biomass is the most important part of the plant. The results showed that shoot biomass of *B. tripartite* in CK_1, T_1 and T_2 were 8.16, 8.41, 5.46 g pot^{-1}, respectively. Compared to control 1(CK_1), the biomass of *B. tripartite* in T_1 was not significantly different, however, its biomass decreased 33.1% in T_2 but the differences of the biomasses between T1 and T2 treatments were not significant ($p > 0.05$). Although the average biomass decreased when exposed to a higher level of Cd, none of them showed any visible toxicity symptoms. This plant also seems to have high resistance to the Cd toxicity, since no visual Cd toxicity symptoms were observed even at higher concentration. Similar positive and neutral responses of biomass to heavy metal stress have also been observed in *Pteris* and other fern species [17].

As for Cd accumulation, the EF and TF values of *B. tripartite* (treatment T_1 or treatment T_2) were all greater than 1 (Table 1). Since accumulating capacity, EF and TF values of *B. tripartite* did not fulfill the criteria for hyperaccumulators with respect to Pb, Cu, and Zn; this species did not exhibit Pb, Cu, and Zn hyperaccumulating capacity [9,11].

Based on the results of tolerance and accumulation properties (Table 1), *B. tripartite* expressed basic characteristics of a Cd-hyperaccumulator [11,18,19].

3.2. Accumulative characteristics of B. tripartite in concentration gradient experiment

Previously it was reported that *B. tripartite* displayed basic properties of a Cd-hyperaccumulator and may be considered as potential Cd-hyperaccumulating plant [20]. For further validation of the report, an experiment based on the concentration gradient is thus needed [21,22]. In concentration gradient experiment, plant height and leaf color of *B. tripartite* did not differ in treatments R_1–R_4 compared to the control (CK_2). There were no significant differences in shoot biomass of *B. tripartite* in all treatments as compared to control, suggesting tolerance characteristics typical of hyperaccumulator [18].

As shown in Table 2, Cd concentrations in *B. tripartite* increased proportionally to soil Cd levels. However, the Cd concentration of plants growing in the soils containing Cd at rates of 10, 25, 50 and 100 mg kg^{-1} did not exceed 100 mg kg^{-1}, although almost all the treatment EFs and TFs were >1 (EFs: 2.59, 1.42, 1.07,0.74; TFs: 1.99, 2.15, 1.61, 1.41).

Though the EFs and TFs is more than 1 in all treatments, however, none of the treated plants accumulated more than 100 mg kg^{-1}. For this reason, *B. tripartite* only considered as Cd accumulator, not a hyperaccumulator [11,18,19].

3.3. Accumulative characteristics of B. tripartite in sewage irrigation area

The cadmium accumulation by *B. tripartite* in sewage irrigation shows in Table 3. All EFs > 1, and all Cd concentration in plant shoots were higher than those in its roots, i.e., TFs > 1. But Cd concentrations in shoots were not greater than 100 mg kg^{-1}. Thus, *B. tripartite* can only be identified as a Cd accumulator [11,18,19]. In the present study, indicating that *B. tripartite* had a strong capability to tolerate the toxicity of Cd, however the maximum accumulation of Cd in above ground biomass was not greater than100 mg kg^{-1}, which did not meet the criteria of a hyperaccumulator [10,22].

It is well known that phytoextraction of heavy metals from a contaminated soil has been done by hyperaccumulators or accumulators [17,22]. However, its effectiveness is determined by the total amount of metal extracted from the contaminated soil and sequestration of heavy metal in the above ground parts of the plants, which is depend upon two major factors: (1) the concentration of the metal in dry biomass; (2) the total biomass produced by the plant. Furthermore, plants used for phytoextraction should be fast growing, deep rooted and easily propagated [23]. There are four indicators to define a hyperaccumulator: (a) the threshold value of metal accumulated in a plant is up to 100 mg kg^{-1}; (b) enrichment factor (EF) index, the ratio of metal concentration in a plant to soil is greater than 1.0; (c) translocation factor (TF) index, the proportion 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 (d) tolerance capability, the

Table 3
Cd accumulating properties of *B. tripartite* in a wastewater irrigation region.

Plant	Plant organ (mg kg^{-1})					EF	TF	Shoot biomass (g plant^{-1})	Soil (mg kg^{-1})	
	Root	Stem	Leaf	Inflorescence	Shoot				Total Cd	Detectable Cd
1	2.5 ± 0.23d	4.5 ± 0.54b	6.5 ± 0.55a	1.9 ± 0.21e	3.6 ± 0.23c	1.91	1.44	5.59	1.88 ± 0.22	1.53 ± 0.12
2	5.3 ± 0.46c	8.5 ± 0.87a	8.6 ± 0.79a	3.2 ± 0.29d	7.7 ± 0.81b	2.96	1.45	6.74	2.6 ± 0.21	2.4 ± 0.25
3	9.3 ± 0.98d	18.3 ± 1.59a	13.7 ± 1.42b	6.4 ± 0.66e	13.4 ± 1.44c	3.37	1.44	7.12	3.98 ± 0.43	3.62 ± 0.34
4	2.9 ± 0.31c	7.5 ± 0.81a	7.1 ± 0.83a	1.8 ± 0.12d	4.8 ± 0.42b	2.24	1.66	13.47	2.14 ± 0.19	1.88 ± 0.17

Note: Different letters in each line denote a significant difference among of treatment means; EF, enrichment factor; TF, translocation factor.

aboveground biomass of a hyperaccumulator should not decrease significantly at the concentration of the critical value that is an important end-point index for judging it as a hyperaccumulator [10,23]. Based on findings from the pot culture and sewage irrigation area experiments, we have identified that *B. tripartite* is a Cd accumulator [24].

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References

[1] Q.X. Zhou, Y.F. Song, Remediation of Contaminated Soils Principles and Methods, Sciences Press, Beijing, China, 2004.
[2] S.D. Cunningham, W.R. Berti, J.W. Huang, Phytoremediation of contaminated soils, Trends Biotech. 13 (1995) 393–397.
[3] S.D. Ebbs, M.M. Lasat, D.J. Brady, J. Cornish, R. Gordon, L.V. Kochian, Phytoextraction of cadmium and zinc from a contaminated soil, J. Environ. Qual. 26 (1997) 1424–1430.
[4] W.H.O. Ernst, Bioavailability of heavy metals and decontamination of soil by plants, Appl. Geochem. 11 (1996) 163–167.
[5] Q.X. Zhou, S.H. Wei, Q.R. Zhang, Ecological Remediation, Chinese Environmental Science Press, Beijing, China, 2006.
[6] J.N. Liu, Q.X. Zhou, T.H. Sun, L.Q. Ma, S. Wang, Growth responses of three ornamental plants to Cd and Cd–Pb stress and their metal accumulation characteristics, J. Hazard. Mater. 151 (2008) 261–267.
[7] S.H. Wei, J.A.T. Silva, Q.X. Zhou, Agro-improving method of phytoextracting heavy metal contaminated soil, J. Hazard. Mater. 150 (2008) 662–668.
[8] S. Karenlampi, H. Schat, J. Vangronsveld, J.A.C. Verkleij, D. van der Lelie, M. Mergeay, A.I. Tervahauta, Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils, Environ. Pollut. 107 (2000) 225–231.
[9] A.J.M. Baker, R.R. Brooks, Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, Ecol. Phytochem. Biorecovery 1 (1989) 811–826.
[10] M. Srivastava, L.Q. Ma, J.A.G. Santos, Three new arsenic hyperaccumulating ferns, Sci. Total Environ. 364 (2006) 24–31.
[11] S.H. Wei, Q.X. Zhou, P.V. Koval, Flowering stage characteristics of cadmium hyperaccumulator *Solanum nigrum* L. and their significance to phytoremediation, Sci. Total Environ. 369 (2006) 441–446.
[12] S.H. Wei, Q.X. Zhou, X. Wang, Characteristics of 1 8 species of weed hyperaccumulating heavy metals in contaminated soils, J. Basic Sci. Eng. 11 (2003) 152–160.
[13] S.H. Wei, Q.X. Zhou, Identification of weed species with hyperaccumulative characteristics of heavy metals, Prog. Nat. Sci. 14 (2004) 495–503.
[14] J.Q. Xia, Detail Explanation on the State Soil-Environment Quality Standard of China, Chinese Environmental Science Press, Beijing, 1996.
[15] Y.Y. Wu, T. Chen, X.X. Zhang, Pollution ecology of Cd in the Zhangshi irrigation area of Shenyang, in: GAO Zheng-min (Editor in Chief). Studies on Pollution Ecology of Soil–Plant Systems, 1986.
[16] Y.H. Ma, Field Experiment and Statistic Methods, Agriculture Press, Beijing, China, 1990.
[17] M. Srivastava, L.Q. Ma, J. Cotruvo, Uptake and distribution of selenium in different fern species, Intern. J. Phytorem. 7 (2005) 33–42.
[18] R.L. Chaney, M. Malik, Y.M. Li, S.L. Brown, E.P. Brewer, J.S. Angle, A.J.M. Baker, Phytoremediation of soil metals, Curr. Opin. Biotechnol. 8 (1997) 279–284.
[19] L.Q. Ma, K.M. Komar, C. Tu, W. Zhang, Y. Cai, A fern that hyperaccumulates arsenic, Nature 409 (2001) 579.
[20] X.E. Yang, X.X. Long, W.Z. Ni, C.X. Fu, *Sedum alfreii* H: a new Zn hyperaccumulating plant first found in China, Chin. Sci. Bull. 47 (2002) 1634–1637.
[21] F.A. Solís-Domínguez, M.C. González-Chávez, R. Carrillo-González, R. Rodríguez-Vázquez, Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system, J. Hazard. Mater. 141 (2007) 630–636.
[22] A.O. Fayiga, L.Q. Ma, J. Santos, B. Rathinasabapathi, B. Stamps, R.C. Littell, Effects of arsenic species and concentrations on arsenic accumulation by different fern species in a hydroponic system, Int. J. Phytoremed. 7 (2005) 231–240.
[23] A.J.M. Baker, Accumulators and excluders-strategies in the response of plants to heavy metals, J. Plant Nutr. 3 (1981) 643–654.
[24] M. Ghosh, S.P. Singh, A comparative study of cadmium phytoextraction by accumulator and weed species, Environ. Pollut. 133 (2005) 365–371.