



## Short Communication

Poultry manured *Bidens tripartite* L. extracting Cd from soil – potential for phytoremediating Cd contaminated soilShuhe Wei<sup>a,\*</sup>, Qixing Zhou<sup>a,b,\*\*</sup>, Jie Zhan<sup>c</sup>, Zhijie Wu<sup>a</sup>, Tieheng Sun<sup>a</sup>, Yelena Lyubu<sup>d</sup>, M.N.V. Prasad<sup>e</sup><sup>a</sup> Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China<sup>b</sup> College of Environmental Science and Engineering, Nankai University, Tianjin 300071, PR China<sup>c</sup> Department of Biotechnology, Liaoning University of Traditional Chinese Medicine, Shenyang 110101, PR China<sup>d</sup> Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov 410049, Russia<sup>e</sup> Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, India

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## ABSTRACT

Pot experiment was conducted in a net house to evaluate the effects of poultry manure on a newly found Cd accumulator *Bidens tripartite* L. phytoextraction potential to soil Cd pollution. The average Cd concentrations in root, stem, leaf, inflorescence and shoots of poultry manured *B. tripartite* were significantly decreased ( $p < 0.05$ ) by 35.5%, 34.4%, 31.0%, 46.5% and 22.6%, respectively, as compared to that of without the addition of poultry manure due to the decrease of extractable Cd in soil. However, Cd extraction capacities ( $\mu\text{g pot}^{-1}$ ) in shoot of *B. tripartite* were significantly increased ( $p < 0.05$ ) due to more than 4-fold increase in shoot biomass. Thus, poultry manure application lowered extractable Cd in soil thereby significantly decreased its uptake, however increased plant biomass and enhanced the Cd phytoextracting efficiency.

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## 1. Introduction

The phytoextraction using hyperaccumulator or accumulator has considerable potential to clean heavy metal from polluted soils. Although there are several new reports on the accumulators, phytoextraction technology has not been gaining momentum for large field application (Wei et al., 2008; Zhang et al., 2010). The main limiting factor insists in the low remediation efficiency of accumulator to heavy metals (Srivastava et al., 2009). To circumvent this situation, some natural and/or synthetic chelators have been used to enhance uptake and translocation of heavy metals from soil and to achieve high removal rates. Notable chelating agents, such as EDTA, CDTA, DTPA, EGTA, EDDHA and NTA have been studied and suggested for field application to mobilize metals and enhance metal accumulation in different plant species (Quartacci et al., 2007; Marques et al., 2008; Munn et al., 2008). However, application of chelators have some negative effects such as toxicity to plant root membranes and soil microorganisms and potential risk of leaching and contamination of ground water with heavy metals. Therefore, an alternative strategy to chelator appli-

cation is to increase heavy metal removal capacity ( $\mu\text{g plant}^{-1}$ ) through enhancing plant shoot biomass (Thayalakumar, 2003; Evangelou et al., 2007). Addition of fertilizer is the most common method for increasing plant production (Yang, 2002). Some investigations about soil amendments such as biosolid, sewage sludge, cattle manure, paper mill sludge and secondary digested sewage sludge have shown good results in various experiments (Adriano et al., 2004; Li et al., 2006; Liu et al., 2009). Poultry manure is the most commonly applied biosolid in China for agricultural production (Yang, 2002). Recently, *Bidens tripartite* L. has been reported as Cd (cadmium) accumulator and had considerable potential for phytoextracting Cd from polluted soil (Wei et al., 2009). However, research on its potential used for extracting Cd from agro-ecosystems is still at infancy. Hence, in this study, pot culture experiment was conducted to assess the effects of poultry manure on *B. tripartite* phytoextracting Cd from contaminated soil.

## 2. Methods

## 2.1. Experimental site information

This research was conducted at the Shenyang Ecological Experimental Station of Chinese Academy of Sciences with 41°31' N and 123°41' E (Wei et al., 2009). The soil is burozem in the station with background concentration of Cd, Pb, Cu and Zn as 0.16, 18.2, 16.7 and 38.3 mg kg<sup>-1</sup>, respectively. Compared to the National

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**Table 1**  
Main properties of tested soil and treated soil with poultry manure.

Treatment	pH	Total Cd (mg kg <sup>-1</sup> )	Organic material (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )
Original soil	6.71 ± 0.09a	0.16 ± 0.02a	17.32 ± 1.05c	0.71 ± 0.03c	10.55 ± 1.14c	88.73 ± 7.62c
Original poultry manure	6.95 ± 0.07a	0.21 ± 0.03a	465.27 ± 3.86a	11.79 ± 1.08a	2017.3 ± 224.72a	310.27 ± 28.16a
Soil added with poultry manure	6.75 ± 0.08a	0.18 ± 0.02a	75.15 ± 6.41b	2.24 ± 0.12b	238.36 ± 21.35b	112.08 ± 9.64b

Means in same columns followed by the same letter were not significantly different at  $p < 0.05$ .

Soil–Environmental Quality Standards of China (NSEQSC, GB 15618, 1995), the soil is a relatively clean.

## 2.2. Pot experiment treatment and plant culture

Tested soil in pot culture experiment were all collected from the surface (0–20 cm) of a field in the station with background concentration of heavy metals. To explore the effects of poultry manure on *B. tripartite* accumulating Cd from different contaminated soils, two groups of pot culture experiments were conducted. In the first group, no poultry manure was added. There were eight treatments, i.e. control 1 (CK<sub>1</sub>) without Cd addition, and treatments T<sub>1</sub>–T<sub>7</sub>, with Cd spiked at 2.5, 5, 10, 25, 50, 100 and 200 mg kg<sup>-1</sup>, respectively. For the second group, 100 g kg<sup>-1</sup> poultry manures were respectively added into the treated soils mentioned above, then got control 2 (CK<sub>2</sub>) added with 100 g kg<sup>-1</sup> poultry manure without Cd addition, and treatments PM<sub>1</sub>–PM<sub>7</sub> with 100 g kg<sup>-1</sup> poultry manure addition and Cd spiked at 2.5–200 mg kg<sup>-1</sup>, respectively. *B. tripartite* is a common one of weed species in agro-ecosystems. In routine agricultural practices in China, 100 g kg<sup>-1</sup> poultry manure is the normal dose recommended (Yang, 2002). Therefore, we used the same dose poultry manure in our experiments.

Collected soil samples and poultry manure were sieved through a 4 mm sieve, then mixed with Cd spiked as CdCl<sub>2</sub>·2.5H<sub>2</sub>O according to designed concentrations and filled plastic pots (∅ = 20 cm, H = 15 cm) with 2.5 kg soil (dry weight), and equilibrated for two months. The two seedlings of *B. tripartite* collected from the station with 3.5 cm in height after growing 15 days were transplanted in each pot during spring. All pots were kept in net house. Loss of water were made up using tap water (no Cd, Pb, Cu and Zn detected) to sustain 80% of soil water-holding capacity. All treatments were replicated three times. The plants were harvested after 95 days of growth.

## 2.3. Sample analysis and data processing

Soil and plant samples were dried by air or in an oven, ground to powder and passed through a sieve size. Then, all samples were digested using a solution containing 87% of HNO<sub>3</sub> and 13% of HClO<sub>4</sub> to determine total heavy metal concentration (Wei et al., 2006). To know extractable Cd and its cation exchange capacity, the concentrations of extractable Cd in soils were extracted by using 1 mol L<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub> (Lu, 2000). Cd concentration was determined by atomic absorption spectrophotometer (AAS, Hitachi 180-80 with a 1.3 nm spectral band width). The measured values of heavy metals were checked by using certified standard reference material (SRM 1547, peach leaves) obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The content of soil organic matter was determined using standard methods (Lu, 2000). The pH was determined using a pH meter (PHS-3B), and the ratio of soil and water was 1:2.5.

The average of three replicates for each treatment and standard deviation (SD) were calculated using Excel and SPSS 11.5 (Ma, 1990). Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests to separate means. Differences were considered significant at  $p < 0.05$  (Ma, 1990).

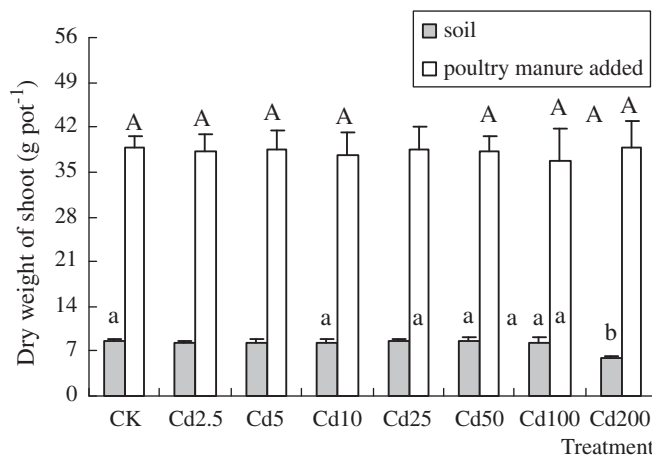
## 3. Results and discussion

### 3.1. Effect of poultry manure on soil physico-chemical properties

Usually, the contents of organic material, total nitrogen (N), available phosphorus (P) and available potassium (K) are the main characteristics of soil fertility (Yang, 2002). Good soil fertility is often showed with higher concentrations of above mentioned indexes. Poultry manure is a kind of organic fertilizer well established for its role in enhancing soil fertility. As showed in Table 1, the contents of soil organic material, total N, available P and available K in the treatments added with 100 g kg<sup>-1</sup> poultry manure were all significantly increased ( $p < 0.05$ ) compared to the soil without poultry manure addition. In addition, the soil pH and total Cd concentration were not obviously changed ( $p < 0.05$ ). Thus, the addition of poultry manure mainly enhanced soil nutrient indices without increased Cd concentration.

### 3.2. Effect of poultry manure on the biomass of *B. tripartite*

Shoot biomass is a very important index to denote plant growth (Yang, 2002). Higher biomass is often with better growth. In phytoremediation, higher biomass also means higher tolerance to heavy metal. When 2.5, 5, 10, 25, 50 or 100 mg kg<sup>-1</sup> Cd were spiked into the soils, *B. tripartite* showed very strong tolerance to Cd, its shoot biomasses were not significantly decreased ( $p < 0.05$ ). However, when 200 mg kg<sup>-1</sup> Cd was spiked, the shoot biomass of *B. tripartite* was decreased significantly ( $p < 0.05$ ), indicating its tolerance to Cd is limited (Fig. 1). Poultry manure addition significantly increased the shoot biomasses of *B. tripartite* and the shoot biomasses increased about four times compared to the treatment without poultry manure added. Furthermore, the tolerance of *B. tripartite* to Cd was also strengthened as shown by the increase of shoot biomass of it in the treatment of 200 mg kg<sup>-1</sup> Cd with poultry manure addition (Fig. 1). Thus, the addition of poultry



**Fig. 1.** Shoot biomass of *B. tripartite* in Cd and poultry manure treatment.

**Table 2**  
Accumulative characteristics of *B. tripartite* phytoextracting Cd (mg kg<sup>-1</sup>).

Treatment	Root	Stem	Leaf	Inflorescence	Shoot	EF <sup>a</sup>	TF <sup>a</sup>	Extractable Cd		Shoot extractive capacity (μg pot <sup>-1</sup> )
								Two month	Harvest	
CK <sub>1</sub>	0.4 ± 0.0 a	0.5 ± 0.0 a	0.7 ± 0.1 a	0.4 ± 0.0 a	0.5 ± 0.1 a	3.3	1.3	0.1 ± 0.0 a	0.1 ± 0.0 a	4.2
CK <sub>2</sub>	0.5 ± 0.1 a	0.5 ± 0.0 a	0.8 ± 0.0 a	0.5 ± 0.0 a	0.5 ± 0.0 a	3.3	1.0	0.1 ± 0.0 a	0.1 ± 0.0 a	19.3
T <sub>1</sub>	3.1 ± 0.2a	8.1 ± 0.5a	8.4 ± 0.6a	4.8 ± 0.3a	6.2 ± 0.5a	2.6	2.0	1.4 ± 0.1a	0.8 ± 0.1a	50.5
PM <sub>1</sub>	2.1 ± 0.1b	5.4 ± 0.3b	5.8 ± 0.5b	2.6 ± 0.2b	4.8 ± 0.3b	1.7	2.3	0.7 ± 0.1b	0.3 ± 0.1b	184.2
% <sup>a</sup>	32.3	33.3	31.0	45.8	22.6	35.9	(14.3)	50.0	62.5	(264.5)
T <sub>2</sub>	5.8 ± 0.4a	14.2 ± 1.1a	17.4 ± 1.2a	10.2 ± 0.8a	12.5 ± 1.1a	2.5	2.2	2.7 ± 0.2a	1.8 ± 0.2a	105.4
PM <sub>2</sub>	3.6 ± 0.2b	9.2 ± 0.7b	11.2 ± 0.9b	5.4 ± 0.5b	9.6 ± 0.6b	2.0	2.7	1.7 ± 0.1b	0.8 ± 0.1b	370.1
%	37.9	35.2	35.6	47.1	23.2	22.4	(23.7)	37.0	55.6	(251.2)
T <sub>3</sub>	13.6 ± 1.1a	33.4 ± 2.9a	39.0 ± 3.2a	17.6 ± 1.4a	26.7 ± 2.3a	2.8	2.0	5.3 ± 0.3a	3.8 ± 0.2a	221.4
PM <sub>3</sub>	8.9 ± 0.7b	20.9 ± 1.8b	24.1 ± 1.9b	10.1 ± 0.8b	21.2 ± 1.9b	2.1	2.4	3.3 ± 0.1b	1.3 ± 0.1b	798.7
%	34.6	37.4	38.1	42.6	20.6	23.0	(21.3)	37.7	65.8	(260.7)
T <sub>4</sub>	21.3 ± 1.9a	52.9 ± 4.9a	53.3 ± 5.1a	22.5 ± 1.9a	37.8 ± 2.8a	1.5	1.8	12.6 ± 0.7a	9.6 ± 0.6a	321.9
PM <sub>4</sub>	14.2 ± 1.1b	34.1 ± 2.8b	37.2 ± 3.2b	11.6 ± 1.0b	29.2 ± 2.1b	1.2	2.1	7.4 ± 0.3b	3.9 ± 0.2b	1126.8
%	33.2	35.4	30.2	48.4	22.8	22.8	(15.7)	41.3	59.4	(250.0)
T <sub>5</sub>	34.0 ± 2.9a	59.1 ± 5.2a	63.9 ± 5.9a	28.8 ± 2.7a	53.4 ± 4.8a	1.1	1.6	25.2 ± 1.9a	17.2 ± 1.1a	459.1
PM <sub>5</sub>	21.8 ± 1.8b	39.2 ± 3.3b	43.3 ± 4.0b	14.7 ± 1.1b	39.8 ± 3.7b	0.8	1.8	14.9 ± 1.0b	6.2 ± 0.4b	1525.9
%	35.8	33.7	32.3	49.0	25.5	24.9	(16.2)	40.9	64.0	(232.4)
T <sub>6</sub>	50.6 ± 4.6a	81.9 ± 7.9a	72.4 ± 6.8a	38.1 ± 3.1a	72.2 ± 7.1a	0.7	1.4	52.9 ± 4.3a	39.4 ± 3.1a	607.8
PM <sub>6</sub>	30.9 ± 2.3b	54.8 ± 5.1b	55.7 ± 4.7b	20.1 ± 1.9b	55.7 ± 3.4b	0.6	1.8	33.2 ± 2.6b	14.9 ± 0.9b	2043.5
%	39.0	33.1	23.1	47.2	22.9	23.2	(26.4)	37.2	62.2	(236.2)
T <sub>7</sub>	75.2 ± 6.4a	81.0 ± 6.9a	77.0 ± 7.2a	38.9 ± 3.4a	70.7 ± 5.5a	0.4	0.9	102.6 ± 9.7a	73.9 ± 5.9a	428.7
PM <sub>7</sub>	48.4 ± 3.7b	54.8 ± 5.3b	56.3 ± 4.9b	21.2 ± 1.9b	56.2 ± 3.8b	0.3	1.2	63.1 ± 4.3b	28.6 ± 1.5b	2229.8
%	35.6	32.3	26.9	45.6	20.5	20.6	(23.4)	38.5	61.3	(420.2)

<sup>a</sup> EF: enhancement factor; TF: translocation factor. Values in parenthesis are the difference ratio of treatment of PM to T, and other values are the ratio of latter to former; Means in same treatment columns followed by different letters were significantly different at  $p < 0.05$ .

manure can increase shoot biomass of the plant and its tolerance to Cd.

### 3.3. Effect of poultry manure on *B. tripartite* phytoextracting Cd

Table 2 showed the characteristics of *B. tripartite* accumulating Cd. The Cd concentrations in root, stem, leaf, inflorescence and shoot of *B. tripartite* under the conditions of poultry manure added (PM<sub>1</sub>–PM<sub>7</sub>) were significantly decreased ( $p < 0.05$ ), compared to the treatments with Cd pollution (T<sub>1</sub>–T<sub>7</sub>) without poultry manure addition, and the average values decreased by 35.5%, 34.4%, 31.0%, 46.5% and 22.6%, respectively. Likewise, the EFs (enhancement factor, ratio of concentration in plant to soil) were all significantly decreased ( $p < 0.05$ ). The main reason may be caused by the reduction of extractable Cd concentration in soil, and the average extractable Cd decreased by 40.4–61.5%. The decrease of extractable Cd concentrations may be caused by the chelation or consolidation of organic materials or phosphorous compounds in poultry manure. However, the TFs (translocation factor, ratio of concentration in shoot to root) and the shoot extractive capacity (μg pot<sup>-1</sup>) were all significantly increased ( $p < 0.05$ ), i.e. the average values increased by 20.1% and 273.6%, respectively. The enhancements of shoot extractive capacities (μg pot<sup>-1</sup>) were caused by the four more times increases of shoot biomasses compared to the treatments without poultry manure addition (Fig. 1).

## 4. Conclusion

The results of this study showed that poultry manure addition not only increased the plant height and shoot biomass of *B. tripartite*, but also decreased its Cd concentration in root, stem, leaf, inflorescence, shoot, and extractable Cd concentration in soil. However, phytoextraction potential (μg pot<sup>-1</sup>) of *B. tripartite* to Cd was finally increased owing to the enhancement of biomass. Thus, poultry manure had two roles: (i) reducing extractable Cd concen-

tration in soil thereby to reduce its concentration in plant; and (ii) enhance phytoextracting efficiency through increasing plant biomass up to four more times.

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