



Bidens pilosa L. hyperaccumulating Cd with different species in soil and the role of EDTA on the hyperaccumulation

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Abstract

Investigating whether the same hyperaccumulator shows a high accumulation potential for different species of the same heavy metal in the soil has rarely been considered until now. In this experiment, Cd accumulation by a hyperaccumulator *Bidens pilosa* L. from soils spiked with 3 and 9 mg Cd kg⁻¹ in the form of Cd(NO₃)₂, CdCl₂, CdBr₂, CdI₂, CdSO₄, CdF₂, Cd(OH)₂, CdCO₃, Cd₃(PO₄)₂, and CdS and effect of soil amendment with EDTA were determined. The results showed that the Cd concentrations in *B. pilosa* for high-solubility species were basically higher. But the enrichment factors (EFs) (shoot to soil Cd concentration ratio) and translocation factors (TFs) (shoot to root Cd concentration ratio) of low-solubility Cd species were all greater than 1, either indicating that there was a high Cd hyperaccumulative potentials of *B. pilosa* without considering on Cd species in soil. EDTA significantly improved *B. pilosa* Cd hyperaccumulation, especially for low-solubility Cd forms in soils. These results can perfectly explain the accumulation properties of one hyperaccumulator to different species of the same heavy metal. Phytoremediation may be applied for a wide scope for different Cd species-contaminated soil. Moreover, the total amount of Cd in soil was important when assessing the risk of Cd-contaminated soils.

Keywords Hyperaccumulative potential · *Bidens pilosa* L. · Cd species

Introduction

Hyperaccumulator is a general term for plants that take up and accumulate very high heavy metal concentrations from the soil. The accumulation properties of hyperaccumulators in

comparison with non-hyperaccumulators were summarized and discussed, i.e., 4 criteria of a hyperaccumulator: threshold criteria, translocation factor (shoot to root concentration ratio, TF > 1), enrichment factor (shoot to soil concentration ratio, EF > 1), and tolerance criterion (biomass was not reduced). A plant characterized by simultaneous TF > 1, EF > 1, and the tolerance criterion was considered an accumulator (Wei and Zhou 2008; van der Ent et al. 2013). Based on these criteria, *Bidens pilosa* L. was confirmed as a Cd hyperaccumulator (Wei and Zhou 2008).

Although more than 100 species of plants have been registered in a new book titled *Phytoremediation resources of heavy metal-contaminated soil* (Xu et al. 2018), there were still very few hyperaccumulator or accumulator species described, including *Noccaea caerulescens* (van der Ent et al. 2013), *Arabidopsis halleri* subsp. gemmifera (Nouet et al. 2015; Jankovska et al. 2016), *Solanum nigrum* L. (Khan et al. 2015), *Sedum alfredii* Hance (Pan et al. 2017), or *Noccaea* spp. (Pavlik et al. 2018). These studies concerned the potential of various hyperaccumulators for the accumulation of different heavy metals, but essentially did not analyze the potential of a single hyperaccumulator for different species of the same heavy metal.

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B. pilosa, collected in Shenyang city of Liaoning province of China, was firstly identified as a Cd hyperaccumulator by comparing 29 plant species of 18 families using a concentration gradient experiment (Wei and Zhou 2008). Later, wild *B. pilosa* collected from the Pb/Zn mine area showed very high Cd concentrations in the leaves or stems (from 112 to 213 mg kg⁻¹) at Cd soil concentrations ranging from 50 to 85 mg kg⁻¹. At the same time, *B. pilosa* EFs and TFs were all higher than 1, indicating that *B. pilosa* collected from natural polluted soil demonstrated all Cd hyperaccumulator characteristics, and could be confirmed as a Cd hyperaccumulator. *B. pilosa* collected from other places in China, such as *B. pilosa* from Beijing city (Li et al. 2009), Chongqing city (Chen et al. 2015), Hanzhong city of Shaanxi province (Dai et al. 2017), Sichuan province (Liu et al. 2017), and Xiangyang city of Hubei province (Zhang et al. 2017) also showed features of a Cd hyperaccumulator. *B. pilosa* collected from Xiangyang city of Hubei province exhibited particularly high phytoremediation potential in a Cd-contaminated field experiment with a 31.3% Cd removal rate (Zhang et al. 2017). Although different *B. pilosa* plants collected from different regions were demonstrated to have similar Cd hyperaccumulation properties, the accumulation potential of *B. pilosa* for different Cd species in the soil was rarely investigated. The aim of this article was to bridge the gap of knowledge by exploring the effect of Cd species in soil on its accumulation by *B. pilosa* and possible improvement role of EDTA on this plant. We hypothesized that (1) there might be significant differences in Cd accumulation by *B. pilosa* from species of different solubilities occurring in soil and (2) EDTA addition to soil may significantly improve Cd uptake by *B. pilosa*.

Materials and methods

Experimental design

Soil pot culture experiment was arranged and conducted in a greenhouse of Shenyang ecological experimental station of Chinese Academy of Sciences (123° 41' E and 41° 31' N). Soil type is meadow burozem and its soil texture is middle loam soil. Basic properties of top soil (0–20 cm) used in this experiment were with pH 6.56, organic material 16.73 g kg⁻¹, total N 0.73 g kg⁻¹, available P 10.33 mg kg⁻¹, available K 89.05 mg kg⁻¹, Cd 0.17 mg kg⁻¹, Pb 14.3 mg kg⁻¹, Cu 12.5 mg kg⁻¹, and Zn 40.0 mg kg⁻¹. Compared with the soil environmental quality risk control standard for soil contamination of agricultural land (SEQ 2018), it was not contaminated by heavy metal and very clean (Yang et al. 2019).

After complete mixing and sieving through a 1-mm nylon sieve, the soil sample was thoroughly stirred and mixed with different Cd species according to the experimental design

(Table 1). The treatment without Cd addition was as controls, i.e., with or without EDTA addition only.

Different Cd chemicals were added to the soil according to a single Cd in 10 different formulas and soil dry weight per pot. Finally, 3 and 9 mg kg⁻¹ (Cd3 and Cd9) Cd in soils were obtained and put into pots $\varphi = 20$ cm and $H = 15$ cm. All pots with different Cd species were equilibrated for 2 months.

Plant culture

B. pilosa was found around different plots and fields in the Shenyang ecological experimental station of the Chinese Academy of Sciences. *B. pilosa* seeds were collected and air dried when they reached the maturity phase in autumn. In spring, these seeds were put into the soil in a seedling tray for germination. When *B. pilosa* seedlings reached the same height of 5 cm, two healthy plants were transplanted to each pot (Table 1). When the transplanted plant grew well, approximately 10 days after transplantation, 0.1 mmol Kg⁻¹ EDTA was added into the soil watering 3 times every 15 days (Cd3 + EDTA, Cd9 + EDTA) (Table 1). Each treatment with different Cd species in the soil was performed in three replications. All pots were randomly placed in the greenhouse, but received well-distributed light. The loss of water in each pot was replenished with tap water and maintained at about 80% of soil water-holding capacity every day (Yang et al. 2019). After 80 days of growth at original maturity, all plants in pots and corresponding soil samples were collected.

Sample analysis and data processing

Roots and shoots of all collected plants were rinsed with tap water and then with deionized water. EDTA was used to soak roots and removed Cd attached to its surface. Oven-dried plant sample (until constant weight) was powdered and filtered through a 2-mm sieve. Concentrated nitric acid and perchlorate (87% HNO₃/13% HClO₄) were used to digest plant and soil samples. Atomic absorption spectrophotometry (AAS, Hitachi 180) was used to determine Cd concentration in digested solutions. All measured values of Cd were verified (QA/QC) by using standard reference material GBW07405 (GSS-5). Extractable Cd concentrations in soils collected when plant samples were harvested were determined after extraction by 1 mol L⁻¹ MgCl₂. Soil pH was measured with a pH meter and electrode (PHS-3B) in soil slurries of a soil: water ratio of 1:2.5. Some physical and chemical characteristics of soil were analyzed by a routine method. Enrichment factor (EF) was calculated with the concentration ratio in plant shoot to soil. Translocation factor (TF) was the ratio of concentration in shoots to roots (Yang et al. 2019).

Data processing and calculations of standard errors were calculated using Microsoft Excel. Duncan's multiple range

Table 1 The treatment of different speciations of Cd in pot culture experiment

Speciation	Solubility (g L ⁻¹)	Treatment			
		Cd3	Cd3 + EDTA	Cd9	Cd9 + EDTA
Cd(NO ₃) ₂	156	Spiked 3 mg kg ⁻¹ Cd of every species Cd in each pot	Spiked 3 mg kg ⁻¹ Cd with 0.1 mmol kg ⁻¹ EDTA for every species Cd in each pot	Spiked 9 mg kg ⁻¹ Cd of every species Cd in each pot	Spiked 9 mg kg ⁻¹ Cd with 0.1 mmol kg ⁻¹ EDTA for every species Cd in each pot
CdCl ₂	120				
CdBr ₂	115				
CdI ₂	86.2				
CdSO ₄	76.7				
CdF ₂	4.36				
Cd(OH) ₂	2.6 × 10 ⁻⁴				
Cd CO ₃	1.72 × 10 ⁻⁴				
Cd ₃ (PO ₄) ₂	6.43 × 10 ⁻⁵				
CdS	1.29 × 10 ⁻¹¹				

After Zhong et al. 2011, Ma et al. 2014 and Song et al. 2015, revised

tests of different treatments at $p < 0.05$ was calculated using the SPSS software (Yang et al. 2019).

Results

B. pilosa biomass and accumulation potential for 10 Cd species

B. pilosa shoot and root biomasses in the controls without EDTA addition were 1.65 g plant⁻¹ and 0.76 g plant⁻¹, and 1.61 g plant⁻¹ and 0.77 g plant⁻¹ for the addition of EDTA, respectively. Basically, there were no significant differences ($p < 0.05$) among them and the other treatments. The data of the controls were now presented in Fig. 1 since it is hard to show.

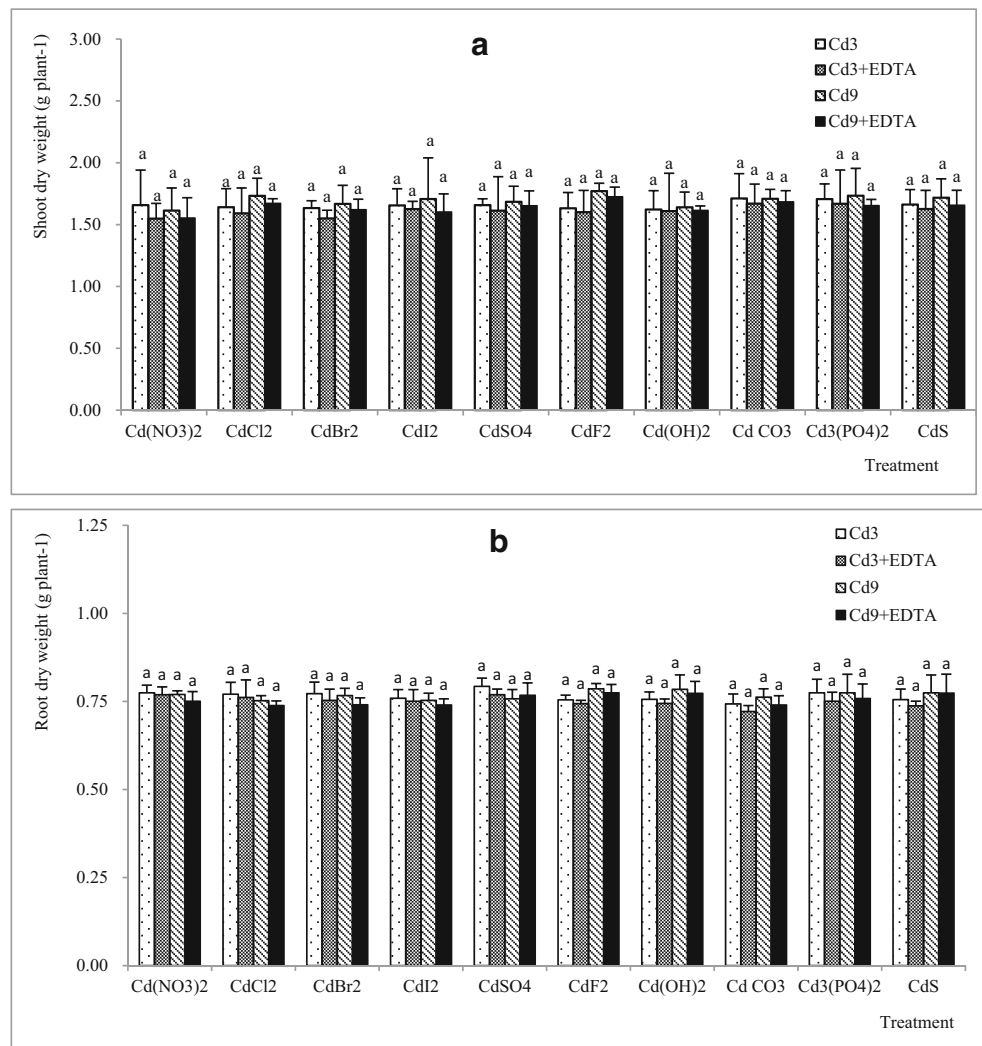
The change trend of *B. pilosa* root biomass was similar to shoot biomass in all treatments (Fig. 1), indicating that the effects of different forms of Cd in the soil on *B. pilosa* biomass were weak. *B. pilosa* shoot biomass, affected by 10 different Cd species, in the treatments of 3 mg kg⁻¹ and 9 mg kg⁻¹ Cd, added with or without EDTA, ranged from 1.61 g plant⁻¹ to 1.77 g plant⁻¹. The differences in *B. pilosa* shoot biomass in 10 Cd species treatments (including Cd3, Cd3 + AS, Cd9, and Cd9 + AS) and the controls were not significant ($p < 0.05$).

The results showed that shoot and root Cd concentrations of *B. pilosa* in the controls without EDTA addition were 0.18 mg kg⁻¹ and 0.78 mg kg⁻¹, and 0.21 g plant⁻¹ and 0.82 g plant⁻¹ for the addition of EDTA, respectively. Basically, both treatments were the same ($p < 0.05$). As shown in Fig. 2A, Cd concentration (24.5 mg kg⁻¹) in *B. pilosa* shoots in case of Cd(NO₃)₂ addition was significantly higher ($p < 0.05$) than that for CdSO₄ (21.7 mg kg⁻¹) when Cd addition level was 3 mg kg⁻¹. However, shoot Cd concentrations were not significantly different ($p < 0.05$) in the treatments with Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂. The same changes

were found between CdI₂ and CdSO₄ treatments. The differences in Cd shoot concentration between CdF₂, Cd(OH)₂, CdCO₃, Cd₃(PO₄)₂, and CdS additions were not significant ($p < 0.05$), but they were all significantly lower ($p < 0.05$) compared with CdSO₄ addition at 3 mg kg⁻¹ Cd. The average Cd concentration in the shoots for Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂ treatments was 23.8 mg kg⁻¹. The average Cd concentration in shoots in the CdI₂ and CdSO₄ additions was 22.4 mg kg⁻¹. The average Cd concentration in the shoots for CdF₂, Cd(OH)₂, Cd CO₃, Cd₃(PO₄)₂ and CdS treatments was 14.9 mg kg⁻¹. The former two were 60.3% and 50.8% higher ($p < 0.05$) than the latter, respectively. Cd concentration in *B. pilosa* shoots was significantly increased ($p < 0.05$) by EDTA added in all Cd treatments compared with the treatments without this compound (Fig. 2A). Cd concentration was on average of 24.7% higher in the samples with Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂, and on average 24.4% higher in the treatments with CdI₂ and CdSO₄, and lastly, it was on average increased by 33.5% in the treatments with CdF₂, Cd(OH)₂, Cd CO₃, Cd₃(PO₄)₂, and CdS at 3 mg kg⁻¹ Cd compared with the treatments without EDTA added.

Cd levels in *B. pilosa* shoots were significantly higher ($p < 0.05$), compared with the treatments with a Cd level at 3 mg kg⁻¹, when Cd concentration added in the soil was increased to 9 mg kg⁻¹ (Fig. 2A). There was the same tendency of changes observed in shoot Cd levels between the treatments with 3 mg kg⁻¹ and 9 mg kg⁻¹ concentrations for all 10 Cd species. The average shoot Cd concentration in the treatments with Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂ was 44.3 mg kg⁻¹, while in cases of CdI₂ and CdSO₄, it was 39.8 mg kg⁻¹. In turn, the average Cd concentration in the shoots treated with CdF₂, Cd(OH)₂, Cd CO₃, Cd₃(PO₄)₂, and CdS was 25.7 mg kg⁻¹. The former two were 72.4% and 54.9% higher than the latter, respectively. EDTA addition also significantly increased ($p < 0.05$) Cd concentrations in *B. pilosa* shoots when 9 mg kg⁻¹ Cd was added compared

Fig. 1 Biomass of *B. pilosa* in different Cd species treatments



with the treatments without EDTA (Fig. 2A). They were on average increased by 17.5% in case of Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂ additions, by 16.8% for CdI₂ and CdSO₄ additions, and by 25.5% in case of CdF₂, Cd(OH)₂, CdCO₃, Cd₃(PO₄)₂, and CdS additions.

The differences in Cd accumulation in *B. pilosa* roots in response to 10 different Cd species (3 and 9 mg kg⁻¹ additions) in the soil and the assisting role of EDTA were generally the same for the shoots (Fig. 2B).

Effects of 10 Cd species on soil pH and extractable Cd concentration in soil

In the treatments of controls, pH were 6.36 and 6.38 without or with EDTA addition, respectively. The soil pH in different treatments ranged from 6.35 to 6.39 (Fig. 3). Basically, these differences were not significant (*p* < 0.05), irrespective of Cd species added (Cd(NO₃)₂, CdCl₂, CdBr₂, CdI₂, CdSO₄, CdF₂, Cd(OH)₂, Cd CO₃, Cd₃(PO₄)₂, and CdS), concentration

applied (3 mg kg⁻¹ and 9 mg kg⁻¹), and with or without EDTA addition.

The changes in trends of extractable Cd concentrations (Fig. 4) were basically the same as those for Cd concentrations in the shoots in all treatments with 3 mg kg⁻¹ and 9 mg kg⁻¹ Cd addition and with or without EDTA addition (Fig. 2A).

When 3 mg kg⁻¹ Cd was added, the average extractable Cd concentration in the treatments with Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂ was 1.6 mg kg⁻¹. Likewise, the average extractable Cd concentration in the treatments with CdI₂ and CdSO₄ was 1.6 mg kg⁻¹, while for CdF₂, Cd(OH)₂, Cd CO₃, Cd₃(PO₄)₂, and CdS, it was 1.3. When EDTA was added, these averages were 29.2%, 28.1%, and 30.9% higher (*p* < 0.05) than the former three without EDTA addition, respectively (Fig. 4).

When 9 mg kg⁻¹ Cd was added, the average extractable Cd concentration in the treatments with Cd(NO₃)₂, CdCl₂, CdBr₂, and CdI₂ was 4.3 mg kg⁻¹. The average extractable Cd concentration in the treatments with CdI₂ and CdSO₄ addition was 4.1 mg kg⁻¹. The average extractable Cd concentration in the treatments with CdF₂, Cd(OH)₂, Cd CO₃, Cd₃(PO₄)₂,

Fig. 2 Cd concentrations in shoots and roots of *B. pilosa*

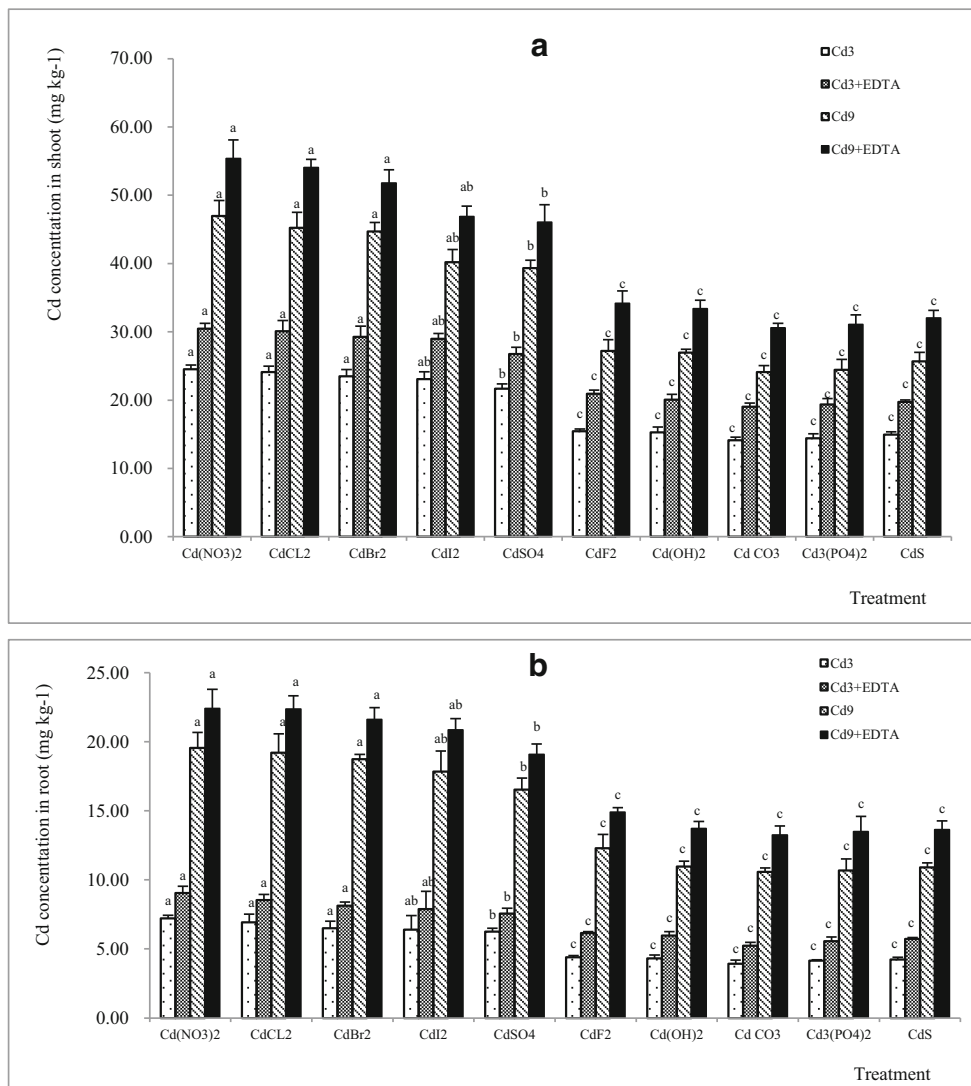


Fig. 3 Soil pH in different treatments

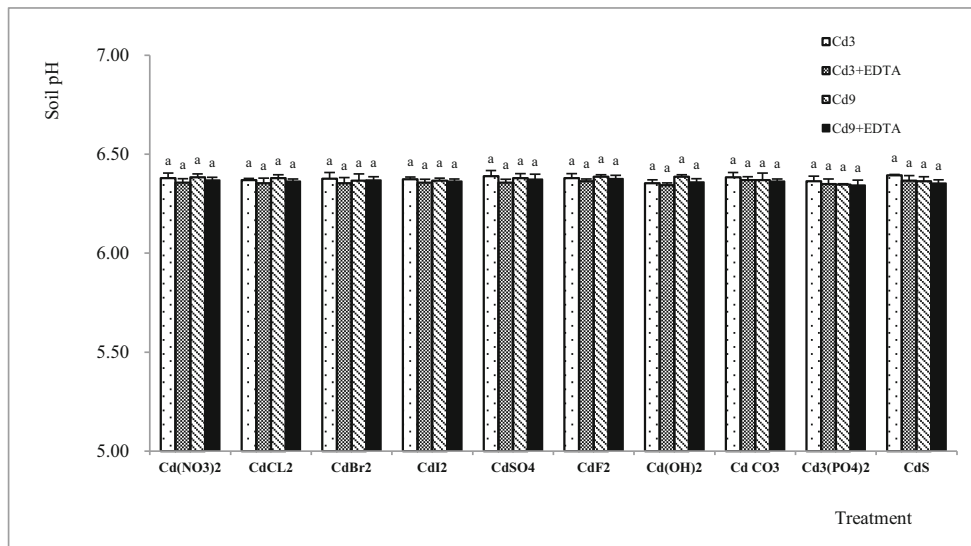
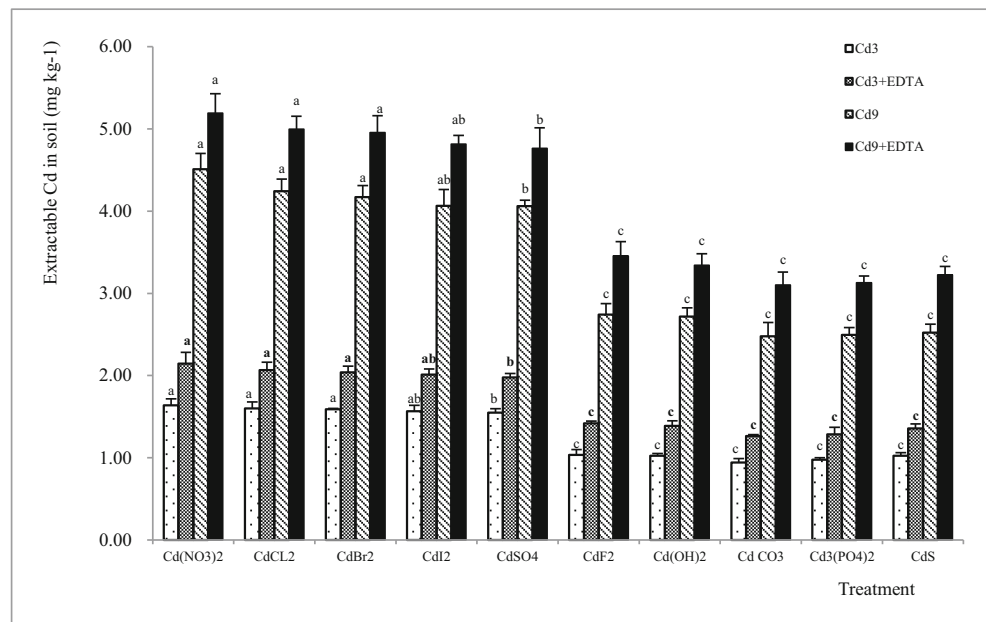


Fig. 4 Extractable Cd concentration in soil



and CdS was 3.4 mg kg⁻¹. The average extractable Cd concentrations when EDTA was added were 17.5%, 18.2%, and 20.5% higher ($p < 0.05$) than the former three without EDTA addition, respectively (Fig. 4).

Effects of 10 Cd species on EF and TF of *B. pilosa*

Various treatments with 10 different Cd species resulted in very similar *B. pilosa* TFs ($p < 0.05$) when Cd was added at 3 mg kg⁻¹ and 9 mg kg⁻¹ with or without EDTA (Fig. 5). However, TFs were significantly higher ($p < 0.05$) in the treatments with 3 mg kg⁻¹ Cd addition than those with 9 mg kg⁻¹ Cd, and the averages of the former and latter were 3.55, 3.52 (Cd3, Cd3 + EDTA) and 2.34, 2.37 (Cd9, Cd9 + EDTA), respectively. In this experiment, also another hyperaccumulation criterion was fulfilled. The enrichment factors EF (shoot-to-growing medium ratio of Cd concentrations) were all > 1 at all treatments. The changes in *B. pilosa* EF trends for 10 different Cd species (Fig. 3) were essentially the same ($p < 0.05$) as those for Cd concentrations in the shoots (Fig. 1A) and extractable Cd concentrations (Fig. 5) in all treatments with 3 mg kg⁻¹ and 9 mg kg⁻¹ Cd as well as with or without EDTA addition. The differences in detail data were also similar to those described above.

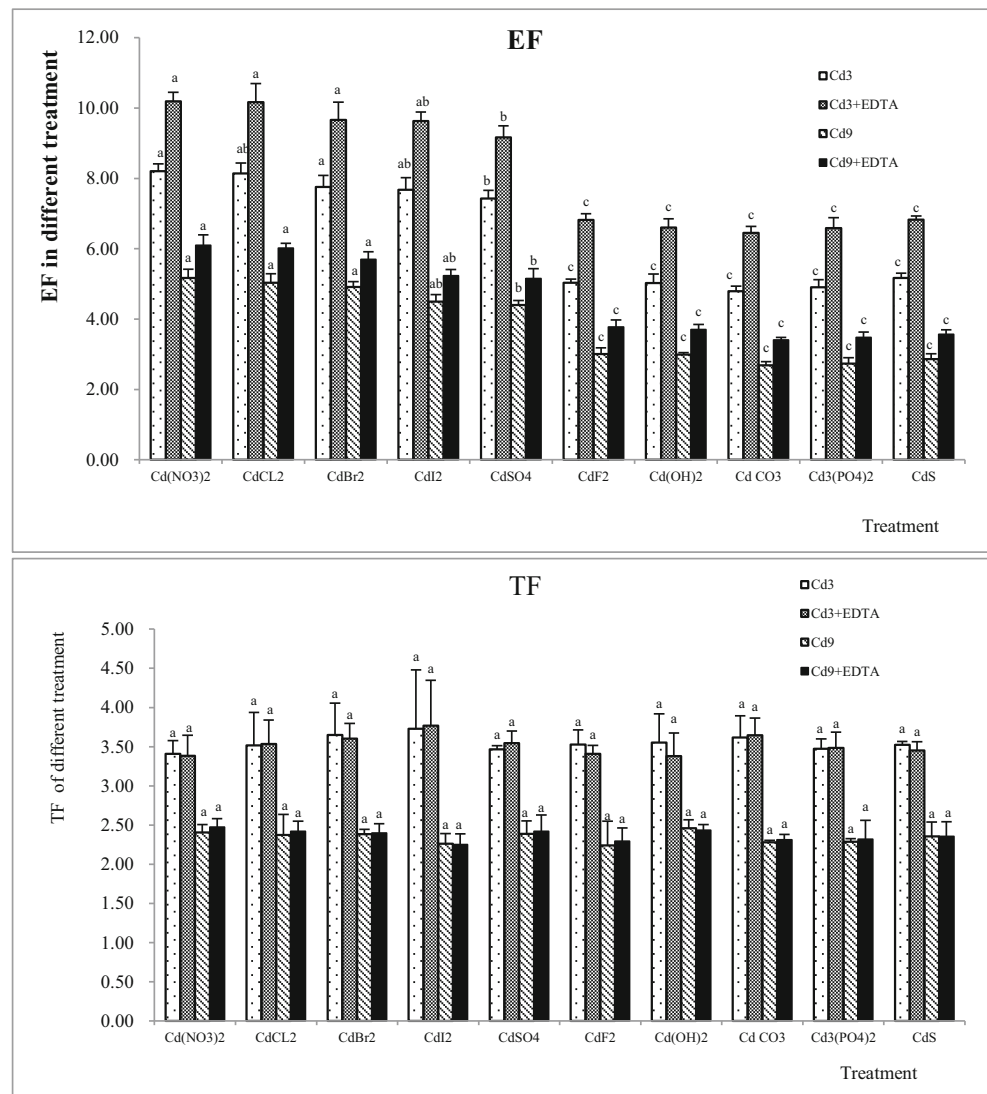
Discussion

One of the important factors reflecting phytoremediation efficiency and tolerance to trace metals in plants is biomass. Unaffected biomass represents tolerance to metal stress of the plant. Root growth inhibition under Cd stress can result from cell wall lignification, which limits cell expansion and

nutrient uptake, and the response of shoot can result from photosynthetic reaction inhibition, which prevents organic accumulation (Baycu et al. 2017). In this experiment, *B. pilosa* biomass was not affected ($p < 0.05$) in the treatments with different Cd forms, indicating that *B. pilosa* showed very strong tolerance (Fig. 1). This result suggested that there may be a wide range for phytoremediating different Cd species-contaminated soil in terms of tolerance.

As shown in Table 1, the solubility of Cd in water in different forms is quite different. The solubilities of Cd(NO₃)₂, CdCl₂, CdBr₂, CdI₂, and CdSO₄ are high, in contrast, and CdF₂, Cd(OH)₂, CdCO₃, Cd₃(PO₄)₂, and CdS are low-solubility Cd species (Table 1). The trends of solubilities were generally consistent with the concentrations of Cd in *B. pilosa* when different Cd forms added in soils, and with the extractable Cd concentrations in different treatments, but not entirely consistent, especially for CdF₂ and CdS (Table 1, Figs. 2 and 4). Basically, Cd concentrations of *B. pilosa* in the treatments with high solubilities of Cd forms (Cd(NO₃)₂, CdCl₂, CdBr₂, CdI₂, and CdSO₄) were higher than those in the treatments with low-solubility Cd species addition (CdF₂, Cd(OH)₂, CdCO₃, Cd₃(PO₄)₂, and CdS) (Fig. 2). But the ratio in *B. pilosa* Cd concentrations between high-solubility and low-solubility species was much lower than between the salt solubilities (Fig. 2), showing that the latter was not a unique factor of the differences. The results presented here (Fig. 4) confirmed that for the so-called low-solubility species of Cd, the extractable Cd concentrations were far from being low and not so different from that of the high-solubility Cd species, confirming that it is not the initial Cd form which mainly controls Cd availability, but its transformation through reactions in soil (Kabata-Pendias 2011). Using a model that simulates the transport by diffusion and convection, Lin et al.

Fig. 5 EF and TF of *B. pilosa* in different treatments



found that the most influential parameter was the initial Cd²⁺ concentration, followed by the soil buffer power for Cd²⁺, the soil water content, and impedance factor (Lin et al. 2016). Therefore, when assessing the risk of heavy metal-contaminated soils, we should not just consider the availability of heavy metals in soils, but the total amount of heavy metals in soils (Lin et al. 2016). This result also showed that phytoremediation was with wide usability for different Cd species in soil.

Usually, EDTA is the commonly used chelator to improve plant accumulating Cd in soil (Eissa 2016; Tananonchai et al. 2019). The application of EDTA in the present experiment played an important supporting role by improving extractable Cd species in soil, in particular of those low-solubility species (Fig. 4), and thus enhanced Cd accumulation of *B. pilosa* (Fig. 2, Eissa 2016; Tananonchai et al. 2019).

EFs and TFs in all treatments were higher than 1 (Fig. 5), indicating *B. pilosa* exhibited characteristics of translocation

factor (TF > 1) and enrichment factor (EF > 1) even for the treatments with low-solubility Cd species addition (Wei and Zhou 2008; van der Ent et al. 2013). Based on all of the above results, *B. pilosa* showed all Cd hyperaccumulator characteristics even for low-solubility Cd species added in soils, indicating a strong Cd accumulation capacity.

Although there were many studies concerned on plants hyperaccumulating Cd, only a few so far that concerning Cd species and most of using high solubility Cd species such as CdCl₂ to explore the effects of endophytic bacterium SaMR12 on *S. alfredii* metal ion uptake (Pan et al. 2017), CdSO₄ in the functional analysis of three HMA4 copies of a metal hyperaccumulator, *A. halleri* (Nouet et al. 2015), and Cd(NO₃)₂ to show the regulation of odd-numbered fatty acid content in the metabolism of *Noccaea* spp. hyperaccumulator adapted to oxidative stress (Pavlík et al. 2018). The hyperaccumulator characteristics of Cd in these plants were often questioned because high-solubility Cd species were used

in these experiments. The results of the present study could perfectly explain the hyperaccumulative properties of a hyperaccumulator to different species of the same heavy metal.

Conclusions

There were significant differences in Cd accumulation by *B. pilosa* from species of different solubilities occurring in soil. The accumulation for high-solubility species was basically high. But the accumulation of *B. pilosa* to low-solubility species also showed all Cd hyperaccumulation characteristics, i.e., with high accumulation potential either. Thus, phytoremediation showed a wide application for different Cd species-contaminated soil based on the results of biomass and Cd accumulation in this experiment. EDTA addition to soil significantly improved different species of Cd uptake by *B. pilosa*, especially for low-solubility species. Furthermore, the total amount of Cd in soil should be considered together with the extractable concentration when assessing the risk of Cd-contaminated soils.

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