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Identification of a Cd accumulator *Conyza canadensis*

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ABSTRACT

One of key steps of phytoremediating heavy metal contaminated soils is still the identification of hyperaccumulator and accumulator. In a former published article, *Conyza canadensis* L. Cronq. expressed some basic properties of Cd-hyperaccumulators. In this study, concentration gradient experiment and two sample-analyzing experiments were used to identify whether this plant is a Cd-hyperaccumulator. When grown on soil spiked with Cd at the rate of 10 and 25 mg kg^{-1} in concentration gradient experiment, *C. canadensis* had both Cd enrichment factor (EF) and Cd translocation factor (TF) greater than 1, while the shoot biomass did not differ significantly as compared to the control. On the other hand, with Cd-spiking rates of 10 and 25 mg kg^{-1}, the Cd concentration in the shoot did not exceed 100 mg kg^{-1}, which is considered as the minimum shoot Cd concentration to qualify as a hyperaccumulator. In the sample-analysis experiments from a Pb–Zn mine area and wastewater irrigation region, *C. canadensis* also showed Cd-accumulator characteristics. Based on the results accomplished, we propose *C. canadensis* as a Cd-accumulator.

1. Introduction

Several methods such as solidification, vitrification, electrokinetic remediation etc can be used to remedy heavy metal contaminated soils [1]. However, these technologies may deteriorate soil quality, cause secondary pollution and often costly [2]. Thus, phytoremediation, which mainly using hyperaccumulator and accumulator plants remove excess heavy metals from contaminated soils, is regarded as a promising cost effective method without major secondary environmental issues especially for the remediation of large areas of contaminated soils with relatively low level of heavy metal contamination [3,4]. Despite the known advantages of phytoremediation over other remediation techniques, only a few Cd hyperaccumulators have been identified, researched and documented. Notably two members of the Brassicaceae family such as *Thlaspi caerulescens* J & C Presl and *Arabidopsis halleri* L. are well known [5,6]. Recently *Viola baoshanensis* Shu, Liu et Lan and *Solanum nigrum* L. have also been reported as Cd hyperaccumulators [7–9]. It is worth mentioning here that screening and evaluation of various plants for metal hyperaccumulator and accumulator should always be an important part of phytoremediation.

Plants are considered as hyperaccumulators when they can accumulate uniquely high quantities of heavy metals. The main characteristics of a hyperaccumulator plant can be summarized as follows: (1) critical concentration, i.e., on a dry mass basis, the suggested critical values in the shoots (stems or leaves) of a hyperaccumulator 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 [10]; (2) translocation factor (TF), which is the ratio of the metal concentration in the shoot to that in root, should be greater than 1 [10]; (3) enrichment factor, i.e., EF (concentration in plant/soil) >1 [11]; (4) tolerance property, a hyperaccumulator should have high tolerance to toxic contaminants. In addition, for the plants tested under experimental conditions, their aboveground biomass should not decrease significantly when growing in contaminated soils [11]. The first item listed above is a unique characteristic of hyperaccumulators, while the rest three features are shared with accumulators. Although shoot concentration of heavy metals is increased by soil concentration, accumulators display consistently lower levels than hyperaccumulators. Accumulators will not exceed the shoot concentrations described above [4,10].

In a previous study, Wei and Zhou evaluated 54 weed species for their hyperaccumulative characteristics [12]. The weed species

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Conyza canadensis showed high tolerance to Cd single and Cd–Pb–Cu–Zn combined pollution as well as met the some properties of Cd-hyperaccumulator under the condition of 10 mg kg^{-1} Cd spiking to the soil. However, it failed to show hyperaccumulative characteristics to Pb, Cu and Zn. In the present study, we further evaluated the Cd accumulation potential of *C. canadensis* through three new experiments in order to identify whether it is a hyperaccumulator or an accumulator.

2. Materials and methods

2.1. Concentration gradient experiment

The pot-culture experiment was conducted at the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (41°31′N and 123°41′E) in spring. The local soil is relatively unpolluted compared to the National Soil-Environmental Quality Standard of China (NSEQSC) (GB 15618, 1995) [13]. Top soil (0–20 cm) was collected from a local field and is a meadow burozem with pH value of 6.6, organic matter of 1.52%, cation exchange capacity of 23.7 cmol kg^{-1} and background concentrations of heavy metals as follows: Cd 0.2; Pb 14.2; Cu 12.4; Zn 40 mg kg^{-1} [12].

Five treatments were tested in the study which includes a control designated as CK (i.e., without external Cd added) and four Cd-spiking treatments designated as T_1, T_2, T_3 and T_4. Each of the five treatments was replicated three times. The four levels of Cd spiking into the soils of T_1, T_2, T_3 and T_4 were 10, 25, 50 and 100 mg kg^{-1}, respectively. The chemical form of Cd used to spike into the test soil was $CdCl_2{\cdot}2.5H_2O$. The collected surface soil was ground and sieved through a 4-mm sieve and homogenized through repeated mixing. The prepared soil was then mixed with the required amount of $CdCl_2{\cdot}2.5H_2O$ in order to achieve levels of Cd-spiking for different treatments, and finally filled into plastic pots (with diameter = 20 cm and height = 15 cm) followed by equilibration for 2 months. Four seedlings of *C. canadensis* with the identical growth stage were transplanted into each pot in spring. Plants in pots were allowed to grow in the natural field. Loss of water from the pots through evaporation was made up using tap water (no Cd detected). No fertilizer was added. All plants were harvested after their seed maturity for determination biomass production and metal analysis.

2.2. Sample-analysis experiment in a Pb–Zn mining site

Qingchengzi Pb–Zn mining area is situated in Fengcheng County, Liaoning Province, China (40°41′N, 123°37′E). The site meteorology is similar to the pot culture experiment site. Secondary forest, sparse brushwood and some transplanted trees are the main vegetation. Marble and micacite are the main host rocks. Brown soil (burozem) is widely distributed in this mining area. The grade of Pb–Zn minerals (galena and sphalante) is up to 70–80%. Cd is an associate metal, mainly compounded within the crystal lattice of sphalante, and its average grade is about 0.034% [14]. The plant samples of *C. canadensis* and its associated soil samples were randomly collected in the mining area in maturity season of autumn (late of August).

2.3. Sample-analysis experiment in a wastewater irrigation area

The experiment was carried out in Zhangshi irrigation region which lies in the west suburb of Shenyang city. Since 1962, most of the farmlands (about 2800 hm^2) in the area have been contaminated due to improper irrigation of rice paddies with Cd-containing industrial wastewater drained from Shenyang Weigong hypaethral trench. Soil Cd occurred mostly in the top soil layer with concentrations ranging from 5 to 7 mg kg^{-1} in gate 1, and 3 to 5 mg kg^{-1} in gate 2 and 3 of the irrigation region [15]. Even though irrigation with industrial wastewater was forbidden right after soil contamination with Cd was discovered in the area, Cd concentration in the contaminated soils has been remaining unchanged till now. We randomly collected seven plants of *C. canadensis* in autumn and their associated soils from the site to see the Cd accumulation characteristics.

2.4. Sample analysis and data processing

Roots, stems, leaves and inflorescences of harvested plant samples were rinsed with tap water to remove dirt, and then carefully washed with deionized water. Nearly half of stems, leaves and inflorescences were mixed together to examine the heavy metal concentration in shoots. The samples were then dried at 105 °C for 5 min, then at 70 °C in an oven until completely dry (about 48 h). The dried plant samples were ground into a powder and passed through a 2-mm sieve. The soil samples were air-dried and ground using a mortar and pestle and passed through a 0.149-mm sieve [9]. The plant and soil samples were digested with an acid mixture containing 13 M HNO_3 plus 1 M $HClO_4$. The extractable heavy metals in soils were extracted with 0.1 mol L^{-1} HCl. The concentrations of heavy metals were determined using an atomic absorption spectrophotometer (Hitachi 180-80; 1.3 nm spectral band width) [9,16]. 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). Soil organic matter content was determined using the methods suggested by Lu [17]. pH was determined electrometrically (PHS-3B meter) using a 1:2.5 ratio of soil:water.

All the values expressed are mean ± S.D. (standard deviation) of the three replicates including three analytical replicates for the same samples in sample-analyzing experiment and sewage irrigation area evaluation experiment. Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests to separate means. Differences were considered significant at $p < 0.05$ [18]. Data were analyzed using the software of Excel and SPSS 11.5.

3. Results

3.1. Cd accumulation potential of C. canadensis in the concentration gradient experiment

The results showed that plant height and leaf color of *C. canadensis* did not differ in treatments T_1–T_4 compared to the control (CK). Its shoot biomasses (2.37, 2.38, 2.35, 2.72 and 2.34 g $plant^{-1}$ for CK and T_1–T_4) were not significantly decreased ($p < 0.05$), suggesting tolerance characteristics typical of hyperaccumulator [12,19,20].

Generally, the Cd concentration in different plant parts did increase with increasing soil Cd levels (Fig. 1). However, Cd concentration in any plant part did not surpass 100 mg kg^{-1} when the soil was spiked with Cd at 10, 25, and 50 mg kg^{-1} (T_1, T_2 and T_3) even though the EF was always higher than 1 (EFs: 2.81,1.92, 1.39 and 0.79 for T_1–T_4, respectively) and TF for T_1 and T_2 was higher than 1 (TFs: 2.00, 1.02, 0.89 and 0.48 for T_1–T_4, respectively). Although the leaf Cd concentration for 100 mg kg^{-1} Cd spiking was greater than 100 mg kg^{-1}, its EF and TF were all less than 1. Thus, *C. canadensis* can be considered merely as a Cd accumulator, not a hyperaccumulator [11,19,20].

3.2. Cd accumulation characteristics of C. canadensis growing in a Pb–Zn mine area

The pH and organic matter content of collected soil samples from the Pb–Zn mine area were 6.5–6.9 and 14.31–15.04 g kg^{-1},

Table 1
Accumulative characteristics of *C. canadensis* to Cd in a Pb–Zn mine area

Plant	Plant (mg kg^{-1})					Shoot EF	Shoot biomass (g plant^{-1})	Soil (mg kg^{-1})	
	Root	Stem	Leaf	Inflorescence	Shoot			TotaL cd	Detectable cd
1	0.5 ± 0.1b	1.1 ± 0.1a	1.2 ± 0.1a	0.5 ± 0.1b	1.1 ± 0.1a	1.10	24.76	1.0 ± 0.1	0.5 ± 0.1
2	0.7 ± 0.1c	1.2 ± 0.1b	2.5 ± 0.3a	1.2 ± 0.1b	1.4 ± 0.2b	1.56	18.14	0.9 ± 0.1	0.6 ± 0.1
3	1.5 ± 0.2b	1.1 ± 0.1b	2.1 ± 0.2a	0.7 ± 0.1c	1.3 ± 0.1b	1.08	44.49	1.2 ± 0.1	0.7 ± 0.1
4	1.2 ± 0.2b	1.8 ± 0.2ab	2.2 ± 0.2a	1.6 ± 0.2a	1.9 ± 0.2a	1.06	12.61	1.8 ± 0.2	0.7 ± 0.1
5	15.4 ± 2.9c	27.2 ± 3.7b	38.3 ± 4.6a	12.5 ± 1.4c	26.8 ± 3.2b	2.27	9.77	11.8 ± 2.3	6.2 ± 0.7
6	3.3 ± 0.3b	4.7 ± 0.5a	4.5 ± 0.5a	1.2 ± 0.1c	4.3 ± 0.4a	1.59	13.84	2.7 ± 0.3	1.2 ± 0.1
7	1.9 ± 0.2c	2.7 ± 0.3b	3.8 ± 0.4a	1.8 ± 0.2c	2.7 ± 0.3b	1.59	25.2	1.7 ± 0.2	1.1 ± 0.1
8	2.0 ± 0.3b	3.6 ± 0.4a	3.2 ± 0.3a	2.2 ± 0.2b	3.3 ± 0.4a	2.06	9.38	1.6 ± 0.2	0.9 ± 0.1

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

Table 2
Cd accumulative properties of *C. canadensis* in a wastewater irrigation oregion

Plant	Plant (mg kg^{-1})					Shoot EF	Shoot biomass (g plant^{-1})	Soil (mg kg^{-1})	
	Root	Stem	Leaf	Inflorescence	Shoot			Total Cd	Detectable Cd
1	5.2 ± 0.6c	8.1 ± 0.9b	11.8 ± 1.4a	2.9 ± 0.3d	8.5 ± 0.9b	3.15	25.82	2.7 ± 0.3	2.5 ± 0.3
2	7.3 ± 0.8b	6.8 ± 0.6b	9.3 ± 1.0a	2.4 ± 0.2c	5.8 ± 0.6b	3.63	48.34	1.6 ± 0.2	1.4 ± 0.2
3	4.6 ± 0.5c	7.4 ± 0.8b	10.8 ± 1.2a	4.4 ± 0.5c	6.9 ± 0.5b	3.83	47.32	1.8 ± 0.2	1.5 ± 0.2
4	3.4 ± 0.6c	6.0 ± 0.7b	10.2 ± 1.3a	2.9 ± 0.3c	5.9 ± 0.6b	2.95	40.4	2.0 ± 0.2	1.8 ± 0.2
5	4.2 ± 0.7c	8.6 ± 0.9b	13.4 ± 1.4a	3.7 ± 0.4c	7.9 ± 0.9b	2.72	57.54	2.9 ± 0.3	2.5 ± 0.3
6	4.2 ± 0.5c	7.4 ± 0.6b	10.8 ± 1.1a	2.8 ± 0.3d	6.5 ± 0.7b	2.83	47.9	2.3 ± 0.3	1.9 ± 0.3
7	4.9 ± 0.5c	7.3 ± 0.7ab	9.6 ± 0.9a	2.7 ± 0.3d	6.3 ± 0.6b	3.15	51.29	2.0 ± 0.2	1.7 ± 0.2

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

respectively. The ranges of total Cd, Pb, Cu and Zn concentrations in the soil samples from the Qingchengzi mining area were 0.9–11.8 mg kg^{-1}, 237.5–4059.4 mg kg^{-1}, 16.4–131.2 mg kg^{-1} and 123.5–3904.5 mg kg^{-1}, respectively. Any kind of toxic symptoms were not noticed in the eight plants of *C. canadensis* collected from the site. The Pb, Cu and Zn concentrations of *C. canadensis* grown in this site are not shown in this paper, since the EFs and TFs for all three metals were below 1 as well as for the sake of brevity. In sharp contrast, both EFs and TFs of Cd were all basically greater than 1 (Table 1). However, Cd concentrations in the shoots were below 100 mg kg^{-1}. Thus, *C. canadensis* from the site showed the properties that meet the criteria of just Cd accumulator [11,19,20].

3.3. *Cd accumulation characteristics of C. canadensis growing in a wastewater irrigation region*

The pH and organic matter content of collected soil samples in Zhangshi irrigation region were basically same as those soils from the Pb–Zn mine area. The soil had total Cd content ranging from 1.6 to 2.7 mg kg^{-1}, while the extractable Cd content ranged from 1.4 to 2.5 mg kg^{-1}. All seven plants collected from this polluted site did not show any toxic symptom either.

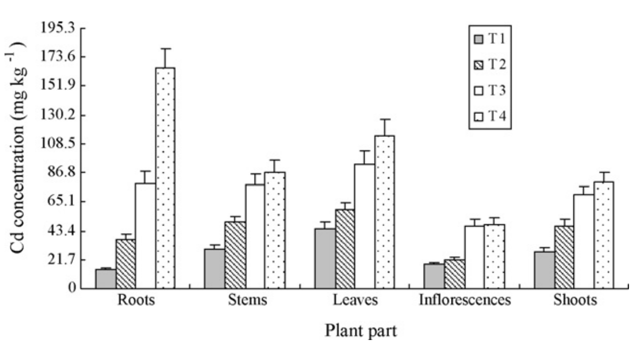

Fig. 1. Accumulation potentials of *C. canadensis* in concentration gradient experiment (mg kg^{-1}).

As shown in Table 2, EFs and TFs for all plants were basically higher than 1. But Cd concentrations in shoots were below 100 mg kg^{-1}. Thus, the plant can be considered as only a Cd accumulator [11,19,20].

4. Discussion and implication

It has been shown that heavy metals can be removed from a contaminated soil by removing hyperaccumulator or accumulator, and this is referred as phytoextraction [3]. However, phytoextraction efficiency is determined by the total amount of metal extracted by the plants, which is consisted in 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 [21]. Compared to Cd accumulator like *Brassica juncea*, even Cd-hyperaccumulator such as *T. caerulescens*, *A. halleri*, *V. baoshanensis* and *S. nigrum*, *C. Canadensis* has larger biomass and other growth merits mentioned above, which showed strong potential to remedy Cd contaminated soil [5–9,22,23].

C. canadensis is an annual farming grass and its height is about 50–100 cm, which is widely distributed and easily found in North America and many countries in Asia [24]. In temperate zone, it usually germinates during April and June and flowers from July to August, and reproduced by its seed. Due to its strong ecological adaptability, many places such as riverside, roadside and farmland are good sites for its growth.

Usually, like *C. Canadensis,* weed species possesses strong tolerant properties especially to light and water stress. They grow fast and often have high biomass [25]. It is possible that the weed species may have strong endurance to heavy metal stress as well. They may have high metal accumulation of heavy metals, and some of them may even hyperaccumulate the heavy metals. Moreover, most of the metal hyperaccumulator plants documented so far such as *Thlaspi caerulescens*, *Phyllanthus serpentines*, *Silene vulgaris* and *Lactuca sativa* belong to weed species or obviously possess the basic properties of weed species [26]. We thus believe that there will be further significant advancement in the screening of accumulators, even hyperaccumulators involving weed species.

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