Journal of Hazardous Materials 159 (2008) 544–547

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

A newly found cadmium accumulator—*Taraxacum mongolicum*

Shuhe Wei[a], Qixing Zhou[a,b,*], Shiny Mathews[c]

[a] Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China
[b] College of Environmental Science and Engineering, Nankai University, Tianjin 300071, PR China
[c] Soil and Water Science Department, University of Florida, Gainesville, FL 32611, United States

ARTICLE INFO

Article history:
Received 8 November 2007
Received in revised form 19 February 2008
Accepted 19 February 2008
Available online 23 February 2008

Keywords:
Phytoremediation
Cadmium
Accumulator

ABSTRACT

Identification of hyperaccumulator and accumulator is still key step of phytoextracting-contaminated soils by heavy metals. In a former published experiment, *Taraxacum mongolicum* showed basic characteristics of hyperaccumulators. In order to confirm if this plant was a Cd-hyperaccumulator, concentration gradient experiment and sample-analyzing experiments were designed and performed. The results showed that Cd enrichment factor and Cd transformation factor of *T. mongolicum* were all higher than 1 in concentration gradient experiment. The shoot biomasses did not reduced significantly ($p < 0.05$) compared with the control without Cd added under the conditions of lower than 25 mg kg^{-1} Cd spiked into soil. However, Cd concentration in shoot of *T. mongolicum* was not higher than 100 mg kg^{-1} the minimum a Cd-hyperaccumulator should have under the conditions of any concentration level of Cd spiked. Thus, *T. mongolicum* should be a Cd-accumulator. In the sample-analyzing experiments settled in a Pb–Zn mine area and Shenyang wastewater irrigation region, *T. mongolicum* also showed that Cd-accumulator characteristics. Based on these results, *T. mongolicum* could be identified as a Cd-accumulator, which may have important implication in plant physiology and gene engineering.

1. Introduction

There are several pathways for heavy metals contaminated soils, two of which are mining and irrigation [1]. In the former, soils around ore can be polluted by metal mine mining due to mineral action in soil under the reaction with environmental factors like water, air and heat for a very long time; caused by leaching during piling of mining waste rock, and by leakage of mining wastewater containing high level of heavy metals [2]. In the latter, industry wastewater irrigation is main reason. Zhangshi irrigation region in Northeast China is a very typical region which soils were contaminated by some heavy metals in a very huge area [3].

There are a number of methods to remediate contaminated soils, such as solidification remediation technology, vitrification remediation technology, electrokinetic remediation and so on. However, these technologies have many shortcomings which include deterioration of soil quality, secondary pollution and high cost. To overcome these disadvantages, phytoremediation is regarded as a promising method, especially for the remediation of large areas of contaminated soil with lower heavy metal concentration [4].

Phytoremediation of contaminated soils by heavy metals means using special plant and its microorganism system in rhizosphere to extract, volatilize or stabilize contaminants in soils, i.e. phytoextraction, phytovolatilization and phytostabilization. Phytoextraction technology is the most engrained method by which contaminated soil is mainly cleaned by hyperaccumulators or accumulators [4]. In other words, heavy metals in contaminated soils can be largely removed from polluted sites by using hyperaccumulators or accumulators. Hyperaccumulators are plants that can exceptionally accumulate high quantities of heavy metals. The main characteristics of hyperaccumulating plants can be summarized as follows: (1) high metal accumulation capacity, i.e. the minimum concentration in the shoots (stems or leaves) of a hyperaccumulator for As, Pb, Cu, Ni and Co should be greater than 1000 mg kg^{-1} dry mass, Zn and Mn are 10,000 mg kg^{-1}, Au is 1 mg kg^{-1} and Cd is 100 mg kg^{-1}, respectively [5]; (2) translocation capability, elemental concentrations in the shoots of a plant should be higher than those in roots [6], i.e. TF > 1 (translocation factor, concentration ratio of shoots to roots); (3) enrichment capability (enrichment factor-EF, concentration ratio of plant shoot to soil), EF value in shoots of plants should be higher than 1 [7]; (4) tolerance capability, a hyperaccumulator should have high tolerance to toxic contaminants [7]. In addition, for the plants tested under experimental conditions, their aboveground biomass should not decrease significantly when growing in contaminated soils. But for the characteristics of accumulators, the last three items above mentioned are same as that of

* Corresponding author at: Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China. Tel.: +86 24 83970373; fax: +86 24 83970436.
E-mail addresses: Zhouqx523@yahoo.com, shuhewei@yahoo.com.cn, shuhewei@hotmail.com (Q. Zhou).

0304-3894/$ – see front matter
doi:10.1016/j.jhazmat.2008.02.052

hyperaccumulator. The main difference is the first item. Though, the heavy metal concentration in shoot is increased with the content in soil, accumulator has lower accumulation capability than the hyperaccumulator, i.e. the maximal concentration in the shoots (stems or leaves) were not greater than the typical concentrations, as for As, Pb, Cu, Ni and Co are not greater than 1000 mg kg^{-1} dry mass, Zn and Mn are for 10,000 mg kg^{-1}, Au is for 1 mg kg^{-1} and Cd is for 100 mg kg^{-1}, respectively [4,5].

Though more than 400 species of hyperaccumulators have been documented in the world [4,8], phytoextraction technology is still not widely used in remediation practices till now. The main reason lies in the low remediation efficiency of hyperaccumulators or accumulators, stemming from both small biomass and long growing seasons [1]. Thus, the identification of hyperaccumulator or accumulator is still the key of phytoextraction.

In a former published article, 54 weed species have been studied aimed at identifying hyperaccumulative characteristic plants [9]. The results showed that *Taraxacum mongolicum* indicated stronger tolerance to Cd single and Cd–Pb–Cu–Zn combined pollution, higher Cd-accumulative ability (EFs were equal to 3.21 and 3.13 under the condition of 10 mg kg^{-1} Cd added), suggesting it possessed basic characteristics of Cd hyperaccumulators. However, the EFs of Pb, Cu and Zn were lower than 1. Thus, they did not own accumulative characteristics to Pb, Cu and Zn. In order to know the potential of *T. mongolicum* accumulating Cd and if it is a hyperaccumulator or accumulator to Cd, we performed three further experiments in this paper.

2. Materials and methods

2.1. Concentration gradient experiment in ecological experiment station

The experiment site, Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (41°31′ N and 123°41′ E) is a temperate zone with semi-moist continental climate. Top meadow burozem soil (0–20 cm) collected from a field was used as the culture medium with pH of 6.6, organic matter of 1.52% and cation exchange content of 23.7 cmol kg^{-1}. The background concentration of Cd, Pb, Cu and Zn are 0.15, 14.2, 12.4 and 39.9 mg kg^{-1}, respectively. Thus, it is very clean soil compared with the National Soil-Environmental Quality Standard of China (NSEQSC) [10].

The site of concentration gradient experiment was arranged in the station. There were five treatments including the control (CK, without external Cd added), T_1, T_2, T_3 and T_4. The concentration of Cd spiked to the tested soils of $T_1 \sim T_4$ were 10, 25, 50 and 100 mg kg^{-1}, respectively. The form of Cd added to the tested soil was $CdCl_2 \cdot 2.5H_2O$.

The tested topsoil samples were sieved through a 4 mm mesh, then mixed with reagents containing heavy metals and filled into plastic pots (ϕ = 20 cm, H = 15 cm), and equilibrated for 2 months. Four seedlings of *T. mongolicum* with the identical growth phase were transplanted into the treated pots. Plants in pots were allowed to grow in the natural field. Loss of water by evaporation from pots was made up using tap water (no Cd detected). No fertilizer was added. All the treatments were replicated three times. All tested plants were harvested after their seed maturity.

2.2. Sample-analyzing experiment in a mine polluted site

The investigated Pb–Zn mining area is situated in Qingchengzi (40°41′ N, 123°37′ E), Fengcheng County, Liaoning Province, China. The site meteorology is similar to the pot culture experiment site. In the mining area, secondary forest, sparse brushwood and some transplanted trees are the main vegetation and marble and micacite are the main host rocks. Brown soil (burozem) is widely distributed. The main host rocks are marble and micacite, and 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% [2].

In maturity season of summer, *T. mongolicum* and its corresponding soil samples were collected in the mining area. Because *T. mongolicum* is a kind of accidentally growing species on the site, any individual plant of it discovered was all collected.

2.3. Sample-analyzing experiment in wastewater irrigation area

Zhangshi irrigation region which lies in the west suburb of Shenyang city is about 35 km to the station. Since 1962, most of farmlands (about 2800 hm^2) of the area have been contaminated by incorrectly irrigating rice paddies with wastewater containing Cd drained from Shenyang Weigong hypaethral trench. An investigation was carried out in 1975, by Institute of Applied Ecology, Chinese Academy of Sciences, and the results showed that the main pollutant of the polluted soil was Cd and the pollutant were mainly distributed in topsoil (0–35 cm). The concentrations of Cd in soil were about 5–7 mg kg^{-1} in gate 1, and 3–5 mg kg^{-1} in gates 2 and 3 of the irrigation region [3]. Though industry sewage irrigation was forbidden since the day of Cd contamination discovered in the area, Cd concentration in contaminated soils has basically not changed till now. Thus, we collected five plants in autumn to see accumulative characteristic of *T. mongolicum* to Cd.

2.4. Sample analysis and data processing

Plant samples (separated into roots, leaves and inflorescences) were firstly rinsed with tap water, followed by deionized water later. The samples were then dried at 105 °C for 5 min and further at 70 °C in an oven until completely dry (near 2 days). Nearly half of leaves and inflorescences were mixed together to be used to examine Cd concentration in shoots. The dried plant samples and collected soil samples were separately ground to a powder and passed through a 2 mm sieve. The plant and soil samples were digested with a solution containing 87% of concentrated HNO_3 and 13% of concentrated $HClO_4$. The concentrations of extractable heavy metals in soils were extracted with 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]. 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 [12]. The pH was determined with the pH meter (PHS-3B), and the ratio of soil and water is 1:2.5 [12].

All the values expressed are mean ± S.D. (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 $p < 0.05$ [13]. 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

3.1. Cd accumulative characteristics of T. mongolicum in concentration gradient experiment

Fig. 1 shows shoot biomasses of *T. mongolicum* in different Cd treatment (CK, T_1–T_4). Compared with the control (CK), the above-

Table 1
Accumulative characteristics of *T. mongolicum* to Cd (mg kg^{-1})

Treatment	Root	Leaf	Inflorescence	Shoot	TF[a]	EF[b]
T_1	8.2 ± 0.9 b	31.9 ± 0.5 a	12.2 ± 2.2 b	31.8 ± 3.4 a	3.9	3.2
T_2	12.0 ± 0.2 b	39.4 ± 4.5 a	14.5 ± 3.1 b	33.0 ± 2.3 a	2.8	1.3
T_3	26.0 ± 2.1 b	45.5 ± 6.5 a	15.3 ± 2.0 c	36.8 ± 4.4 a	1.4	0.7
T_4	41.9 ± 3.9 b	60.3 ± 5.4 a	18.0 ± 2.6c	57.2 ± 6.2 a	1.3	0.6

Note: Different letter in each row means a significant difference among different plant parts.
[a] TF, transformation factor, concentration ratio in shoots to roots.
[b] EF, enrichment factor, concentration ratio in plant shoot to soil.

Table 2
Heavy metal concentration in soil samples collected from Pb–Zn mine area

Soil sample	Heavy metal (mg kg^{-1})							
	Cd	Extractable Cd	Pb	Extractable Pb	Cu	Extractable Cu	Zn	Extractable Zn
1	9.4 ± 0.9	4.0 ± 0.3	792.7 ± 89.2	285.0 ± 30.2	34.9 ± 3.2	12.0 ± 1.7	1291.1 ± 110.7	356.0 ± 38.7
2	0.5 ± 0.1	0.2 ± 0.0	237.5 ± 28.7	155.3 ± 16.7	16.4 ± 2.7	6.2 ± 0.9	123.5 ± 10.5	58.2 ± 44.9
3	1.1 ± 0.2	0.4 ± 0.0	1562.1 ± 154.3	603.0 ± 66.2	64.2 ± 7.8	37.3 ± 4.3	271.4 ± 22.8	110.7 ± 15.4
4	2.2 ± 0.2	0.9 ± 0.1	3577.0 ± 389.5	957.0 ± 98.4	93.2 ± 9.6	4.7 ± 0.5	428.4 ± 39.8	185.0 ± 20.9
5	6.1 ± 0.7	4.1 ± 0.3	722.5 ± 88.1	328.7 ± 33.8	107.0 ± 11.2	45.8 ± 4.4	1461.5 ± 144.3	609.2 ± 66.5
6	10.0 ± 0.9	3.7 ± 0.4	880.7 ± 90.6	201.2 ± 21.1	58.4 ± 60.6	4.4 ± 0.3	1364.6 ± 128.9	114.8 ± 12.2
7	26.2 ± 2.3	16.0 ± 1.9	4059.4 ± 467.2	668.7 ± 70.0	131.2 ± 12.7	14.5 ± 2.6	3904.5 ± 400.0	1372.8 ± 130.8

Table 3
Accumulative characteristics of *T. mongolicum* to Cd in Pb–Zn mine area

Plant	Part	Heavy metal (mg kg^{-1})				Cd EF[a]	Pb, Cu and Zn EF	Cd TF[b]	Shoot biomass (g plant^{-1})
		Cd	Pb	Cu	Zn				
1	Root	3.7 ± 0.4	19.5 ± 20.5	10.2 ± 1.1	141.4 ± 15.2	2.45	<1	6.2	25.40
	Shoot	23.0 ± 3.2	572.2 ± 60.3	22.6 ± 2.4	12.0 ± 1.4				
2	Root	0.6 ± 0.1	15.2 ± 2.2	8.1 ± 0.7	25.1 ± 3.4	4.40	<1	3.7	3.47
	Shoot	2.2 ± 0.3	167.2 ± 17.5	8.2 ± 0.8	122.2 ± 10.2				
3	Root	0.5 ± 0.0	28.3 ± 3.2	17.8 ± 2.0	76.2 ± 6.8	1.73	<1	3.8	7.37
	Shoot	1.9 ± 0.3	587.2 ± 60.2	17.8 ± 1.6	69.1 ± 7.6				
4	Root	0.9 ± 0.1	42.3 ± 4.3	13.0 ± 1.2	35.4 ± 4.3	2.18	<1	2.7	1.98
	Shoot	2.4 ± 0.2	298.0 ± 30.1	17.0 ± 1.9	146.7 ± 12.9				
5	Root	2.0 ± 0.2	5.8 ± 5.5	6.0 ± 0.5	36.8 ± 4.4	1.02	<1	3.1	0.9
	Shoot	6.2 ± 0.7	55.9 ± 6.5	10.8 ± 1.2	160.9 ± 18.7				
6	Root	4.1 ± 0.4	23.4 ± 3.4	9.5 ± 0.8	39.6 ± 4.0	1.73	<1	4.2	3.36
	Shoot	17.3 ± 2.2	77.0 ± 8.2	8.7 ± 0.9	134.0 ± 14.2				
7	Root	3.5 ± 0.4	84.6 ± 9.0	54.7 ± 6.6	105.8 ± 11.7	1.02	<1	7.6	1.37
	Shoot	26.7 ± 3.3	1327.7 ± 140.2	23.2 ± 3.2	131.0 ± 15.2				

Note: Different letter in each row means a significant difference among different plant parts.
[a] TF, transformation factor, concentration ratio in shoots to roots.
[b] EF, enrichment factor, concentration ratio in plant shoot to soil.

Table 4
Accumulative characteristics of *T. mongolicum* to Cd in a wastewater irrigation region

Plant	Root Cd (mg kg^{-1})	Shoot Cd (mg kg^{-1})	Soil total Cd (mg kg^{-1})	Soil extractable Cd (mg kg^{-1})	Cd EF[a]	Cd TF[b]	Shoot biomass (g plant^{-1})
1	1.8 ± 0.2	3.2 ± 0.3	2.6 ± 0.2	2.1 ± 0.1	1.23	1.78	4.37
2	0.8 ± 0.1	3.0 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	4.29	3.75	6.06
3	1.0 ± 0.1	2.7 ± 0.3	2.0 ± 0.2	1.6 ± 0.2	1.35	2.7	2.85
4	2.4 ± 0.3	3.8 ± 0.5	1.5 ± 0.1	1.2 ± 0.2	2.53	1.58	3.16
5	0.7 ± 0.1	2.5 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	1.19	3.57	3.27

Note: Different letter in each row means a significant difference among different plant parts.
[a] TF, transformation factor, concentration ratio in shoots to roots.
[b] EF, enrichment factor, concentration ratio in plant shoot to soil.

ground dry weights of *T. mongolicum* did not significantly decrease ($p < 0.05$) under the conditions of 10, 25,50 and 100 mg kg^{-1} Cd spiked, which indicates strong tolerance of the plant to Cd contamination [7].

Cd concentrations in plant different parts were listed in Table 1. When spiked Cd concentrations were lower than 25 mg kg^{-1}, the EFs were higher than 1, 3.2 for 10 mg kg^{-1} Cd added (T_1) and 1.3 for 25 mg kg^{-1} Cd added (T_2). But for treatment T_3 (50 mg kg^{-1} Cd added) and T_4 (100 mg kg^{-1} Cd added), the EFs were lower than 1. Furthermore, Cd concentrations in leaves or shoots were not higher than the minimum 100 mg kg^{-1} of what Cd-hyperaccumulator should have [5]. Thus, *T. mongolicum* can only be regarded as a Cd-

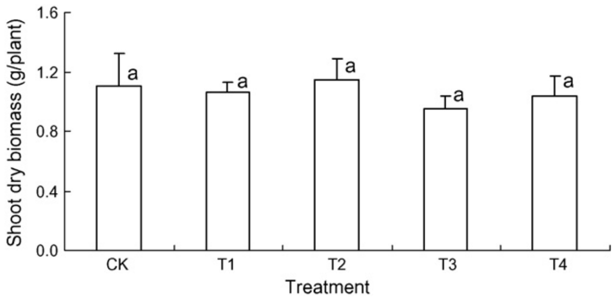

Fig. 1. Shoot biomass of *T. mongolicum* in different Cd treatment (same letter in columns means a non-significant difference among different treatment, $p < 0.05$).

accumulator, though TFs were higher than 1 and Cd concentration in plant increased with soil [4,5].

3.2. Heavy metal accumulative characteristics of T. mongolicum growing in a Pb–Zn mine area

Toxic symptoms were not observed in plants collected from the Pb–Zn mine area. The pH and organic matter content of collected soil samples were 6.53–6.95 and 14.31–15.04 g kg^{-1}, respectively. According to the NSEQSC (GB 15618, 1995), soil samples collected from the mining area can be classified into three types: (1) Cd single pollution, including sample 2; (2) Cd–Pb combined pollution, including soil samples 3 and 4; (3) Cd–Pb–Zn combined pollution, all the other soil samples (Table 2). The ranges of total Cd, Pb, Cu and Zn concentrations in the soil samples from the Qingchengzi mining area were 0.5–26.2 mg kg^{-1}, 237.5–4059.4 mg kg^{-1}, 16.4–131.2 mg kg^{-1}, 123.5–3904.5 mg kg^{-1}, respectively [10].

Seven plants of *T. mongolicum* were collected from Qingchengzi Pb–Zn mine area. As shown in Table 3, EFs and TFs of Cd were all greater than 1. EFs of Pb, Cu and Zn were all lower than 1. Heavy metal concentrations in plants were basically increased with soils. In addition, any heavy metal concentrations in plant shoots were not higher than typical concentration what hyperaccumulator should have. Thus, *T. mongolicum* only showed Cd accumulative property [4,5].

3.3. Cd accumulative characteristic of T. mongolicum growing in a wastewater irrigation region

The pH and organic matter content of collected soil samples in Zhangshi irrigation region were 6.53–6.95 and 14.31–15.04 g kg^{-1}, respectively. Total five *T. mongolicum* plants were collected. Cd concentrations in soil samples were listed in Table 4. The total Cd contents were 0.7–2.6 mg kg^{-1} and the extractable contents were 0.6–2.1 mg kg^{-1}. All plants collected from this polluted site did not show any toxic symptom either.

As shown in Table 4, EFs and TFs for all plants were higher than 1. Cd concentrations in plants were basically increased with soils too. But Cd concentrations in shoots were not greater than 100 mg kg^{-1} what Cd hyperaccumulator should have [4]. Thus, the plant can only be identified as Cd accumulator [4,5].

All in all, based on the results of three experiments, *T. mongolicum* can be basically regarded as a Cd accumulator.

4. Conclusion and implication

According to the three experiments discussed in this research, *T. mongolicum* showed Cd accumulative characteristics in an unpolluted site (Shenyang Station of Experimental Ecology), a Pb–Zn mine pollution area (Qiyingzi Pb–Zn mine) and in wastewater irrigation region (Zhangshi irrigation region). Therefore, it can be validated as a newly found Cd-accumulator.

T. mongolicum is one of weed species which possesses such properties as strong anti-stress, light contest and water contest. They grow fast and often have high biomass [14]. Based on these characteristics, it is possible that weed species may have strong endurance and high accumulation of heavy metals, and some of them may hyperaccumulate heavy metals. Moreover, according to the botanic species of the hyperaccumulators documented, we can find that most of these plants such as *Thlaspi caerulescens*, *Phyllanthus serpentines*, *Silene vulgaris* and *Lactuca sativa* belong to weed species or obviously possess the basic properties of weed species [15]. We thus believe that there will certainly be breakthrough progresses in the screening of accumulators even hyperaccumulators based on weed species.

T. mongolicum is one of perennial herbage weed species with 10–25 cm height. Thus, the remediation efficiency may be increased by cutting several times in 1 year [16].

Acknowledgements

The research was supported by hi-tech research and development program of China (No. 2006AA06Z386), Sino-Russion Joint Research Center on Natural Resources and Eco-Environmental Sciences.

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