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Potential of *Taraxacum mongolicum* Hand-Mazz for accelerating phytoextraction of cadmium in combination with eco-friendly amendments

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ABSTRACT

Phytoextraction and phytostabilization are well-established sub-processes of phytoremediation that are being followed for *in situ* remediation of soils contaminated with toxic metals. *Taraxacum mongolicum* Hand-Mazz, a newly reported Cd accumulator has shown considerable potential for phytoextracting Cd. This paper investigated the effects of urea and chicken manure on *T. mongolicum* phytoextracting Cd from soil using pot culture experiments. The results showed that urea application did not affect the Cd concentrations in root, leaf, inflorescence and shoot of *T. mongolicum*, but chicken manure significantly decreased them ($p < 0.05$) by 23.5%, 31.5%, 24.8% and 30.4% owing to decreased extractable Cd. Urea and chicken manure significantly increased ($p < 0.05$) the phytoextraction capacities ($\mu g\,pot^{-1}$) of *T. mongolicum* to Cd by 3–5-fold due to the increase in shoot biomass (increased 4–7 folds). Further, addition of urea and chicken manure increased organic matter, nitrogen, phosphorus and potassium, the microorganism count, urease and phosphatase activities of soil indicating their eco-friendly function. Urea is ideal for optimizing phytoextraction of *T. mongolicum* to Cd, while chicken manure is appropriate for phytostabilization.

1. Introduction

Cd pollution is one of the widespread global problems. Remediation of this persistent pollutant is necessary but very difficult. Phytoremediation is a promising technique to remediate heavy metal contaminated soils for safe utilization [1]. Phytoextraction mainly using hyperaccumulator or accumulator to remove heavy metal from contaminated soil and phytostabilization using low accumulating plant to safely product agricultural production in polluted soil are two main methods of phytoremediation [2]. Several strategies for Cd minimization in agro-ecosystems have been adopted. The notable ones are the use of several types of chemicals and biological materials. Soil amendments such as lime, biosolid, sewage sludge, cattle manure, poultry manure, paper mill sludge and secondary digested sewage sludge were reportedly used in various experiments [3]. The main mechanism of stabilization was to decrease extractable concentrations of heavy metals in soil, and then to decrease the extracts of vegetables or other food sources [4]. Using hyperaccumulators or accumulators to phytoextract heavy metals are currently considered the most promising pathway of phytoremediation. Though the reports about hyperaccumulator or accumulator are increasing, phytoextraction technology has not widely been used in remediation practice [2]. The key factor of limiting phytoextraction is low remediation efficiency, which insists on the limited accumulating efficiency, i.e. the multiplication of heavy metal concentration in shoot and shoot biomass [2]. Thus, some natural and/or synthetic chelators were used to increase uptake of heavy metals from soil thus achieving higher removal rates. Some amendments such as EDTA, EDDS, EGTA, NTA and organic acids have been studied for their abilities to mobilize metals and increase metal accumulation in different plant species [5–8]. Unfortunately, most of these chelating agents showed some negative effects on soil environment such as elevated toxicity to both plants and soil microorganisms, and the potential risk of leaching into ground water [7,9]. Thus, some environmental friendly amendments to strengthen phytoextraction or phytostabilization were developed. Particularly, an alternative approach of increasing accumulator's phytoextraction capacity by enhancing plant shoot biomass has shown good potential [10,11].

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Urea and chicken manure are two main chemical and organic fertilizers utilized in agricultural production in China, which is the most common method for increasing plant production [12]. *Taraxacum mongolicum* Hand-Mazz is a newly discovered Cd accumulator and shows great phytoextraction potential [13]. In this article, we will study the effects of urea and chicken manure on *T. mongolicum* accumulating Cd using pot culture experiments.

2. Materials and methods

2.1. Background of experimental site

The experiments were conducted in a net house at the Shenyang Ecological Experimental Station of Chinese Academy of Sciences (41°31′ N and 123°41′ E). This site is a temperate zone with the semi-moist continental climate, 3100–3400 °C of the annual accumulative temperature higher than 10 °C, 520–544 kJ cm^{-2} total annual solar radiation, 650–700 mm of average annual precipitation, and about 127–164 days of frost free days per year. The average annual temperature is 5–9 °C. The coldest month is January (average −14 °C) and the warmest month is July (average 24 °C). The soil is burozem with background concentration of Cd, Pb, Cu and Zn as 0.16, 18.2, 16.7 and 38.3 mg kg^{-1}, respectively. Compared to the National Soil-Environmental Quality Standards of China (NSEQSC, GB 15618, 1995), the soil is relatively clean.

2.2. Experiment with different Cd concentrations but same fertilizer level

In order to determine the effects of urea and chicken manure on *T. mongolicum* accumulating Cd potentials, 3 Cd concentration gradient experiments were carried out simultaneously. In the first group, 5 treatments without fertilizers were added including control 1 (CK_1), and treatments of Cd2.5, Cd5, Cd10 and Cd25 with Cd spiked at 2.5, 5, 10 and 25 mg kg^{-1}, respectively. In the second group, 1 g kg^{-1} of urea was added, i.e. the control 2 (CK_2, added with 1 g kg^{-1} urea without Cd addition), and treatments of U2.5, U5, U10 and U25 with Cd spiked at 2.5, 5, 10 and 25 mg kg^{-1} and added with 1 g kg^{-1} urea, respectively. Similarly, in the third group, 100 g kg^{-1} of chicken manure was added in control 3 (CK_3) as well as in treatments of C2.5, C5, C10 and C25 spiked with Cd at 2.5, 5, 10 and 25 mg kg^{-1}, respectively. *T. mongolicum* is a weed species, and the response of this species to fertilization rates is not well known till now. Typically, the addition of 1 g kg^{-1} of urea and 100 g kg^{-1} of chicken manure are a normal level for agriculture production, and thus we selected these rates for this experiment [14].

2.3. Experiment with same Cd concentration but different fertilizer levels

In order to explore the effects of different levels of urea and chicken manure on Cd accumulation in *T. mongolicum*, 3 fertilizer concentration gradient experiments were carried out simultaneously. Cd treatment concentration under different levels of fertilizers was spiked at 25 mg kg^{-1}. Urea and chicken manure were amended at 0.5 g kg^{-1} (U0.5), 1 g kg^{-1} (U1), 2 g kg^{-1} (U2), and 50 g kg^{-1} (C50), 100 g kg^{-1} (C100) and 200 g kg^{-1} (C200). The control was same to the treatment given in Section 2.2, i.e. Cd25 .

2.4. Pot process and plant culture

Soil and chicken manure were sieved through a 4 mm sieve. Soil was mixed with different concentrations of urea and chicken manure, then spiked with Cd (added as $CdCl_2 \cdot 2.5H_2O$) according to design. The mixture was filled in plastic pots (ϕ = 20 cm, H = 15 cm) with 2.5 kg soil (dry weight) per pot and equilibrated for two months. Four seedlings of *T. mongolicum* collected from the station (3 cm in height) were transplanted in each pot during spring. All pots were put in the net house in the station. Tap water was added throughout the experiment (no detectable Cd, Pb, Cu and Zn) to sustain 80% of soil water-holding capacity. All treatments in all experiments were replicated three times. All plants were harvested after growing 80 days.

2.5. Sample determination and data processing

Plant samples were separated into roots, leaves and inflorescences, then rinsed with tap water and carefully washed with deionized water later. These samples were oven-dried initially at 105 °C for 5 min, thereafter at 70 °C until completely dried (near 2 days). Near half of leaves and inflorescences were mixed together to examine Cd concentration in shoots. The dried plant samples were ground to a powder and passed through a sieve. Soil samples were air-dried and ground using a mortar and pestle and passed through a 1 mm sieve. Plant and soil samples were digested using a solution containing 87% of HNO_3 and 13% of $HClO_4$ to determine total metal concentration [15]. The concentrations of extractable Cd in soils were extracted using 1 mol L^{-1} NH_4NO_3 [16]. Cd concentration was determined using an atomic absorption spectrophotometer (AAS, Hitachi 180-80 with a 1.3 nm spectral band width). The measured values of trace metals were verified 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, total nitrogen (N), available phosphorus (P) and available potassium (K) were determined using standard methodology [17]. The value of pH was determined using a pH meter (PHS-3B) with the ratio of soil and water at 1:2.5. Soil microorganism count was determined by using dilution plate counting method. Soil urease and neutral phosphatase activities were analyzed by spectrophotometric method and the disodium phenyl phosphate method [18–20]. All data of three replicates for each treatment were calculated using Excel and SPSS 11.5 [21]. The enhancement factor (EF) was calculated as the ratio of Cd concentration in plant to concentration of Cd in soil, and the translocation factor (TF) was calculated by the ratio of Cd concentration in shoots to concentration of Cd in roots. Data were analyzed by one-way ANOVA with the Duncan's multiple range tests to separate means. Differences were considered significant at $p < 0.05$ [21].

3. Results and discussion

3.1. Effects of urea and chicken manure on soil physico-biological environment

Main indexes of soil fertility are usually indicated by the concentrations of organic matter, total N, available P and available K [12]. As shown in Table 1, in the treatments added with 0.5, 1 and 2 g kg^{-1} urea, and 50, 100 and 200 g kg^{-1} chicken manure, these indexes were almost significantly ($p < 0.05$) higher than similar soil without fertilizer addition. Furthermore, the soil pH and total Cd concentration were not significantly changed ($p < 0.05$). Thus, the addition of fertilizer was good for the soil's nutritional indices.

Usually, soil microorganism count and some enzyme activities can roughly reflect heavy metal toxicity to soil biologisms [22]. The results showed that urea addition did not affect bacteria, fungi and actinomycete counts but chicken manure addition increased their counts (Table 2). Soil urease and phosphatase activities were often impacted by urea and organic matter addition. The results showed that soil urea activities increased after urea was added and phosphatase activities were enhanced due to chicken manure addition (Table 2). Both physical indexes (concentrations of organic mat-

Table 1
Physico-chemical properties of soil and treated soil with fertilizer.

Treatment	Addition level	pH	Total Cd ($mg\,kg^{-1}$)	Organic matter ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	Available P ($mg\,kg^{-1}$)	Available K ($mg\,kg^{-1}$)
Soil	Original	6.7 a	0.17 a	17.38 e	0.87 c	10.83 e	90.26 e
Chicken manure	Original	6.9a	0.21a	475.72 a	12.36 a	2087.46 a	316.73 a
Soil added with urea	0.5 $g\,kg^{-1}$	6.8 a	0.16 a	17.61 e	0.98 g	11.02 e	89.32 e
	1 $g\,kg^{-1}$	6.72a	0.15 a	17.28 e	1.19 e	10.79 e	88.95 e
	2 $g\,kg^{-1}$	6.8 a	0.18 a	17.73 e	1.31 d	9.97 e	89.63 e
Soil added with chicken manure	50 $g\,kg^{-1}$	6.8 a	0.17 a	43.64 d	1.13 f	133.92 d	97.15 d
	100 $g\,kg^{-1}$	6.8 a	0.18 a	77.54 c	2.68 c	242.65 c	114.28 c
	200 $g\,kg^{-1}$	6.8 a	0.18 a	134.83 b	2.89 b	436.07 b	128.04 b

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

Table 2
Soil microorganism count and enzyme activities.

Cd addition level	Fertilizer addition level	Bacteria ($10^6\,g^{-1}$ soil)	Fungi ($10^4\,g^{-1}$ soil)	Actinomycete ($10^5\,g^{-1}$ soil)	Urease ($\mu mol\,NH_3\,g^{-1}\,h^{-1}$)	Phosphatase (μg phenol g^{-1} soil)
Cd0	Soil	3.9 b	1.7 b	1.9 b	2.7 c	487.4 b
	U1	4.0 b	1.8 b	2.0 b	4.8 b	488.2 b
	C100	7.6 a	2.8 a	5.9 a	6.2 a	758.3 a
Cd2.5	Soil	3.8 b	1.6 b	1.8 b	2.5 c	458.2 b
	U1	4.1 b	1.7 b	1.9 b	4.6 b	463.7 b
	C100	7.5 a	2.7 a	5.8 a	5.9 a	761.5 a
Cd5	Soil	3.9 b	1.6 b	1.7 b	2.4 c	465.8 b
	U1	3.8 b	1.6 b	1.8 b	4.5 b	472.6 b
	C100	7.1 a	2.6 a	6.1 a	5.8 a	759.4 a
Cd10	Soil	2.8 b	1.1 b	1.3 b	2.1 c	479.1 b
	U1	2.9 b	0.8 b	1.4 b	4.2 b	482.6 b
	C100	5.2 a	2.4 a	5.3 a	5.7 a	747.5 a
Cd25	Soil	2.6 d	0.9 d	1.1 d	2.6 c	481.4 d
	U0.5	2.7 d	1.0 d	1.2 d	3.5 b	485.9 d
	U1	2.8 d	1.1 d	1.3 d	4.3 ab	487.3 d
	U2	2.9 d	1.2 d	1.4 d	5.1 a	483.7 d
	C50	5.5 c	2.1 c	4.2 c	5.2 a	537.3 c
	C100	6.8 b	2.6 b	5.1 b	5.6 a	725.6 b
	C200	7.9 a	3.1 a	6.3 a	5.9 a	982.4 a

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

ter, total N, available P and available K) and biological indexes (soil microorganism count and some enzyme activities) were partly expressed that urea and chicken manure were eco-friendly amendments to Cd polluted soil [22].

3.2. Urea and chicken manure promoted the growth of T. mongolicum

In the experiment of different Cd concentrations with same fertilizer level, all shoot biomass in the treatments added with urea and chicken manure was significantly increased ($p < 0.05$) compared to that of controls (CK_1–CK_3) (Fig. 1). The shoot biomass increased in average by 4–5-folds for urea addition and 6–7 folds for chicken manure addition due to improved soil fertility (Fig. 1, Table 1). Similar results were found in the experiment of same Cd concentration with different fertilizer levels, i.e. shoot biomass increased in average by 4–7 folds for urea addition at 0.5, 1, 2 $g\,kg^{-1}$ and 7–9 folds for chicken manure addition at 50, 100, 200 $g\,kg^{-1}$ compared to the control 2 (Fig. 2). Thus, the addition of these fertilizers increased plant shoot biomass, which partly indicated they were environmental-friendly [22].

3.3. Effects of same dose fertilizers on T. mongolicum accumulating Cd

Cd concentrations in roots, leaves, inflorescences and shoots of *T. mongolicum* were increased with the higher spiked Cd without any fertilizer addition, indicating the accumulation characteristics of it to Cd (Table 3). When 1 $g\,kg^{-1}$ urea was added to soil, Cd concentrations in roots, leaves, inflorescences and shoots of *T. mongolicum* were not affected ($p < 0.05$, Table 3). This might be because that urea amendment did not affect the concentrations of organic matters, phosphorous compounds, pH and soil extractable Cd (Tables 1 and 3). However, the Cd concentrations in roots, leaves, inflorescences and shoots of *T. mongolicum* under the conditions of Cd pollution (C2.5–C25) were significantly decreased by the added chicken manure ($p < 0.05$), compared to the control 1 (Table 3). The

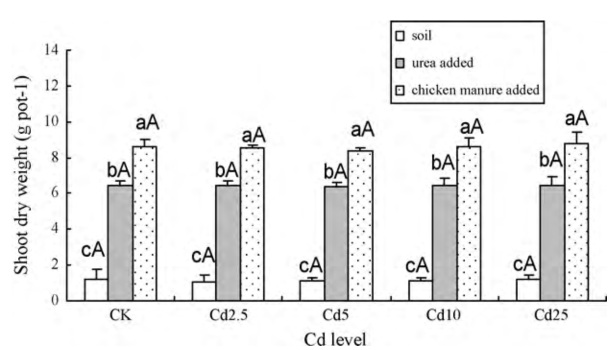

Fig. 1. Shoot biomass of *T. mongolicum* in 3 Cd concentration gradient experiments. CK, without addition of Cd. Treatments of Cd2.5, Cd5, Cd10 and Cd25 were with Cd spiked at 2.5, 5, 10 and 25 $mg\,kg^{-1}$, respectively. Vertical bars represent standard deviations. The means within same Cd treatments followed by the same letter (a–c) were not significantly different at $p < 0.05$. The means among different Cd treatments followed by the same letter (A–B) were not significantly different at $p < 0.05$ either.

Table 3
Effects of same fertilizer level on *T. mongolicum* accumulating Cd (mg kg^{-1}).

Treatment		Root	Leaf	Inflorescence	Shoot	EF	TF	Extractable Cd		Shoot extraction capacity (μg pot^{-1})
								Two month	Harvest	
Cd2.5	Soil	2.9 a	10.4 a	5.9 a	7.3 a	2.94 a	2.52 a	1.4 a	0.9 a	7.9 c
	Urea	2.8 a	10.9 a	6.2 a	7.8 a	3.13 a	2.79 a	1.3 a	1.1 a	50.4 a
	Manure	2.1 b	7.6 b	4.1 b	5.4 b	2.18 b	2.57 a	0.8 b	0.3 b	45.9 b
Cd5	Soil	6.2 a	16.3 a	10.2 a	13.2 a	2.65 a	2.13 a	2.5 a	1.7 a	15.4 c
	Urea	5.9 a	16.8 a	9.7 a	14.1 a	2.83 a	2.39 a	2.6 a	1.5 a	90.1 a
	Manure	5.1 b	11.4 b	6.8 b	9.2 b	1.85 b	1.80 a	1.5 b	0.8 b	77.0 b
Cd10	Soil	15.0 a	28.3 a	10.5 a	25.7 a	2.57 a	1.71 a	5.5 a	3.9 a	29.7 c
	Urea	14.8 a	27.9 a	11.2 a	26.1 a	2.62 a	1.76 a	5.2 a	4.1 a	168.1 a
	Manure	11.2 b	18.3 b	8.6 b	16.3 b	1.63 b	1.46 a	3.2 b	1.3 b	140.9 b
Cd25	Soil	21.3 a	41.2 a	13.6 a	34.5 a	1.38 a	1.62 a	12.2 a	9.8 a	42.3 c
	Urea	19.4 a	39.7 a	13.9 a	35.1 a	1.40 a	1.81 a	11.9 a	9.5 a	225.0 a
	Manure	16.3 b	27.3b	11.2 b	24.7 b	0.99 b	1.51 a	6.9 b	4.0 b	217.2 b

Note: 1 g kg^{-1} urea or 100 g kg^{-1} was added; EF: enhancement factor; TF: translocation factor. Means in same Cd treatment columns followed by different letters were significantly different at $p < 0.05$.

Table 4
Effect of different fertilizer levels on *T. mongolicum* accumulating Cd (mg kg^{-1}).

Treatments	Roots	Leaf	Inflorescence	Shoot	EF*	TF*	Extractable Cd in soil		Shoot extraction capcity (μg pot^{-1})
							Two months	Harvested time	
Cd25	21.3 a	41.2 a	13.6 a	34.5 a	1.38 a	1.62 a	12.2 a	9.8 a	42.3 f
U0.5	20.5 a	40.8 a	13.2 a	33.8 a	1.35 a	1.65 a	11.2 a	9.1 a	164.3 e
U1	19.4 a	39.7 a	13.9 a	35.1 a	1.40 a	1.81 a	11.9 a	9.5 a	225.0 b
U2	21.1 a	41.7 a	12.8 a	34.9 a	1.39 a	1.65 a	11.5 a	9.3 a	262.4 a
C50	18.2 b	32.4 b	12.1 b	26.1 b	1.04 b	1.43 b	10.1 a	5.6 b	185.8 d
C100	17.4 c	27.3 c	11.2 c	24.7 c	0.99 b	1.42 b	6.9 b	4.0 c	217.2 c
C200	15.1 d	24.9 d	10.1 d	22.5 d	0.90 b	1.49 b	5.4 c	3.1 d	200.7 cd

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

average values decreased by 23.5%, 31.5%, 24.8% and 30.4%, respectively. The main reason for its decrease may be the reduction of extractable Cd concentrations in soil, as the average extractable Cd decreased by 42–61.4% by the added manure. Likewise, the enhancement factor (EF, ratio of concentration in plant to concentration in soil) was significantly decreased ($p < 0.05$). The decrease of extractable Cd concentrations may be caused by the chelation or consolidation of organic matters or phosphorous compounds in chicken manure (Tables 1 and 3). Yet the shoot Cd extraction capacities (μg pot^{-1}) were increased by urea and chicken manure amendment in all Cd doses due to their increase of the shoot biomass, and increased 3–4 and 4–5-folds compared to the controls (Table 3). Particularly, the Cd extraction capacities (μg pot^{-1}) increased by the addition of urea were significantly higher than those of the addition of chicken manure (Table 3).

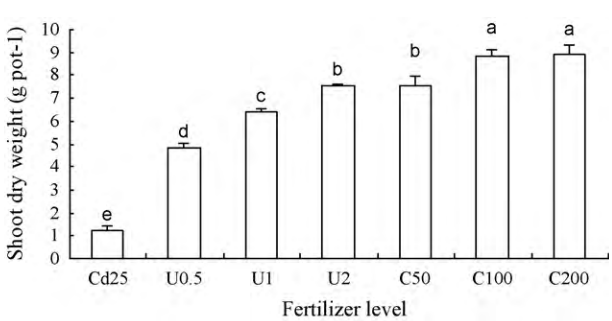

Fig. 2. Shoot biomass of *T. mongolicum* in fertilizer concentration gradient experiments. Vertical bars represent standard deviations. The means followed by the same letter (a–e) were not significantly different at $p < 0.05$.

3.4. Effects of different doses of fertilizer on T. mongolicum accumulating Cd

Cd concentrations in roots, leaves, inflorescences and shoots of *T. mongolicum* were not significantly affected by increasing urea level, under the condition of 25 mg kg^{-1} Cd pollution ($p < 0.05$, Table 4). However, with the addition of chicken manure at different levels, Cd concentrations in roots, leaves, inflorescences and shoots of *T. mongolicum* were significantly decreased due to the reduction of extractable Cd concentrations in soil ($p < 0.05$, Tables 1 and 4). As far as phytoextraction amount is concerned, with the increase of fertilizer addition level, extraction capacities (μg pot^{-1}) of *T. mongolicum* in shoot were basically increased due to their increased shoot biomass, because the extraction capacities (μg pot^{-1}) were calculated by multiplying the shoots Cd concentrations with their dry weights (Fig. 2, Table 4).

4. Conclusion

The results showed that urea and chicken manure addition did not show negative effect to soil and increased the shoot biomass of *T. mongolicum,* but chicken manure decreased Cd concentration in roots, leaves, inflorescences and shoots as well as extractable Cd concentrations in soil. However, the extent of biomass enhancement was higher than the reduction of Cd concentrations in plant tissue with a final result of increased extraction capacity (μg pot^{-1}) of Cd in the *T. mongolicum* treated with chicken manure. As for urea, it did not affect Cd concentration in the plant tissue of *T. mongolicum* and Cd extractable concentration in soil, but increased the plant shoot biomass, and finally increased extraction capacity (μg pot^{-1}). Thus, the roles of chicken manure in phytoremediation may be beneficial to be used in phytostabilization. Urea is better to be used in phytoextraction.

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