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Tolerance and accumulation characteristics of cadmium in Amaranthus hybridus L.

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ABSTRACT

Because of its toxicity to animals and humans, cadmium (Cd) is an environmentally important heavy metal. Consequently, researchers are interested in using hyperaccumulator and accumulator plants to decontaminate Cd polluted soils. To investigate Cd tolerance, uptake and accumulation by *Amaranthus hybridus* L., Cd concentration gradients were applied to a soil (at rates of 0, 30, 60, 90, 120, 150 and 180 mg kg⁻¹) and hydroponics solutions (at rates of 0, 5, 10, 15, 20, 30, and 40 mg L⁻¹) following a field survey. *A. hybridus* grew normally at added Cd concentrations \leq 90 mg kg⁻¹ and \leq 20 mg L⁻¹ in the soil culture and in the hydroponics solutions, respectively. In the hydroponics solutions, peroxidase activity showed a quadratic relationship and catalase activity changed irregularly with increasing Cd concentrations. The highest Cd concentration and accumulation in shoots were 241.56 mg kg⁻¹ and 1006.95 μ g pot⁻¹ in the soil culture, and 354.56 mg kg⁻¹ and 668.42 μ g pot⁻¹ in the hydroponics experiment. Bioconcentration factors in soil culture and hydroponics solutions were 0.58–1.22 and 5.18–17.55, and translocation factors were 0.64–1.50 and 0.33–0.92, respectively. *A. hybridus* has potential phytoremediation capability in Cd polluted soils.

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1. Introduction

Heavy metal pollution of soil is an important global environmental problem [1]. Heavy metals are not subject to degradation processes and remain almost indefinitely in the environment. Cadmium (Cd) is a non-essential heavy metal that is highly toxic to living cells at very low concentration [2]. It is consumed by animals and humans in their diet and can cause serious diseases [3]. In recent years soil Cd contents have dramatically increased because of inputs from anthropogenic sources, including smelters, agricultural applications of fertilizer and sewage sludge [4,5]. Consequently there is an important and urgent need to develop methods to clean up Cd-contaminated soils [6].

Many efforts have been undertaken to find methods of removing heavy metals from soil [7], such as land filling, fixation and leaching. All these methods may be very helpful in contributing to the restoration of metal-polluted soils. However, they are usually expensive [8]. In contrast, the use of plant species to stabilize or remove pollutants from soils, generally defined as phytoremediation, offers the great advantage of being inexpensive, environmentally friendly and does not alter the soil matrix [9]. Phytoremediation usually uses hyperaccumulator or accumulator plants which accumulate especially high concentrations of heavy metals in their tissues to clean up soils contaminated by heavy metals.

Recent research has studied the tolerance and accumulation of heavy metals in plants. For instance, Kirkham [10] studied Cd in plants growing on polluted soils; Zu et al. [11] investigated the accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulators in a lead–zinc mine area; Zhang et al. [12] reported a cadmium accumulator – *Malva sinensis*, and Lefèvre et al. [13] studied cadmium tolerance and accumulation in a noxious weed [13]. Meanwhile, many Cd-hyperaccumulators have been identified, including *Brassica juncea* [14], *Viola baoshanensis* [15], *Arabis gemmifera* [16] and *Lonicera japonica* [6], but some of the plants are small and slow growing [3]. Screening new hyperaccumulators or accumulators and identifying their tolerance, uptake and accumulation to heavy metals are key processes of phytoremediation.

Amaranthus hybridus L. was found at the Fuquan lead–zinc mine area in Hanyuan, Sichuan, China. A field survey revealed that the plant grew well and was widely distributed in the mining area where the mean Cd concentration of rhizosphere soil reached 16.06 mg kg⁻¹. Consequently the plant could have potential for reducing heavy metal pollution in soil. Researchers have reported the nutritive value [17], endophytic fungi [18] and acetolactate synthase [19] of *Amaranthus* species but there are few reports of the tolerance and accumulation characteristics of Cd in *A. hybridus*.

The aim of this study was to assess the growth and physiological response, and investigate the uptake and accumulation of Cd by *A*. *hybridus* growing in a soil culture and hydroponics experiment.

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2. Materials and methods

2.1. Plant description

A. hybridus L., commonly called Smooth Amaranth, Smooth Pigweed, or Slim Amaranth, is a species of annual flowering plant. The plant is extremely variable, and it is believed that many other amaranth species are natural hybrids derived from *A. hybridus*. It was originally a pioneer plant in North, Central and South America but is naturalized in many other places with warm climates. In China, the plant is mainly distributed in the south including Sichuan, Hunan, Jiangsu and Guizhou. *A. hybridus* grows from a short taproot and can be up to 2.5 m in height. It is distinguished by a vertical stem, and terminal, glabrous or glabrescent panicles. The plant grows in many different places, including mining wastelands, tailings, barrens and other disturbed habitats.

2.2. Site description and field survey

Plant seeds in this experiment were collected from the Fuquan lead–zinc Mine in Hanyuan, Sichuan ($29^{\circ}5'N$, $102^{\circ}16'E$). The mine site is in the temperate zone and has a humid subtropical monsoon climate. The altitude is 858 m; the annual mean temperature is 17.9 °C and the mean total precipitation is 780 mm. The mean soil pH, organic matter and total N were 7.52, 23.12 g kg^{-1} and 1.05 g kg^{-1} , and available N, Olsen P, available K, total Pb and Zn were 90.2, 10.7, 107.3, 532.1 and 1929.0 mg kg⁻¹, respectively. Seven plant samples were selected in the field survey, two from the wasteland, three from the grassland and two from mine tailings.

2.3. Soil culture

The soil was ground to pass through a 4-mm mesh. Each plastic pot (40 cm length, 20 cm width, 15 cm height) was filled with 6.0 kg of ground soil that was mixed with seven levels of Cd in solution (prepared by dissolving analytical grade CdCl₂·2.5H₂O): 0 (control), 30, 60, 90, 120, 150, and 180 mg kg^{-1} . The soil used in the study is a Typic Aqui-Alluvic Primosol with $168 \,\mathrm{g \, kg^{-1}}$ clay, $390 \,\mathrm{g \, kg^{-1}}$ silt, and $425 \,\mathrm{g \, kg^{-1}}$ sand. The physicochemical properties of the soil include 24.82 g kg⁻¹ organic matter, 1.16 g kg⁻¹ total N, 127.5 mg kg⁻¹ available N, 17.6 mg kg⁻¹ Olsen P, 165.3 mg kg⁻¹ available K, a pH of 6.35, and a Cd concentration of 0.14 mg kg⁻¹. The soil was incubated for 4 weeks, after which three uniform A. hybridus seedlings (5–6 cm high with 3–4 fronds) were transplanted into each pot. Three replicates were run for each treatment and the experiment was arranged in a completely randomized design. The pots were placed in a net house with transparent polythene sheets to protect them from rainwater leaching. No fertilizer was added during the experiment. The pots were watered with tap water (no Cd detected) according to water loss during the experiment.

The plant samples were harvested 90 days after transplanting and washed thoroughly with running tap water followed by distilled water. The samples were divided into roots, stems and leaves, and the fresh weight (FW) immediately determined. The samples were dried at 80 °C for 48 h and the dry weight (DW) measured. The dried plant samples were finely ground and sieved through a 1-mm nylon sieve.

2.4. Hydroponics experiment

Healthy seedlings with 6–7 fronds were selected and their roots soaked in 0.1% KMnO₄ for 10 min to sterilize and accelerate root growth. The seedlings were then transferred to covered plastic pots ($30 \text{ cm} \times 20 \text{ cm} \times 10 \text{ cm}$) containing 4000 mL of 1/4 diluted Hoagland nutrient solution (pH 5.8) and with one seedling in each

of 10 holes through the cover. After 10 days of cultivation to allow the seedlings to adapt to the diluted Hoagland nutrient solution, Cd as CdCl₂·2.5H₂O was added to the solution. There were eight treatments with the Cd concentrations for each treatment being 0 (control), 1, 5, 10, 15, 20, 30, 40 mg L⁻¹. Three replicates were used for each treatment. The nutrient solution was aerated continuously and replaced every 4 days, and the pH was maintained at 5.8 adjusted with 0.1 mol L⁻¹ NaOH or HCl. The growth temperature was controlled at an average of $25 \pm 3 \,^{\circ}$ C and $18 \pm 3 \,^{\circ}$ C during the day and night, respectively. The growth responses of plants were measured, and the plants were harvested after 30 days.

Harvested plant samples were washed thoroughly first with running tap water followed by deionized water. The roots were immersed into 0.01 mM L⁻¹ HCl for 10 min to remove any metals adhering to the root surface before washing in deionized water. They were divided into roots and shoots, and the fresh weight determined immediately. The fresh samples were dried at 80 °C for 48 h, and the dry weights (DW) measured. The dried plant samples were finely ground and sieved through a 1-mm nylon sieve.

2.5. Determination of enzyme activity

All enzyme activity data are related to plant fresh weight (FW). About 1.0g of leaf sample was ground in an ice-cooled mortar with 5 mL of ice-cooled 50 mM Na-phosphate buffer (pH 7.8, containing 0.1 mM EDTA) and polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant used for enzyme activity determinations.

The activities of peroxidase (POD) were determined as described by Wu and von Tiedemann [20]. One unit of enzyme activity was expressed as an increase of 0.01 unit of absorbance at 470 nm and expressed as $U g^{-1}$ FW.

Catalase (CAT) activity was determined using a method described by Cao et al. [21]. One unit of enzyme activity was defined as the amount of the enzyme that reduced 50% of the H_2O_2 in 60 s at 25 °C.

2.6. Translocation factor and bioconcentration factor

The bioconcentration factor (BCF) is the ratio of metal concentration in plant roots or shoots to that in the soil or solution (only for the added Cd treatments). The translocation factor (TF) is defined as the ratio of metal concentration in plant shoots to that in plant roots. BCF and TF provide two indexes of the ability of the plant to accumulate a particular metal from the soil or solution.

2.7. Plant and soil analyses

Plant samples (0.3 g) were digested with a solution of 4/1 (v/v) HNO₃/HClO₄. A soil sample was taken from each pot, air-dried at room temperature for 15 days, and then ground to pass through a 2-mm nylon sieve. Soil samples (1.0 g) were digested in 1:2:2 (v:v:v) HNO₃:HCl:HClO₄ mixture to obtain the total heavy metal content. Concentrations of Cd in plants and soil were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, IRIS Intrepid II, Thermo Electron Corporation, USA). CdCl₂ was used as a standard material for assurance control.

2.8. Statistical methods

Data analyses were performed using SPSS version 13.0. Variance analysis, Pearson's correlation, and regression analysis were performed. The least significant difference (LSD) was used at P<0.05 for separating means. One-way analysis of variance (ANOVA) was performed for the various plant indexes.

Table 1

Cd concentration and accumulation, and growth parameters of A. hybridus in the field survey.

Sample	Plant	Root	Biomas	s(gDW)				Concen	tration (m	ng kg ⁻¹)				Accumul	ation (µg)
110,	(cm)	(cm)	Root	Stem	Leaf	Flower	Shoot	Soil	Root	Stem	Leaf	Flower	Shoot	Root	Shoot
1	104.0	30.3	4.02	12.04	1.68	6.33	20.06	8.46	11.38	8.64	5.26	6.67	7.73	45.75	155.08
2	86.3	19.7	2.77	6.56	1.15	1.30	9.01	15.25	9.73	15.64	8.24	5.50	13.23	26.95	119.22
3	68.0	19.8	1.66	5.28	5.39	4.38	15.05	7.35	8.36	13.53	5.40	6.76	8.65	13.88	130.15
4	83.0	12.3	3.60	14.68	1.53	1.99	18.21	18.05	8.90	20.08	8.75	6.88	18.26	32.04	332.42
5	86.0	18.8	2.10	5.53	1.36	0.70	7.59	15.18	7.97	25.67	6.74	5.31	20.40	16.74	154.84
6	267.1	30.5	45.28	85.52	29.93	27.08	142.53	25.45	16.05	38.52	21.62	25.65	32.15	726.74	4582.34
7	135.5	25.4	14.84	127.42	44.60	40.35	212.36	22.69	19.21	35.97	22.22	21.08	28.37	285.11	6024.65

3. Results

3.1. Cd concentrations of soil and plant tissues in the field survey

The field survey of heavy metal concentrations in *A. hybridus* was conducted in September 2008 after plants had grown for about 3 months. The greatest plant height was 267.1 cm and the largest shoot biomass was 212.36 g (Table 1). The mean Cd concentration of the rhizosphere soil was 16.06 mg kg⁻¹. The highest Cd concentration and accumulation in plant shoots were 32.15 mg kg⁻¹ and 6024.65 μ g, respectively. These results indicate that *A. hybridus* has a high biomass and Cd-accumulation capacity in this soil area which is polluted by heavy metals.

3.2. Growth responses of A. hybridus in the soil culture and hydroponics experiment

In the soil culture, plant height and root length decreased with increasing soil Cd stress levels (Table 2). The shoot biomass of treated plants also decreased along with increasing soil Cd concentrations. Root and shoot biomasses were significantly greater than that of the control when the Cd concentration was $\leq 60 \text{ mg kg}^{-1}$ for shoots and $\leq 90 \text{ mg kg}^{-1}$ for roots, respectively. However, their biomasses decreased significantly at Cd levels $\geq 120 \text{ mg kg}^{-1}$ (Table 2).

After 30 days exposure to Cd stress in the hydroponics experiment, there were no significant differences for plant heights at Cd levels $\leq 10 \text{ mg L}^{-1}$ (P < 0.05, Table 2) but heights decreased at levels $\geq 15 \text{ mg L}^{-1}$. Root lengths in the Cd treated plants were greater than in the control at Cd levels $\leq 20 \text{ mg L}^{-1}$, and decreased obviously at the Cd concentrations $\geq 30 \text{ mg L}^{-1}$ (Table 2). Shoot and root biomass decreased with increasing solution Cd concentrations. Compared with the control, biomass was significantly lower at solution Cd concentrations $\geq 15 \text{ mg L}^{-1}$ for the root biomass and > 10 mg L^{-1} for the shoot biomass.

3.3. Enzyme activities of plants in the hydroponics experiment

Fig. 1 shows the enzyme activities of *A. hybridus* under the different Cd concentration treatments. POD activities increased with increasing Cd concentration up to 15 mg L^{-1} and then decreased sharply by 16.74-39.43% at Cd concentrations of $20-40 \text{ mg L}^{-1}$ (Fig. 1(A)). Compared with the control, irregular but significant (*P*<0.05) changes in CAT activity were observed with increasing Cd concentrations (Fig. 1(B)).

3.4. Cd concentration and accumulation by A. hybridus in the soil culture and hydroponics experiments

Fig. 2 shows the concentration and accumulation in plant shoots and roots at the different Cd concentration treatments in the soil culture. Cd concentrations in roots and shoots increased with increasing Cd stress levels (Fig. 2(A)). Cd concentrations in roots and shoots exceeded 100 mg kg⁻¹ at soil Cd concentrations of 90 mg kg⁻¹ and 120 mg kg⁻¹ and reached their maximum of 229.96 and 241.56 mg kg⁻¹, respectively, at 180 mg kg⁻¹. Cd concentration was greater in shoots than in roots at Cd stress levels \geq 120 mg kg⁻¹. Shoots accumulated more Cd than roots (Fig. 2(B)) accounting for 80.5–94.8% of the total plant accumulation. The highest accumulation of Cd in shoots was 1006.95 µg pot⁻¹ at the Cd level of 150 mg kg⁻¹.

In the hydroponics experiment, Cd concentrations in roots and shoots also increased with increasing Cd stress levels (Fig. 3(A)). Concentrations in roots and shoots exceeded 100 mg kg⁻¹ at Cd levels of 10 and 20 mg L⁻¹. At the solution Cd concentration of 40 mg L⁻¹, Cd concentrations in plant roots and shoots reached maximum values of 587.03 and 354.56 mg kg⁻¹, respectively. Cd concentrations in plant shoots were less than in roots at all Cd concentration treatments. At the same Cd concentration, Cd accumulation in shoots reached a maximum of 668.42 µg pot⁻¹ (Fig. 3(B)). Cd accumulation in shoots was 24.4–62.6% of that in the whole plants.



Fig. 1. Enzyme activities of the plant leaves in the hydroponics experiment: (A) POD and (B) CAT. Means with different letters are significantly different from each other (P<0.05) according to the LSD test.



Fig. 2. Concentrations and accumulation of Cd in A. hybridus in the soil culture: (A) concentrations and (B) accumulation. Means with different letters are significantly different from each other (P<0.05) according to the LSD test.

4. Discussion

4.1. Tolerance

Cadmium can cause adverse effects on photosynthesis and the antioxidant enzymes of plants, resulting in various symptoms of phytotoxicity, such as chlorosis and reduced biomass, inhibition of root elongation, and death [22]. Tolerance of adverse conditions is a basic trait for phytoremediation. In this study, plant heights, root lengths and biomass in two pot experiments showed a linear relationship with increasing Cd concentration treatments (Table 3). Compared with the control, A. hybridus grew normally at Cd concentrations $< 90 \text{ mg kg}^{-1}$ in soil culture and $< 10 \text{ mg L}^{-1}$ in the hydroponics experiment (Table 2). At the same Cd stress levels, the plants did not show visible Cd toxicity symptoms in leaves (yellow spots and stripes). Shoot biomass in the two experiments decreased with increasing Cd stress levels. However, at Cd levels \leq 90 mg kg⁻¹ in soil culture and \leq 10 mg L⁻¹ in the hydroponics experiment, they did not decrease significantly (P > 0.05, Table 2), reducing by only 3.3% and 20.6%, respectively.

Cd is known to induce a burst of active oxygen species in plant tissues, leading to oxidative stress [23]. Among various enzymes, POD and CAT play an important part in active oxygen species [6,24]. Changes in POD and CAT activities with increasing Cd concentrations were mainly observed in the hydroponics experiment. POD activity showed a quadratic relation with the Cd concentration treatments according to the regression equation:

$$Y = 907.246 + 32.514X - 1.176X^2 \quad (R^2 = 0.389, P < 0.01)$$
(1)

where *Y* is the POD activity of leaf in the hydroponics experiment, and *X* is the Cd concentration in solution. The equation indicates that Cd concentrations in solution had significant effects

on POD activity in plant leaves (P < 0.01) similar to *Triticum aestivum* [24]. However, compared with the control, CAT activities in leaves did not change systematically with increasing Cd concentrations though they decreased by 7.4–26.0% (Fig. 1(B)). This is different to that found for *Bruguiera gymnorrhiza* and *Kandelia candel* [25] suggesting that further studies are needed to investigate changes in CAT activity in relation to Cd stress.

Plant height, high biomass and enzyme activities showed that *A*. *hybridus* had great tolerance of Cd polluted areas.

4.2. Cd concentration and accumulation

Various plants have unique physiologies allowing them to take up heavy metals [26]. After 90 and 30 days exposure to different Cd stress, Cd concentrations in A. hybridus shoots in the two experiments showed a quadratic relationship with increasing Cd concentrations, indicating that Cd concentrations in shoots were significantly affected by concentrations of the metal in soil and solution (P < 0.001, Table 3). At Cd concentrations up to 120 mg kg⁻¹ in the soil culture and 20 mg L^{-1} in the hydroponics experiment (Figs. 2(A) and 3(A)), Cd concentrations in shoots became more than 100 mg kg⁻¹ which is regarded as the threshold value of Cd-hyperaccumulators [27]. The highest Cd concentrations in shoots in the soil culture and hydroponics experiment were 241.56 and 354.56 mg kg⁻¹, respectively. Comparison with other species showed that both values were lower than that for Viola baoshanensis (1168 mg kg $^{-1}$) [15]. However, A. hybridus had greater biomass than Viola baoshanensis and would therefore accumulate more Cd from polluted areas. In addition, at Cd levels $> 120 \text{ mg kg}^{-1}$ in the soil culture, Cd concentrations in shoots were higher than in roots. However, in the hydroponics experiment they were lower in shoots than in roots. A similar result was observed in Dittrichia viscose [28].



Fig. 3. Concentrations and accumulation of Cd in *A. hybridus* in the hydroponics experiment: (A) concentration and (B) accumulation. Means with different letters are significantly different from each other (*P*<0.05) according to the LSD test.

Treatment		Plant height		Root length		Root biomass		Shoot biomass	
Soil culture (mg kg ⁻¹)	Hydroponics (mgL ⁻¹)	Soil culture (cm)	Hydroponics (cm)	Soil culture (cm)	Hydroponics (cm)	Soil culture (g)	Hydroponics (g)	Soil culture (g)	Hydroponics (g)
0	0	60.3 ± 7.6bc	26.0 ± 1.0a	15.8 ± 1.3a	16.5 ± 2.8c	1.19 ± 0.16b	3.13 ± 0.59ab	$12.11 \pm 0.76c$	$3.74 \pm 0.18a$
30	1	$88.0 \pm 3.0a$	$23.5 \pm 3.1 ab$	16.3 ± 2.5a	27.7 ± 6.7a	$2.24\pm0.20a$	$3.60 \pm 0.97a$	$21.33 \pm 0.9a$	$3.56\pm0.16ab$
60	5	$65.00 \pm 4.2 bc$	$23.3 \pm 4.2ab$	$13.2 \pm 1.0b$	$23.4 \pm 2.0ab$	$2.46 \pm 0.16a$	$3.01 \pm 0.72ab$	$15.88 \pm 2.8b$	$3.21 \pm 0.45 ab$
06	10	$57.67 \pm 2.5b$	$24.7 \pm 3.1a$	$12.3 \pm 0.8 bc$	$23.5 \pm 0.5 ab$	$1.43 \pm 0.15b$	$2.45 \pm 0.32b$	$11.71 \pm 1.13c$	$2.97 \pm 0.02b$
120	15	$49.33 \pm 2.5c$	$19.2 \pm 0.8b$	$10.8 \pm 1.3 \mathrm{bc}$	$20.7 \pm 1.5 bc$	$0.51\pm0.35c$	$2.22 \pm 0.55 bc$	$5.71 \pm 1.54d$	$2.59 \pm 0.57 bc$
150	20	$41.67 \pm 4.2d$	$19.7 \pm 2.1b$	$10.3 \pm 0.8c$	$20.0 \pm 1.0 bc$	0.46+0.10c	$1.42 \pm 0.26c$	$5.62 \pm 1.5d$	$2.43 \pm 0.44 \text{bc}$
180	30	$35.33 \pm 2.5d$	$16.0 \pm 2.0 \mathrm{bc}$	$10.0 \pm 1.7c$	$14.7 \pm 5.5c$	$0.38\pm0.08\mathrm{c}$	$0.72\pm0.17c$	$1.78\pm0.58e$	$2.21 \pm 0.19c$
	40		$13.6 \pm 1.2c$		$14.3 \pm 3.1 d$		$0.72\pm0.06c$		$1.89\pm0.38c$
^a Means with d	ifferent letters are sigr	nificantly different from	i each other $(P < 0.05)$ accoi	rding to the LSD test.					

Table 2 Plant height, root length and biomass of *A. hybridus* in the soil culture and hydroponics experiment.^a.

Table 3

Relationship of the Cd concentrations in soil (mg kg⁻¹, X) and solution (mg L⁻¹, X) with growth parameters, Cd concentrations and accumulation of A. hybridus (Y).

	Equation	R^2	Р
Soil culture			
Plant height	Y = 76.238 - 0.217X	0.638	< 0.001
Root length	Y = 16.101 - 0.038X	0.730	< 0.001
Root biomass	Y = 2.090 - 0.009X	0.495	< 0.001
Shoot biomass	Y = 18.355 - 0.087X	0.661	< 0.001
Root concentration	$Y = 8.808 + 0.589X - 0.003X^2$	0.911	< 0.001
Root accumulation	$Y = 20.763 + 2.035X - 0.010X^2$	0.425	< 0.01
Shoot concentration	$Y = 2.240 + 0.367X - 0.005X^2$	0.995	< 0.001
Shoot accumulation	$Y = 28.780 + 15.335X - 0.070X^2$	0.691	< 0.001
Hydroponics			
Plant height	Y = 25.183 - 0.293X	0.738	< 0.001
Root length	Y = 23.586 - 0.231X	0.325	< 0.01
Root biomass	Y = 3.288 - 0.075X	0.785	< 0.001
Shoot biomass	Y = 3.497 - 0.044X	0.770	< 0.001
Root concentration	$Y = 23.744 + 13.365X - 0.010X^2$	0.933	< 0.001
Root accumulation	$Y = 94.944 + 32.516X - 0.679X^2$	0.535	< 0.001
Shoot concentration	$Y = 1.095 + 6.289X + 0.066X^2$	0.987	< 0.001
Shoot accumulation	$Y = 8.022 + 20.357X - 0.089X^2$	0.920	< 0.001

A. hybridus took up Cd from contaminated soil or solution and accumulated it in its tissues. Cd accumulation in shoots also showed a quadratic relationship with increasing Cd concentrations in both soil and solution (Table 3). Cd accumulation in shoots increased with increasing Cd concentrations in the hydroponics experiment, but this was not observed in the soil culture (Figs. 2(B) and 3(B)). There are many factors that influence Cd accumulation in plant tissue, including different concentrations, surroundings and biomasses. All of them can cause distinct changes in the accumulation of heavy metals in plant shoots. The highest shoot accumulations of Cd in the soil culture and hydroponics experiments were 1006.95 and 668.42 µg pot⁻¹, respectively, when the Cd concentrations reached 150 mg kg^{-1} in the soil culture and 40 mg L^{-1} in the hydroponics solutions. Moreover, Table 1 shows that Cd accumulation of A. hybridus reached 6024.65 µg in the field.

Table 4

TF and BCF of *A. hybridus* in the soil culture and hydroponics experiment, and in the field survey.^a.

	TF	BCF in shoot	BCF in root
Soil cu	llture (mg kg ⁻¹)		
0	$0.84\pm0.63b$	$0.72 \pm 0.41 bc$	$0.93\pm0.17 bc$
30	$0.75\pm0.03b$	$0.75 \pm 0.06 bc$	$1.00{\pm}0.04b$
60	$0.64\pm0.06b$	$0.58\pm0.04c$	$0.91 \pm 0.05 bc$
90	$0.73\pm0.06b$	$0.79\pm0.02bc$	$1.08\pm0.06 ab$
120	$1.12\pm0.06ab$	$0.93\pm0.03b$	$0.84\pm0.03c$
150	$1.50\pm0.14a$	$0.93\pm0.04b$	$0.62\pm0.07d$
180	$1.05\pm0.11b$	$1.22\pm0.05a$	$1.17\pm0.14a$
Hydro	ponics (mg L ⁻¹)		
0	$0.92\pm0.04a$		
1	$0.53\pm0.07c$	$17.55 \pm 1.82a$	$33.08 \pm 2.91a$
5	$0.34\pm0.05d$	$5.18\pm0.77d$	$15.43\pm0.16c$
10	$0.33\pm0.01d$	$7.17 \pm 0.37c$	$21.43\pm0.98b$
15	$0.39\pm0.04d$	$5.96 \pm 0.19 cd$	$15.52\pm1.15c$
20	$0.55\pm0.07c$	$8.65 \pm 1.03 bc$	$15.73\pm0.65c$
30	$0.79\pm0.03b$	$8.36 \pm 0.06 bc$	$10.54\pm0.30d$
40	$0.60\pm0.03c$	$8.86 \pm 0.42b$	$14.67\pm0.20c$
Field s	urvey (sampling number)		
1	0.68	0.91	1.35
2	1.36	0.87	0.64
3	1.03	1.18	1.14
4	2.05	1.01	0.49
5	2.56	1.34	0.53
6	2.63	1.66	0.63
7	1.63	1.30	0.85

^a Means with different letters are significantly different from each other (P < 0.05) according to the LSD test.

The BCF was used to evaluate the metal accumulation efficiency in plants. Most BCFs of shoots were lower than 1.0 in the soil culture (Table 4), but when the Cd concentration reached 180 mg kg⁻¹, it was higher than 1.0 which is the threshold value for a Cdhyperaccumulator [29]. In the hydroponics experiment, BCFs were always greater than 1.0 under the different Cd treatments (Table 4). A similar conclusion was also reported for *Lonicera japonica* [6]. BCFs in the field survey were similar to those in the soil culture experiment. However not all of them exceeded 1.0.

The translocation factor (TF) demonstrated that *A. hybridus* had the ability to tolerate and translocate Cd to shoots (Table 4). TFs were 0.64–1.50 for the soil culture, 0.33–0.92 for the hydroponics solutions, and 0.68–2.63 for the field survey. Most TFs in the soil culture and hydroponics solutions were lower than 1.0.

From the observed Cd concentrations, accumulation, and BCF and TF values of *A. hybridus* in the two experiments and the field survey, *A. hybridus* is a Cd-accumulator, with potential for accumulating Cd and consequent Cd removal by phytoremediation.

5. Conclusion

Overall, consideration of Cd tolerance, concentrations, accumulation, and BCF and TF values of Cd in shoots, confirms that *Amaranthus hybridus* L. is a Cd-accumulator which possesses the advantages of high biomass, easy cultivation, extensive competitive ability and wide geographical distribution. Thus, this plant has great potential to be used for phytoremediation of soils contaminated with Cd.

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