

Uptake and Translocation of Cd and Pb in Four Water Spinach Cultivars Differing in Shoot Cd and Pb Concentrations

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Abstract

A pot experiment was conducted to study the differences of uptake and translocation of Cd and Pb in four water spinach cultivars, including two low-Cd-Pb cultivars and two high-Cd-Pb cultivars. The results showed that the characteristics of low-Cd-Pb and high-Cd-Pb accumulation were stable in the two types of cultivars, respectively. The differences in shoot Cd concentration between low-Cd-Pb and high-Cd-Pb cultivars were attributable to the differences of Cd uptake and translocation from root to shoot, but the differences of shoot Pb concentration appeared to be driven by whole-plant Pb uptake rather than by Pb translocation from root to shoot.

Keywords: cadmium (Cd), lead (Pb), water spinach, uptake, translocation

Introduction

Cadmium (Cd) and lead (Pb) are highly toxic heavy metals to human beings and other living creatures [1, 2]. Because of anthropogenic activities, such as mining, smelting, electroplating and wastewater irrigation, Cd and Pb concentrations in soils have increased continuously during the last 100 years [3, 4]. Plants can absorb and accumulate harmful trace elements via roots and leaves, which may pose a potential risk to human health through bioaccumulation and biomagnification along the food chain [5]. Therefore, there is increasing concern about the safety of Cd and Pb in agricultural products.

Differences in Cd and Pb accumulation among crop cultivars (cv.) have been investigated in rice (*Oryza sativa* L.) [6, 7], Chinese cabbage (*Brassica pekinensis* L.) [3, 4], asparagus bean (*Vigna unguiculata* subsp. *sesquipedalis* L.) [8] and more. Based on the genetic differences, some

researchers have proposed that selective breeding for cultivars with a low heavy metal accumulating trait, which is highly inheritable and could minimize heavy metal accumulation in edible parts of the crops to levels below the proposed international limits [5].

Water spinach (*Ipomoea aquatica* Forsk.), an important leafy vegetable in southern Asia, India, and southern China, is vulnerable to Cd and Pb pollution [9, 10]. In our previous studies, two low-Cd-Pb and two high-Cd-Pb water spinach cultivars were obtained [10-12]. Nevertheless, it is not still known how the differences in shoot Cd and Pb concentrations, whether it is due to increased uptake from the soil, or to differences in translocation of total Cd and Pb in the plant. In durum wheat, cultivars differed in grain Cd concentration attributable to differences in translocation from the root to the shoot and within the shoot, rather than to differences in root uptake [13]. But genetic differences in the Cd concentration of lowland rice seemed to be driven by plant Cd uptake rather than by differential Cd distribution among plant parts [14]. The aim of this study was to inves-

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tigate the mechanisms of differences in shoot Cd and Pb concentrations in the four water spinach cultivars. We hypothesized that the differences among water spinach cultivars in shoot Cd and Pb concentrations were due to differences in the translocation of Cd and Pb from root to shoot, rather than to differences in the plant uptake of Cd and Pb.

Experimental Procedures

Plant Materials

Based on our previous studies, four water spinach cultivars differing in shoot Cd and Pb concentrations were used in this study. The four cultivars were two low-Cd-Pb cultivars, cv. YQ and cv. QLQ, and two high-Cd-Pb cultivars, cv. GDB and cv. T308, respectively. Shoot Cd and Pb concentrations (fresh weight, FW) of cv. YQ and cv. QLQ were both significantly lower (by at least 40%) than those of cv. GDB and cv. T308 when grown in contaminated soils with Cd (0.58-1.26 mg·kg⁻¹ dry weight, DW) and Pb (85.4-327.6 mg·kg⁻¹, DW) [10, 12].

Soil Treatments and Experiment Design

This study was conducted as a pot experiment in an experimental garden of the Hunan Institute of Technology (112°41'E, 26°52'N), Hengyang, Hunan, China. The experimental soil was collected from a vegetable farm of the institute, and was air-dried for 7 days and ground to pass through a 5 mm sieve. The soil physical and chemical properties were analyzed according to the analytical methods of soil and agricultural chemistry [15]. The soil pH, organic matter, cation exchange capacity (CEC), total Cd, and total Pb were 6.38, 1.82%, 8.7 cmol·kg⁻¹, 0.22 mg·kg⁻¹ (DW), and 35.3 mg·kg⁻¹ (DW), respectively. Four treatments, including low Cd, high Cd, low Pb, and high Pb, were carried out by adjusting soil Cd and Pb concentrations. The soils spiked at 0.4 and 1.0 mg·kg⁻¹ of Cd in the form of Cd(NO₃)₂·4H₂O and 40, and 80 mg·kg⁻¹ of Pb in the form of Pb(CH₃COO)₂·3H₂O were designated low Cd, high Cd, low Pb, and high Pb, respectively. Each of the four soils were placed in a large basin, watered, and left to balance outdoors under a waterproof tarpaulin for three months. The final soil Cd concentrations were 0.58 and 1.14 mg·kg⁻¹ (DW) for low Cd and high Cd, respectively, and soil Pb concentrations were 72.4 and 122.6 mg·kg⁻¹ (DW) for low Pb and high Pb, respectively. According to the Farmland Environmental Quality Evaluation Standards for Edible Agricultural Products (HJ 332-2006) of China, the maximum levels (MLs) for Cd and Pb are 0.3 and 50 mg·kg⁻¹ (DW), respectively; all the soils were contaminated by Cd or Pb in different degrees. Plastic pots, with diameters of 18 cm (top) and 13 cm (bottom) and height of 15 cm, were each filled with 2.5 kg (DW) of the prepared soil. Three pots (n=3) were used for each cultivar for each treatment. Eight seeds per pot were sown into the soil. The experiment was laid out as a completely randomized design.

After germination, seedlings were thinned to 4 plants·pot⁻¹ left in a week and watered with tap water daily. Solid compound fertilizer (N:P:K = 15:15:15) was applied into the soils for 3.0 g·pot⁻¹ on the 15th day after germination.

Plant Sampling and Chemical Analysis

On the 40th day after germination, whole water spinach plants were harvested and washed thoroughly with tap water and then with deionized water. The plants were divided into roots and shoots. All the samples were dried at 105°C for 20 min and then at 70°C to constant weight. The dried plant samples were crushed to pass through a 0.149 mm sieve for chemical analysis after their dry weights were recorded. Cd and Pb concentrations of the dry samples were determined by atomic absorption spectrometry (Hitachi Z-2300) after digestion with HNO₃:H₂O₂ (10:3, v/v) in a microwave oven (Microwave digester MDS-6, Shanghai Sineo Microwave Chemistry Technology Co., Ltd., China). A Certified Reference Material (CRM) of plant GBW07605 (provided by the National Research Center for CRM, China) was used for quality control in the Cd and Pb analytical procedures of the plant samples.

Statistical Analysis

Data were statistically processed on a computer using SPSS 13.0 and Excel 2003 and analyzed by one-way ANOVA with the least significant difference (LSD) test. All the correlations were assessed using Pearson product-moment correlation. Translocation rate was calculated as follows:

$$\text{Translocation rate} = \left[\frac{\text{total Cd (or Pb) in shoot}}{\text{total Cd (or Pb) in the whole plant}} \right] \times 100\%$$

Results and Discussion

Cd Accumulation and Distribution

The shoot Cd concentrations of low-Cd-Pb cultivars (cv. YQ and cv. QLQ) were always significantly lower ($p < 0.05$) than those of high-Cd-Pb cultivars (cv. GDB and cv. T308) (Fig. 1a). The results were consistent with the results of our previous research [10, 11]. However, the root Cd concentrations of cv. GDB and cv. T308 were lower than those of cv. YQ and cv. QLQ, except that there was no obvious difference ($p > 0.05$) among cv. YQ and the two high-Cd-Pb cultivars under high Cd exposure (Fig. 1a). This indicated that the low-Cd-Pb cultivars of water spinach could better restrict the Cd absorbed by roots to be transported to shoots in comparison with the high-Cd-Pb cultivars. Similarly, maize (*Zea mays* L.) hybrids Maikada and Reduta exhibited higher root Cd concentrations than did Helena and Quintal, but shoot Cd concentrations of the former two hybrids were obviously lower than those of the latter two [16]. It was also reported that low-Cd soybean cultivars had higher root Cd concentrations than did high-Cd

Table 1. Variations among water spinach cultivars on distribution portions (%) of Cd and Pb quantities in shoots and roots.

Organ	Treatment	Cultivar			
		YQ	QLQ	GDB	T308
Shoot	Low Cd	59.5 b	59.5 b	74.5 a	76.2 a
	High Cd	60.3 b	57.2 b	72.3 a	75.0 a
	Low Pb	42.9	39.7	42.9	36.9
	High Pb	30.7	30.0	35.1	30.5
Root	Low Cd	40.5 A	40.5 A	25.5 B	23.8 B
	High Cd	39.7 A	42.8 A	27.7 B	25.0 B
	Low Pb	57.1	60.3	57.1	63.1
	High Pb	69.3	70.0	64.9	69.5

Different lowercase (shoot) and capital (root) letters in the same row indicate significant differences among the cultivars at $p < 0.05$ level.

cultivars [17]. Shoot Cd accumulation in the two low-Cd-Pb cultivars also were significantly lower ($p < 0.05$) than those in the two high-Cd-Pb cultivars (Fig. 1b). However, no significant differences in root Cd accumulation were found between cv. GDB and the two low-Cd-Pb cultivars under low Cd exposure, and between cv. QLQ and the two high-Cd-Pb cultivars under high Cd exposure (Fig. 1b). But the total Cd accumulations in the two low-Cd-Pb cultivars were obviously lower ($p < 0.05$) than those in the two high-Cd-Pb cultivars in the two Cd treatments. Moreover, the low-Cd-Pb cultivars had a greater ($p < 0.05$) proportion of Cd in the roots than did the high-Cd-Pb cultivars, but there were no differences ($p > 0.05$) in Cd distribution portion between the same type of cultivars (Table 1). These results implied that water spinach cultivars differed in shoot Cd concentration attributable to differences not only in Cd uptake but also in root-to-shoot Cd translocation. Similarly, the differences in grain Cd concentration among rice cultivars occurred in plant Cd uptake and Cd transport from root

to shoot to grain, but root Cd concentrations and accumulations in high-Cd rice cultivars were still significantly higher than those in low-Cd cultivars [7]. Perhaps the mechanisms of low-Cd cultivars accumulating Cd were associated with crop species.

Pb Accumulation and Distribution

Under the two Pb exposures, Pb concentrations and accumulations in shoots and roots of low-Cd-Pb cultivars (cv. YQ and cv. QLQ) were all significantly lower ($p < 0.05$) than those of high-Cd-Pb cultivars (cv. GDB and cv. T308) (Fig. 2a, b). This suggests that the mechanisms of uptake, translocation and accumulation of Pb and Cd were different in water spinach cultivars. In addition, no conspicuous differences in root Pb concentrations and accumulations were observed between the two low-Cd-Pb cultivars as well as the two high-Cd-Pb cultivars, except that Pb concentration of cv. GDB was significantly lower ($p < 0.05$) than that of cv. T308 under low Pb exposure (Fig. 2a). And there also were no significant differences ($p > 0.05$) in Pb distribution portion in the same organ among the four cultivars (Table 1). These results illustrated that the differences in shoot Pb concentrations among water spinach cultivars appeared to be driven by plant Pb uptake rather than by root-to-shoot Pb translocation. It is worth noting that 23.8-42.8% of Cd absorbed by water spinach was restrained in roots, but the Pb proportions in roots reached 57.1-70.0% (Table 1). A similar report was that more than 60% of Pb in 30 Chinese cabbage cultivars distributed in the roots in the Pb treatment 500 mg·kg⁻¹ and there was no significant difference in Pb distribution among the cultivars [4]. This demonstrated that, compared with Cd, Pb was more difficult to be transferred from root to shoot.

Correlations between Shoot Metal Concentrations and Metal Accumulations and Translocation Rates

Shoot Cd concentrations were significantly and positively correlated ($p < 0.001$) with Cd accumulations in

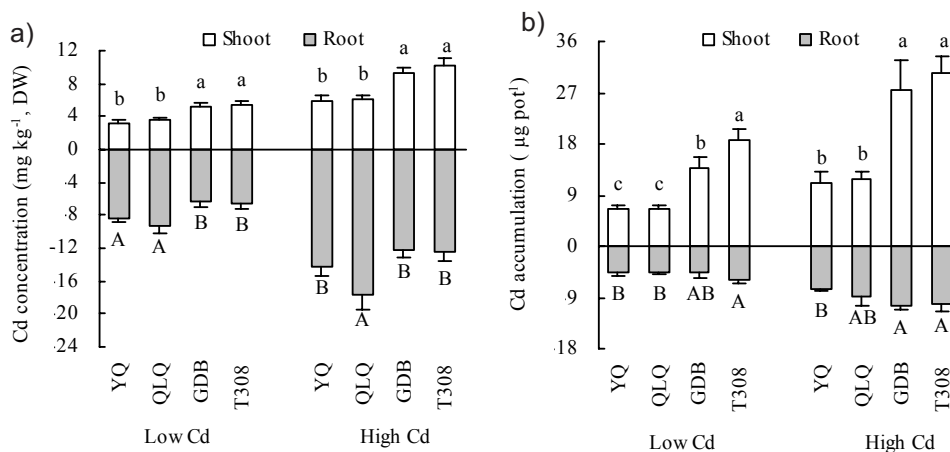


Fig. 1. Cd concentrations (a) and accumulations (b) in shoots and roots of water spinach cultivars under Cd exposure. Different lowercase (shoot) and capital (root) letters indicate significant differences at $p < 0.05$ level among the cultivars in the same treatment; error bars represent SD ($n = 3$).

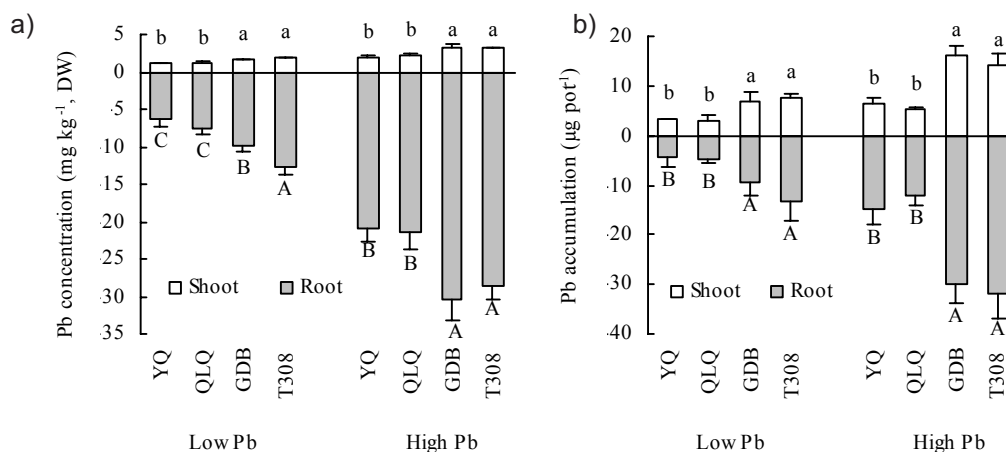


Fig. 2. Pb concentrations (a) and accumulations (b) in shoots and roots of water spinach cultivars under Pb exposure. Different lowercase (shoot) and capital (root) letters indicate significant differences at $p < 0.05$ level among the cultivars in the same treatment; error bars represent SD ($n = 3$).

water spinach plant (Fig. 3a). Similarly, shoot Pb concentrations correlated highly ($r = 0.934$, $n = 24$) with total Pb accumulations (Fig. 3b). Especially, there also existed positive and significant correlations ($p < 0.001$) between shoot Cd concentrations and translocation rates of Cd under the two Cd exposures (Fig. 4a). However, no correlations were observed between shoot Pb concentrations and translocation rates of Pb (Fig. 4b). The results showed again that shoot Cd concentration in water spinach was controlled by plant Cd uptake and the translocation of Cd from root to shoot, but shoot Pb concentration was associated with total Pb accumulation in water spinach rather than the Pb transport from root to shoot. In our previous study, it was found that the expression increment of T156 (a multidrug resistance associated protein gene and related with Cd transport) in the shoot of cv. T308 was greater than that of cv. QLQ under Cd exposure [18]. However, it is still unclear about the molecular mechanisms of differences in shoot Pb concentrations among water spinach cultivars.

Conclusions

Cadmium was predominantly accumulated and distributed in water spinach shoots while Pb was in roots. Translocation rates of Cd were greater than those of Pb, showing that Cd was more easily transported to shoots of water spinach than Pb. The difference in shoot Cd concentration between low-Cd-Pb and high-Cd-Pb cultivars was associated with the uptake and translocation of Cd. However, shoot Pb concentration was driven by Pb uptake but not by Pb translocation.

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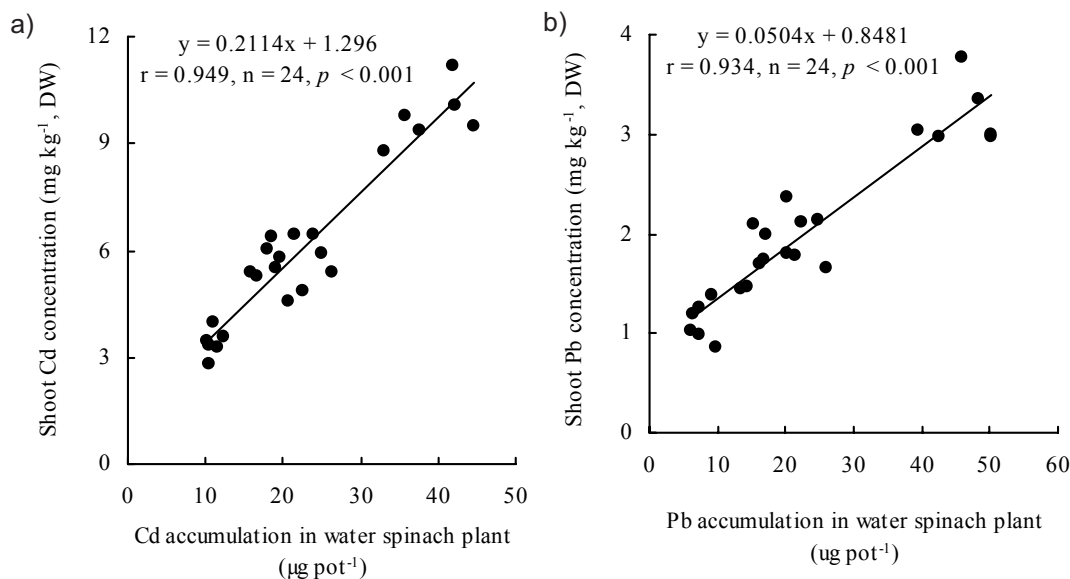


Fig. 3. Correlations between shoot Cd concentrations and total Cd accumulation (a), and between shoot Pb concentrations and total Pb accumulation (b) in water spinach.

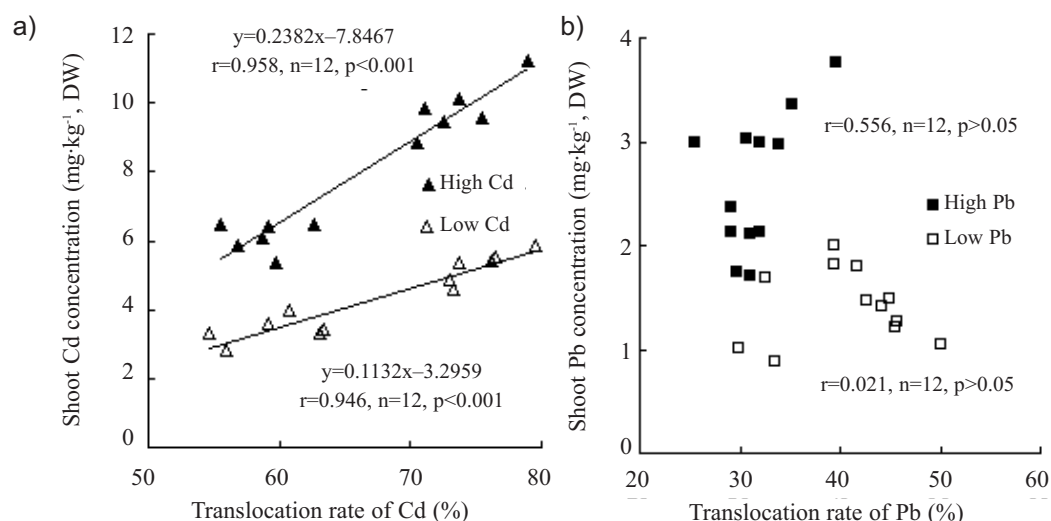


Fig. 4. Correlations between shoot Cd concentrations and Cd translocation rates (a), and between shoot Pb concentrations and Pb translocation rates (b).

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