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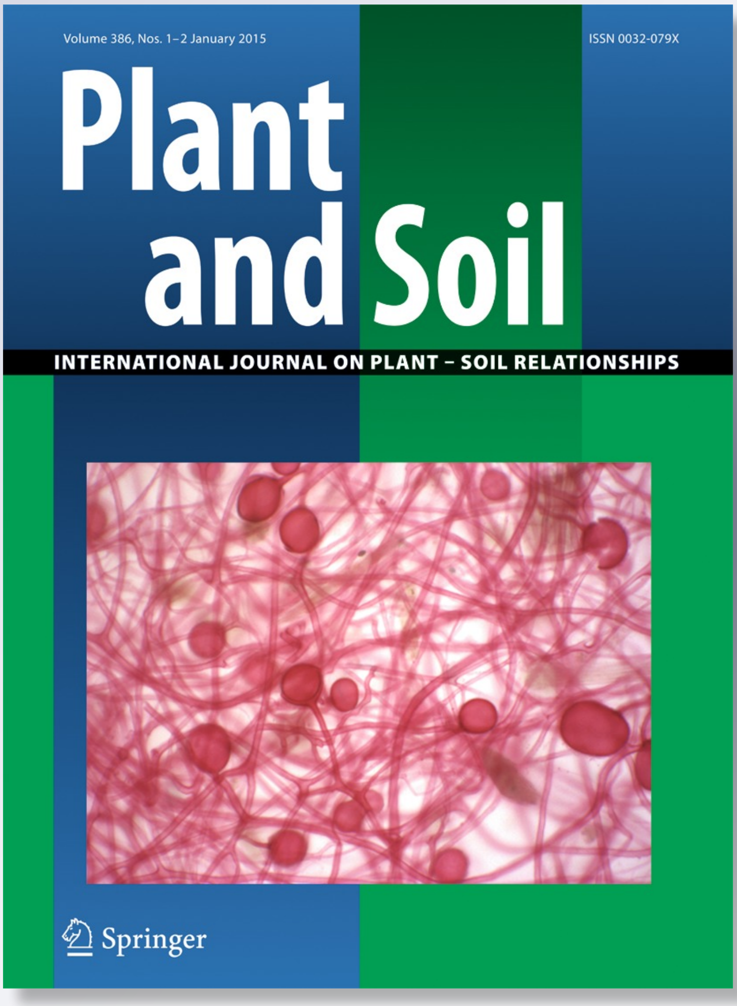

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REGULAR ARTICLE

Cultivar-specific differences in heavy metal (Cd, Cr, Cu, Pb, and Zn) concentrations in water spinach (*Ipomoea aquatic* 'Forsk') grown on metal-contaminated soil

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Abstract

Purpose This study aimed to investigate the cultivar differences and the involved rhizosphere mechanisms in multiple heavy metal (i.e., Cd, Cr, Cu, Pb, and Zn) accumulation in water spinach.

Methods Pot experiments were performed on long-term contaminated soil to determine heavy metal accumulation in 15 water spinach cultivars. A hydroponics experiment was extended using Ca channel blocker $LaCl_3$.

Results Nearly two-fold variations of heavy metal concentrations were found among the 15 cultivars. Cd, Cr, Cu, and Pb concentrations positively correlated with Ca and Zn concentrations. $LaCl_3$ significantly reduced the phyto-uptake of Cd and Cr. The cultivar differences in heavy metal accumulation coincided with the concentration variation of metals activated by low molecular weight organic acids (LMWOAs) in the rhizosphere. Temperature and soil salinity clearly affected the cultivar differences in heavy metal accumulation.

Conclusions Ca uptake and LMWOA secretion serve a crucial function in the variation of heavy metal accumulation among water spinach cultivars. Temperature and soil salinity should be prioritized in cultivar screening.

Keywords Combined heavy metals · Major metals · Cultivar difference · *Ipomoea aquatic* 'Forsk' · Ca channel · Low molecular weight organic acids

Introduction

Heavy metal contamination in agricultural soils is an emerging problem given its effect on farm product safety (Huang et al. 2007; Wei and Yang 2010). Controlling heavy metal content in crops can help reduce potential health risks. Applicable strategies mainly include decreasing heavy metal content in root layer soil, immobilizing heavy metals in soil, and cultivating crops with low heavy metals accumulation (Bhargava et al. 2012). Cultivation of crops with low heavy metal accumulation has attracted considerable attention for the possibility of utilizing slightly and/or moderately contaminated soils as a long-term effective and economical approach (Liu et al. 2009). Crop cultivar differences in terms of heavy metal accumulation have been well-documented. The characteristic of heavy metal

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accumulation is genotype-dependent (Wang et al. 2007). Cultivars with low heavy metal accumulation have been achieved in common crops, such as rice (Zeng et al. 2008), wheat (Stolt et al. 2006), soybean (Arao and Ishikawa 2006), potato (Dunbar et al. 2003), lettuce (Thomas and Harrison 1991), barley (Chen et al. 2007), asparagus bean (Zhu et al. 2007), and cabbage (Liu et al. 2009).

The worldwide heavy metal contamination in soil mostly results from a mixture of metals rather than a single element (Gowd et al. 2010; Khan et al. 2008; Luo et al. 2011; Mico et al. 2007). More importantly, the combined risk of multiple toxic metals on human health should not be neglected. Therefore, an applicable cultivar with low heavy metal accumulation can simultaneously minimize the accumulation of co-existing toxic metals. However, most of the available studies often focus on single metals (Zeng et al. 2008). Significant differences in multiple heavy metal accumulation have recently been reported in cultivars of asparagus bean (Zhu et al. 2007) and rice (Liu et al. 2003; Yoshihara et al. 2010). However, the intrinsic mechanism involved in cultivar differences has not been elucidated.

Nonessential metals for agriculture crops are assumed to be taken up through transport systems for essential metals because of the insufficient specificity of these systems (Lu et al. 2010; Yoshihara et al. 2010). Therefore, transport mechanisms for major essential metals (i.e., K, Na, Ca, and Mg) may serve an important function in nonessential heavy metal uptake of crops. The metal concentration in the rhizosphere solution determines the concentration gradient of metal across cytoplasmic membrane and bioavailable metal amounts, thereby affecting the transmembrane rate of the metal and accumulation capacities of plants (Li and Cheng 2007). The metal concentration variation in the rhizosphere solution for a specific soil is attributed to metal activation by rhizosphere exudates (Dessureault-Rompre et al. 2008).

Aside from genetic control, heavy metal uptake by a specific crop cultivar may be affected by growing conditions, such as temperature, soil characteristics (i.e., pH, redox status, cation exchange capacity, organic matter content, and salinity), and soil heavy metal concentration (Grant et al. 2008; Li et al. 2010). Among these factors, temperature and soil salinity directly affect heavy metal translocation by altering plant transpiration rate and plasma membrane permeability (Liu et al. 2010; Li et al. 2012). The consistency of cultivar-specific differences in uptake of multiple metals under relatively low temperature or salinity conditions has not been tested.

Water spinach, a leafy vegetable, is very popular in Southeast Asia and easily polluted by Cd, Cr, Cu, Pb, Zn, and Hg (Li et al. 2010; Xin et al. 2010). Water spinach is often planted during April to October and harvested during June to December in Southern China. Therefore, this study investigates (1) the cultivar difference in heavy metal accumulation (i.e., Cd, Cr, Cu, Pb, and Zn) of water spinach; (2) rhizosphere mechanisms of cultivar differences in heavy metal accumulation by analyzing the relationships of heavy metal concentrations in shoots with major metal (i.e., K, Na, Ca, and Mg) uptakes, as well as rhizosphere concentrations of dissolved heavy metal and exudate among different cultivars; and (3) effect of season and soil salinity on cultivar-specific differences in heavy metal accumulation.

Materials and methods

Pot experiment

The soils used in the pot experiment were obtained from farmlands in the suburbs of Guangzhou City (Guangdong Province, China). The soils were irrigated by wastewater 20 years ago and contaminated with heavy metal. The soil pH was 6.38, and soil salinity was 0.15 %. Soil organic matter content was 35.4 g/kg and soil cation exchange capacity was 20.86 cmol/kg. The metal element concentrations are listed in Table 1. Cd, Pb, and Zn exceeded the limits set by the Farmland Environmental Quality Evaluation Standard for Edible Agricultural Products (HJT 332–2006, China). Water spinach seeds of 15 common cultivars (Fig. 1) were sowed directly into the pots (diameter, 22 cm; depth, 15 cm) with 1.5 kg sieved soil and cultivated in a greenhouse at the Jinan University campus, Guangzhou (Guangdong Province, China). The pots were arranged in a randomized complete block design with three replicates. The seedlings were thinned to 25 plants per pot. Moisture content of the soil was maintained at 75 % of the field water-holding capacity using deionized water. All plants were harvested after being cultured for 50 d. The fresh weights (FWs) of the whole plants and the shoots (stem and leaf) in each pot were recorded after cleaning with deionized water. All samples were oven-dried at 105 °C for 0.5 h, and then dried to a constant weight at 76 °C. The samples were ground to fine powder that can pass through a 60-mesh sieve in a pre-cleaned steel grinder after recording their dry weights. The samples were then stored in polythene zip-bags. The pot experiments were conducted during the warm season (June to August, average daily temperature 24 °C–31 °C) and cool season (October to December, average daily temperature 10 °C–18 °C).

The uptake of heavy metal by crops grown in cool season is lower than that in warm season because of lower transpiration (Li et al. 2003; Liu et al. 2010; Tani and Barrington 2005). However, soil salinity increases heavy metal uptake by crops (Li et al. 2010, 2012). Under soil salinity stress, growing crops during cool season may be more beneficial to food safety than during warm season. Therefore, the pot experiments using 0.3 % NaCl-pretreated soil were performed only during cool season to investigate the effect of soil salinity on the cultivar-specific differences in heavy metal accumulation.

Hydroponics experiment

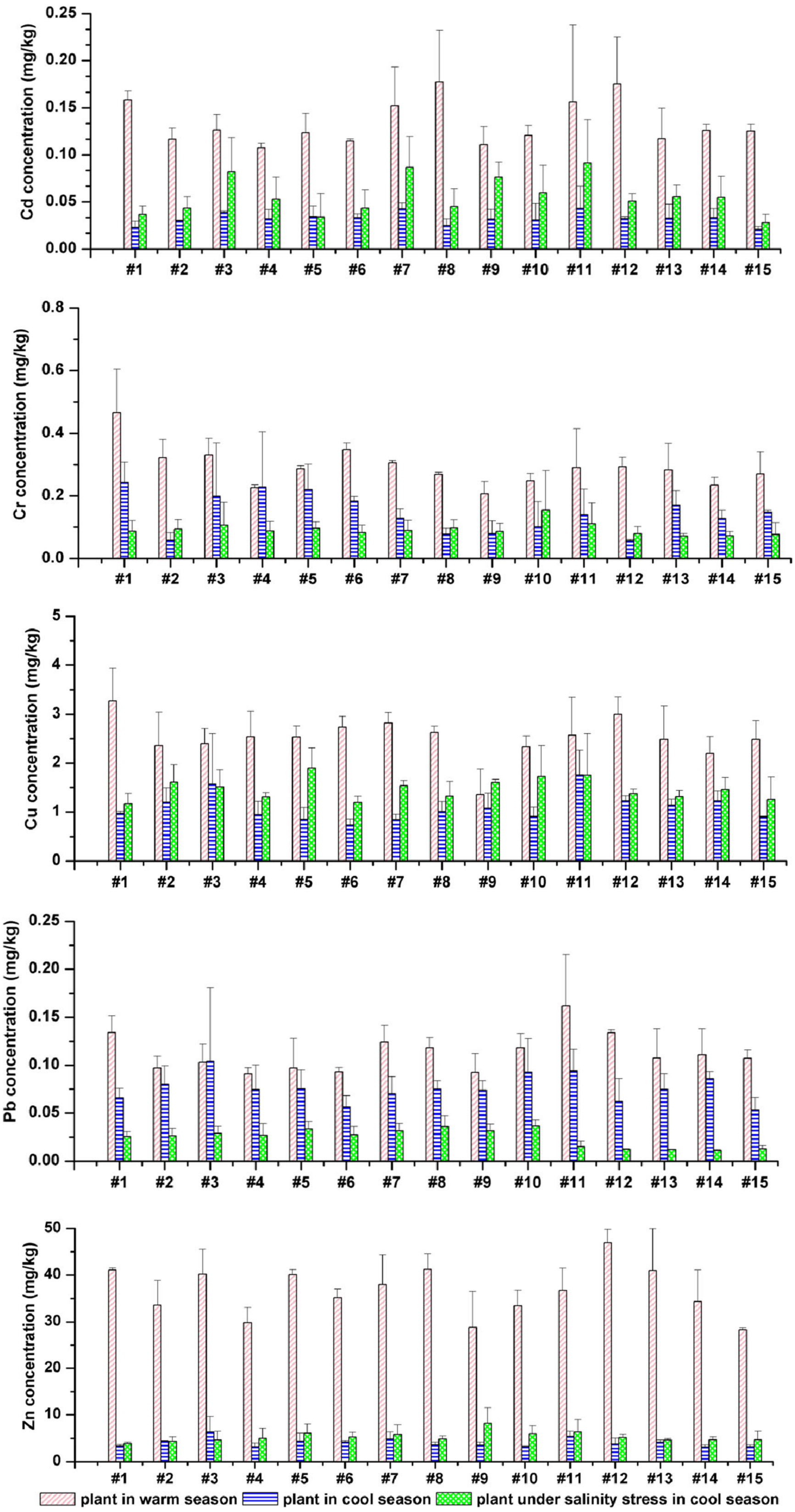
Cultivars #1 and #15 were randomly selected for hydroponics experiment in warm season (June to August, average daily temperature 24 °C–31 °C) to investigate the common mechanisms involved in the transmembrane transport of heavy metal in water spinach cultivars. Six uniform seedlings for each cultivar were transferred to a 1.5 L modified 0.3 strength Hoagland nutrient solution (pH=5.5, buffered with Mes-Tris) for 4 d in a plastic vessel, and then to 0.5 strength for 8 d, 0.8 strength for 8 d, and finally to full strength to adapt a nutrient solution. The nutrient solution was aerated continuously and replaced every 4 d. The full-strength Hoagland nutrient solution was composed of 4.0 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 mM $MgSO_4 \cdot 7H_2O$, 5.0 mM KNO_3, 1.0 mM NH_4NO_3, 1.0 mM KH_2PO_4, 0.132 mM $MnSO_4 \cdot 4H_2O$, 0.1 mM H_3BO_3, 0.03 mM $ZnSO_4 \cdot 7H_2O$, 0.1 μM $CuSO_4 \cdot 5H_2O$, 0.1 μM $CoCl_2$, 1.0 μM $Na_2MoO_4 \cdot 2H_2O$, 5.0 μM KI, 0.1 mM $FeSO_4 \cdot 7H_2O$, and 0.1 mM EDTA–Na_4. Subsequently, 1 mM Ca ion channel blocker $LaCl_3 \cdot 7H_2O$, 2 μM $Cd(NO_3)_2$, 5 μM $Pb(OAC)_2 \cdot 3H_2O$ and 50 μM $CrCl_3 \cdot 6H_2O$ were added to the nutrient solution after the plants were cultured for 40 d in the nutrient solution with pH adjusted to 5.5 by 2 mM Mes-Tris. The plants were harvested after 8 h of exposure to La^{3+} and heavy metal treatment. Blank experiments in the solution without La^{3+} were also run in parallel and served as controls. The plant

Table 1 Concentrations of metal elements in soil (dry weight)

	K (g/kg)	Na (g/kg)	Ca (g/kg)	Mg (g/kg)	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Total	28.46±1.42	162.90±22.49	37.21±6.04	15.65±1.43	1.81±0.10	44.80±6.24	50.62±7.61	94.48±9.43	338.55±67.17
Standard[a]	–	–	–	–	0.30	150.00	50.00	50.00	200.00
Exchangeable	–	–	–	–	0.27±0.00	0.40±0.06	0.22±0.03	0.16±0.02	9.41±1.87

a, National Standard of PR China, Farmland Environmental Quality Evaluation Standard for Edible Agricultural Products (HJT 332–2006). Data are means±SD (*n*=3)

Fig. 1 Heavy metal concentrations in the shoots of water spinach (FW). The grown cultivars were Guangxiliuyebai (#1), Jinyan (#2), Importation Tailand (#3), Sihainongwangliuyebaigu (#4), Liuyeyougu (#5), Sihainongwangliuye (#6), Chunbaigudaye (#7), Tailand Zhuye (#8), Sihainongwangdabaigeng (#9), Liuyeqingu (#10), Tailand Baigenliuye (#11), Hongkongdaye (#12), Jianxiyuanzhongdaye (#13), Taiwanbaiguliuye (#14), and Panyuchunbailiuye (#15). Bars represent±SD (*n*=3)



roots were cleaned with deionized water and desorbed with 15 mM EDTA–NH_4 to further analyze the Na, Ca, Mg, Cd, Cr, Cu, Pb, and Zn uptake.

Analysis of heavy metals in water spinach

Samples (root or shoot) were digested with concentrated nitric acid in a microwave digesting apparatus (CEM MARs XPRSS, USA). The digested samples were cooled to room temperature, evaporated to remove acid, and finally diluted with deionized water to 25 mL. The K, Na, Ca, Mg, Cd, Cr, Cu, Pb, and Zn concentrations in the solution were determined by Atomic Absorption Spectrometry (AA7000, Shimadzu, Japan). Standard reference materials of the plant [GBW07602 (GSV-1)] and blanks were carried through digestion and analyzed as part of the quality control protocol. Analysis results were accepted when the measured concentrations in the reference materials were within one standard deviation of the certified values.

Rhizosphere solution collection and analysis

The soil adhering to the roots of plants grown in warm season was shaken down, loaded in a syringe, and then centrifuged to collect the rhizosphere solution. The collected solution was filtered through a 0.45 μm membrane, and the metal concentrations (i.e., K, Na, Ca, Mg, Cd, Cr, Cu, Pb, and Zn) in the filtered solution were measured by inductively coupled plasma mass spectrometry. Subsequently, the repeated samples of each cultivar were mixed to obtain enough solution for dissolved organic carbon (DOC) and low molecular weight organic acids (LMWOAs) measurements. DOC analysis was performed on a SHIMADZU TOC-VCSH. LMWOAs (including acetic, malic, tartaric, maleic, oxalic, citric, succinic, fumaric, formic, and propionic acids) were analyzed using an ion chromatograph (ICS-900, Dionex, America) coupled with an Ion IonpacAS11-HC column and an IonpacAG11-HC guard column (50 mm×4 mm i.d.). The mobile phase was a KOH solution at 1 ml/min flow rate and 30 °C temperature. The gradient elution process was 1 mmol/L for 10 min, 45 mmol/L for 25 min, and 1 mmol/L for the final 5 min.

Data analysis

The low (the first three) and high (the last three) heavy metal accumulators were defined in this paper according to the ranking of the heavy metal content in the edible part (shoot) of all the tested water spinach cultivars. SPSS 17.0 and Origin 8.0 were used to analyze the data and plots, respectively. Pearson correlation analyses were carried out on the metal contents in the shoots of different cultivars. P values were two-tailed, and two significant levels were using $P=0.05$ and 0.01. The outcomes of the control and treatment groups in the hydroponics experiment were compared using a T test at the 0.05 probability level. The metal decrease ratios caused by the inhibition of La^{3+} were calculated using Equation (1).

$$\text{Ratio} = \frac{\text{Control}-\text{Treatment}}{\text{Control}} \times 100\% \qquad (1)$$

Control: Metal concentration in water spinach in hydroponics.

Treatment: Metal concentration in water spinach exposed to $LaCl_3$ in hydroponics.

Results

Cultivar variations of heavy metal concentrations in shoots of water spinach grown in soil

The heavy metal concentrations (i.e., Cd, Cr, Cu, Pb, and Zn) in the shoots of the 15 cultivars are shown in Fig. 1. The concentrations in warm season were as follows: Cd (0.107 mg/kg to 0.177 mg/kg), Cr (0.207 mg/kg to 0.467 mg/kg), Cu (1.355 mg/kg to 3.275 mg/kg), Pb (0.091 mg/kg to 0.162 mg/kg), and Zn (28.83 mg/kg to 46.91 mg/kg). Obvious variations in heavy metal concentrations were observed among the water spinach cultivars. The average contents in the cultivars (#4, #9, and #13) with lower accumulation of heavy metals were as follows: Cd (0.112 mg/kg), Cr (0.239 mg/kg), Cu (2.127 mg/kg), Pb (0.097 mg/kg), and Zn (33.21 mg/kg). The values in the cultivars (#1, #8, and #12) with higher heavy metal accumulation were as follows: Cd (0.170 mg/kg), Cr (0.343 mg/kg), Cu (2.967 mg/kg), Pb (0.129 mg/kg), and Zn (43.06 mg/kg).

In cool season, the concentrations of heavy metals in the shoots of different cultivars grown were as follows: Cd (0.021 mg/kg to 0.043 mg/kg), Cr (0.057 mg/kg to 0.243 mg/kg), Cu (0.733 mg/kg to 1.759 mg/kg), Pb (0.053 mg/kg to 0.094 mg/kg), and Zn (3.178 mg/kg to 5.454 mg/kg) (Fig. 1). The cultivars with low and high heavy metal accumulation were identified and listed in Table 2. The high accumulators (#1 and #8) of Cd became low accumulators under the effect of lower temperature. The low Cd-accumulation characteristic of cultivars (#4, #9, and #13) disappeared. The cultivar ranks in the concentrations of other metals (i.e., Cr, Cu, Pb, and Zn) also clearly changed. Heavy metal concentrations in the shoots of different cultivars under salinity stress in cool season were as follows: Cd (0.028 mg/kg to 0.091 mg/kg), Cr (0.072 mg/kg to 0.154 mg/kg), Cu (1.167 mg/kg to 1.900 mg/kg), Pb (0.011 mg/kg to 0.036 mg/kg), and Zn (3.883 mg/kg to 8.192 mg/kg). The cultivar difference in terms of Cd-accumulation exhibited no obvious alteration, but the cultivar ranks in the accumulation of the other heavy metals (i.e., Cr, Cu, Pb, and Zn) significantly changed compared with the above ranks in cool season.

Cross-correlations of metal concentrations in the shoots of different cultivars

The correlation coefficients among the metal concentrations in different cultivars grown in warm season are listed in Table 3. A total positive correlation was found between the major metals and the heavy metals. K was positively correlated with Cd, Cr, Cu, Pb, and Zn at the 0.01 level. Na had a strong positive correlation with Cr and Cu at the 0.01 level. Ca had a significant correlation with Cd ($P<0.05$), Cr ($P<0.01$), Cu ($P<0.01$), Pb ($P<0.05$), and Zn ($P<0.01$). Mg was also correlated with Cr ($P<0.01$). Zn was positively correlated with Cd, Cr, Cu, and Pb at the 0.01 level. Cd was positively correlated to Cr ($P<0.05$), Cu ($P<0.01$), Pb ($P<0.01$), and Zn ($P<0.01$). Table 4 shows heavy metals concentrations in the shoots of water spinach related to Ca content. The average Ca content of high-Cd accumulators (#1, #8, and #12) was 2.28-fold that of the low-Cd accumulators (#4, #9, and #13).

Table 5 shows the correlations among the metal concentrations of shoots in cool season. Strong positive correlations between Ca and Cd, as well as Cd and Pb, diminished under lower temperature. Positive correlation is observed between Ca and Pb, as well as Zn and Pb. Under salinity stress in cool season, Na was positively correlated with Cd, Cr, and Cu, instead of Ca. The correlations among the heavy metals strengthened. Zn had a significantly positive correlation with Cd, Cr, Pb, and Cu at the 0.01 level.

Table 2 Cultivars with low (L) and high (H) accumulation of heavy metals grown in warm and cool season

Season	Cd		Cr		Cu		Pb		Zn	
	L	H	L	H	L	H	L	H	L	H
Warm season	#4, #9, #13	#1, #8, #12	#4, #9, #14	#1, #3, #6	#9, #10, #14	#1, #7, #12	#4, #6, #9	#1, #11, #12	#4, #9, #15	#1, #8, #12
Cool season	#1, #8, #15	#3, #7, #11	#2, #8, #12	#1, #4, #15	#5, #6, #7	#3, #11, #14	#1, #6, #15	#3, #10, #11	#4, #10, #14	#3, #7, #11
Cool season (under salinity stress)	#1, #5, #15	#3, #7, #11	#13, #14, #15	#3, #10, #11	#1, #6, #15	#5, #10, #11	#12, #13, #14	#5, #8, #10	#1, #2, #3	#5, #9, #11

Table 3 Correlation coefficients of the metals contents of shoots in warm season

	K	Na	Ca	Mg	Cd	Cr	Cu	Pb	Zn
K	1	0.292	0.376*	0.084	0.553**	0.586**	0.739**	0.518**	0.525**
Na	0.292	1	0.316*	0.548**	0.262	0.687**	0.391**	0.023	0.248
Ca	0.376*	0.316*	1	0.377*	0.380*	0.475**	0.415**	0.319*	0.391**
Mg	0.084	0.548**	0.377*	1	0.247	0.394**	0.286	0.041	0.099
Cd	0.553**	0.262	0.380*	0.247	1	0.297*	0.463**	0.657**	0.619**
Cr	0.586**	0.687**	0.475**	0.394**	0.297*	1	0.758**	0.331*	0.452**
Cu	0.739**	0.391**	0.415**	0.286	0.463**	0.758**	1	0.471**	0.525**
Pb	0.518**	0.023	0.319*	0.041	0.657**	0.331*	0.471**	1	0.443**
Zn	0.525**	0.248	0.391**	0.099	0.619**	0.452**	0.525**	0.443**	1

N=45; ** Significance at 0.01 level; * Significance at 0.05 level

Inhibition of metal uptake in water spinach by La^{3+}

Although cultivars #1 and #15 were selected randomly for the hydroponic experiment, they can well represent water spinach because of a high accumulation (#1) and a low accumulation (#15, no significant difference among cultivars #13, #14, and #15) of most of heavy metals. $LaCl_3$ acts as a Ca channel blocker and restrains Ca influx across the root-cell plasma membrane of a plant (Moyen and Roblin 1997). Metal concentrations (i.e., Na, Ca, Mg, Cd, Cr, Cu, Pb, and Zn) in the roots and shoots of the cultivars (#1 and #15) exposed to $LaCl_3$ are listed in Table 6. The ratios of Na, Ca, Cd, and Cr in the roots of cultivar #1 decreased by 14.20, 16.93, 57.94, and 81.50 %, respectively. The ratios of Na, Ca, Mg, Cd, and Cr in the roots of cultivar #15 decreased by 16.79, 22.03, 18.87, 45.56, and 87.02 %, respectively. La^{3+} had no obvious effect on Cu, Pb, and Zn accumulation in both cultivars.

Cultivar difference in dissolved heavy metals and exudates in rhizosphere

The rhizosphere solution concentrations of heavy metals, LMWOA, and DOC are presented in Table 7. The high accumulators (#1, #8, and #12) had higher concentrations of heavy metals (i.e., Cd, Cr, Cu, Pb, and Zn), LMWOA, and DOC in rhizosphere than the low accumulators (#4, #9, and #13). The average concentrations of the high accumulators in the rhizosphere solution were 1.63-, 2.04-, 1.70-, 2.85-, and 1.63-folds concentrations of the low accumulators for Cd, Cr, Cu, Pb, and Zn, respectively. These results agreed well with the proportional relation of metal concentration in the shoots of water spinach between the high and low accumulators. The concentrations in the shoots of the high accumulators were 1.52-, 1.44-, 1.39-, 1.33-, and 1.30-folds concentrations of the low accumulators for Cd, Cr, Cu, Pb, and Zn, respectively.

Table 4 Metals concentrations in shoots of high-Cd cultivars compared to low-Cd cultivars grown in warm season (mg/kg, FW), Data are means±SD (*n*=3)

	Cultivar	Ca	Cd	Cr	Cu	Pb	Zn
Low accumulation	#4	382.9±52.2	0.107±0.005	0.226±0.009	2.540±0.527	0.091±0.006	29.83±3.26
	#9	525.5±99.4	0.111±0.019	0.207±0.039	1.355±0.523	0.093±0.019	28.83±7.72
	#13	551.2±157.9	0.117±0.032	0.283±0.085	2.486±0.686	0.108±0.031	40.99±8.98
	average	486.6	0.112	0.239	2.127	0.097	33.21
High accumulation	#1	1023±62	0.159±0.009	0.467±0.138	3.275±0.667	0.134±0.018	41.07±0.44
	#8	996±85	0.178±0.055	0.269±0.006	2.625±0.127	0.118±0.010	41.22±3.34
	#12	1309±105	0.175±0.049	0.294±0.030	3.001±0.352	0.134±0.003	46.91±2.89
	average	1109	0.170	0.343	2.967	0.129	43.06
Fold		2.28	1.52	1.44	1.39	1.33	1.30

Table 5 Correlation coefficients of the metals contents of shoots in cool season

	K	Na	Ca	Mg	Cd	Cr	Cu	Pb	Zn
K	1	0.448**	0.320*	0.291	0.096	0.393**	0.307*	0.236	0.284
Na	−0.060	1	0.582**	0.586**	0.199	0.337*	0.395**	0.350*	0.659**
Ca	−0.266	0.533**	1	0.842**	0.231	0.314*	0.682**	0.565**	0.680**
Mg	0.198	0.467**	0.660**	1	0.281	0.180	0.774**	0.729**	0.723**
Cd	0.176	0.325*	0.238	0.346*	1	−0.008	0.117	0.214	0.324*
Cr	0.255	0.511**	−0.117	0.022	0.537**	1	0.081	0.177	0.176
Cu	0.235	0.398**	0.092	0.245	0.464**	0.633**	1	0.565**	0.512**
Pb	0.539**	0.208	−0.254	0.075	0.253	0.522**	0.358*	1	0.657**
Zn	0.545**	0.291	0.005	0.356*	0.614**	0.557**	0.596**	0.455**	1

N=45; ** Significance at 0.01 level; * Significance at 0.05 level

□Represents the coefficients in cool season; □ Represents the coefficients under salinity stress in cool season

Discussion

Cultivar difference in multiple heavy metal uptakes and accumulation

A nearly two-fold variation range of the heavy metal concentrations (i.e., Cd, Cr, Cu, Pb, and Zn) was observed among the cultivars of water spinach. The strong positive correlations between the nutritive elements (i.e., K, Na, Ca, Mg, and Zn) and heavy metals (i.e., Cd, Cr, Cu, and Pb) suggested that these heavy metals are transported together with the nutritive metals in the plant of water spinach (Solti et al. 2011). Ion transportation across the membrane is the primary approach by which metals enter a plant cell, and ion channels are the most important regulatory mechanism (White 2000; Yoshihara et al. 2010). K and Ca channels are still acknowledged as the main transportation routes of major metals in plants until now. Na can traverse root-cell plasma membrane both through K and Ca channels (Mei et al. 2014; Roberts and Tester 1997; White et al. 2002). Ca channels have weak selectivity and allow the influx of divalent cations such as Mg^{2+} (Li and Cheng 2007). Consequently, Ca channels may be employed by heavy metals to pass through the plasma membrane. The strong inhibition of Na, Cd, and Cr uptake by La^{3+} validated that Na, Cd, and Cr could enter root cells of water spinach through the Ca channels in the plasma membrane, and channel selectivity was created by their affinity for specific metals (Moyen and Roblin 1997). This condition resulted in a close correlation between Ca and the two metal (i.e., Cd and Cr) concentrations in the shoots of different cultivars (Table 3).

Apart from the ion channel, transporter is another important mechanism of metal entry into plant cells across the cytoplasmic membrane. A strong positive correlation was also found among Zn and other metals (i.e., Cd, Cr, Cu, and Pb). Cd uptake in plants also

Table 6 Inhibition of metal uptake in water spinach roots by $LaCl_3$ (means±SD, *n*=3)

Cultivar		Na	Ca	Mg	Cd	Cr	Cu	Pb	Zn
#1	Control (mg/kg, FW)	1458±31a	663±30a	210±17a	2.83±0.44a	25.27±2.44a	7.00±1.52a	6.62±1.25a	50.72±11.27a
	Treatment (mg/kg, FW)	1251±40b	551±36b	171±20a	1.19±0.38b	4.68±0.84b	5.49±0.42a	4.96±0.42a	40.50±6.68a
	Decrease (%)	14.20	16.93	18.59	57.94	81.50	21.56	25.14	20.15
#15	Control (mg/kg, FW)	1549±29a	626±144a	211±14a	1.94±0.39a	49.09±7.54a	4.79±0.57a	9.19±1.94a	44.57±13.12a
	Treatment (mg/kg, FW)	1289±115b	488±37b	171±9b	1.06±0.15b	6.37±0.70b	4.11±0.61a	6.44±2.95a	36.58±2.31a
	Decrease (%)	16.79	22.03	18.87	45.56	87.02	14.32	30.00	21.84

Table 7 Concentrations of heavy metals and exudates in the rhizosphere solution of cultivars grown in warm season

	Cultivar	Cd (μg/kg)	Cr (μg/kg)	Cu (μg/kg)	Pb (μ/kg)	Zn (mg/kg)	LMWOA (mg/kg)	DOC (mg/kg)
Low accumulation	#4	2.24±0.08	7.49±1.15	31.36±0.45	12.22±1.59	2.58±0.40	53.75	75.28
	#9	2.54±0.05	20.98±7.06	61.55±3.17	23.24±1.00	3.78±0.60	64.67	98.33
	#13	2.80±0.10	12.13±1.95	58.80±3.24	10.83±1.74	2.65±0.13	66.28	82.61
	Average	2.53	13.53	50.57	15.43	3.00	61.57	85.41
High accumulation	#1	4.18±0.09	19.54±1.10	58.53±2.39	39.54±2.13	4.96±0.34	69.86	82.21
	#8	4.27±0.13	35.41±0.31	102.29±0.44	46.87±4.29	4.33±0.38	80.44	107.03
	#12	3.94±0.12	27.93±0.50	96.96±0.87	45.31±1.95	5.39±0.39	76.18	100.27
	Average	4.13	27.63	85.93	43.91	4.89	75.49	96.50
Fold		1.63	2.04	1.70	2.85	1.63	1.23	1.13

The concentrations refer to the mass of metals and exudates per 1 kg of dry soil. Data are means±SD (n=3)

occurred partly through the Fe and Zn pathways because they had similar electron configurations, as well as chemical and physical properties (Yoshihara et al. 2010). Recent molecular studies have identified a number of gene families involved in heavy metal uptake, transport, and homeostasis within plants, including zinc-regulated transporters and iron-regulated transporter-like protein (ZIP) family, natural resistance-associated macrophage protein (Nramp) family, and heavy metal P-type ATPases (Pedas et al. 2009). ZIP proteins from plants are capable of transporting Zn^{2+}, Fe^{3+}/Fe^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, and Cd^{2+}. Nramp transports a broad range of divalent metals, including Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, and Pb^{2+} (Nevo and Nelson 2006). P_{1B} ATPases have a fundamental role in the homeostasis and biotolerance of transition metal ions and exhibit two substrate specificities: either Cu^{2+}/Ag^{2+} or $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ (Axelsen and Palmgren 2001).

Therefore, high-Ca cultivars exhibit higher heavy metal (Cd, Cr, Cu, Pb, and Zn) uptake capacity than low-Ca cultivars (Table 4). The cultivar differences in heavy metal accumulation may partly depend on the abundance and activity variations of Ca channels and Zn transporters among different water spinach cultivars. However, the differences in the Ca channels and Zn transporters among the cultivars of water spinach require further research at the molecular level.

Heavy metal uptake and accumulation by plants depend not only on the transport capacity of plant, but also on the available metal concentration in the rhizosphere soil. The cultivar difference in the heavy metal (i.e., Cd, Cr, Cu, Pb, and Zn) concentrations of water spinach shoots was also positively associated with the variation of dissolved heavy metal concentrations in the rhizosphere of different cultivars. This difference was ascribed to root-induced exudates, including LMWOA, sugar, amino acid, and other dissolved organic matters. The cultivars with higher concentrations of LMWOA and DOC had high concentration of heavy metal in the rhizosphere solution. These soluble organic matters could stimulate growth of rhizosphere microorganisms. The increasing microorganisms influenced heavy metal speciation and solubility through biosorption and biotransformation, and increased the DOC concentration in the rhizosphere by microbial extracellular secretion (De Maria et al. 2011). In various rhizosphere exudates, LMWOA had strong heavy metal activation effect by acidifying soil and directly chelating metal (Dessureault-Rompre et al. 2008; Evangelou et al. 2006). Hence, LMWOA was believed to serve a vital function in heavy metal dissociation from the solid phase and diffusion from the bulk soil to the root surface. The concentration of detected LMWOA is presented in Table SM-2 (Supplementary materials), including acetic, malic, tartaric, maleic, oxalic, citric, succinic, fumaric, formic, and propionic acid. The main components of LMWOA were acetic, tartaric, and maleic, and maleic acid mainly contributed to the variation of the total LMWOA. The variation of heavy metal concentrations in the rhizosphere solution is in the order Pb>Cr>Cu>Cd=Zn (Table 7), indicating that LMWOA had a different degree of activation on each metal. However, the adsorption-desorption of heavy metals in soil was a complex ion competition process of multiple metals, and the present experiment design did not allow us to evaluate the link between the specific metal and organic acid. The experiment used long-term contaminated soil to approach a realistic scenario. Heavy metal activation by organic acids in aged soil is greater than that in spiked soil.

In conclusion, variations of heavy metal accumulation in water spinach mainly contributed to the cultivar differences in the Ca and Zn uptake characteristics, as well as the LMWOA in the rhizosphere. This work suggests the following points: (1) cultivars with low heavy metal accumulation also have low uptake of nutrient elements such as Ca and Zn; (2) heavy metals sharing the same transportation system can be minimized simultaneously through cultivar screening.

Effect of temperature and soil salinity on variation of heavy metal uptakes among different cultivars

Cool season did not interfere with the regular growth of water spinach. However, transpiration rate of plant in cool season is far lower than that in warm season (Li et al. 2003). Transpiration can affect heavy metal uptake by controlling water flow in xylem (Liu et al. 2010). Therefore, all the heavy metal concentrations in shoots were far below those in the warm season. Clear variations in heavy metal (i.e., Cd, Cr, Cu, Pb, and Zn) concentrations still exist in the shoots of the different cultivars of water spinach; however, the ranks of the cultivars and cross-correlations among metals significantly changed in cool season. This change was partly attributed to differential decrease of transpiration among the cultivars (Liu et al. 2010). In addition, the plasma membrane and osmotic adjustable substances of plant cells must undergo changes to tolerate cool temperature (Bai 2009). Cultivars differed in response to cool temperature, thus resulting in different degrees of variations in the plasma membrane. The variations in the membrane permeability and transpiration led to a differential alteration of metals transportation among different cultivars.

Soil salinity affects heavy metal availability to plant, as well as physiological and biochemical processes, such as plasma membrane permeability and transpiration in plants. This effect is closely related to the uptake and translocation of heavy metals in crops (Mei et al. 2014). Shoot Cd concentration in all cultivars increased because Cd is more easily mobilized and transferred from saline soil to the plants than other heavy metals under salinity stress (Acosta et al. 2011; Li et al. 2012). The cultivar difference in heavy metal concentrations slightly changed, but the correlations were significantly altered. The average content of Na increased from 1761.8 mg/kg to 2932.2 mg/kg (control in cool season), and the value of Ca decreased from 1712.8 mg/kg down to 641.6 mg/kg. These results indicate that Ca channels were occupied by large quantities of Na (Li and Cheng 2007). Our previous research shows that Na can compete with Ca for Ca channels (Mei et al. 2014). Thus, more heavy metals could only cross root-cell membrane via Zn or Fe transporters (Solti et al. 2011), which led to stronger positive correlations between Zn and other heavy metals (i.e., Cd, Cr, Cu, and Pb). Changes also occurred in the rhizosphere exudates and microorganisms, the root cell walls and plasma membrane, and transpiration in response to salt stress, resulting in a variation of the uptake mechanisms of metals (Ashraf and Bashir 2003; Galvez et al. 2012; Nandwal et al. 2000). Salt-sensitive and salt-resistant genotypes had different responses to salinity, which led to variations in the cultivar differences in heavy metal accumulation in water spinach. Temperature and soil salinity clearly changed the cultivar differences in the combined heavy metal accumulation by water spinach. Both factors should be considered in screening cultivars with low heavy metal accumulation. However, the limited available cultivars indicate that a possibly larger cultivar difference may be found through selection in more cultivars. We can thus attain consistent cultivars with low heavy metal accumulation across environmental stresses.

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