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# Responses of different water spinach cultivars and their hybrid to Cd, Pb and Cd–Pb exposures

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ABSTRACT

A pot experiment was conducted to investigate the stability of Cd and/or Pb accumulation in shoot of Cd and Pb pollution-safe cultivars (PSCs), the hereditary pattern of shoot Cd accumulation, and the transfer potentials of Cd and Pb in water spinach (*Ipomoea aquatica* Forsk.). A typical Cd-PSC, a typical non-Cd-PSC (Cd accumulative cultivar), a hybrid from the former two cultivars, and two typical Cd + Pb-PSCs were grown in seven soils with different concentrations of Cd and Pb. The results showed that concentrations of Cd and Pb in shoot of the PSCs were always lower than the non-PSC and the highest Cd and Pb transfer factors were also always observed in the non-PSC, indicating the stability of the PSCs in Cd and Pb accumulation. Shoot Cd concentration seemed to be controlled by high Cd dominant gene(s) and thus crossbreeding might not minimize Cd accumulation in water spinach. Interaction between Cd and Pb in soils affected the accumulations of the metals in shoot of water spinach. Under middle Cd and Pb treatments, the presence of higher Pb promoted the accumulation of Cd. However, under high Pb treatment, accumulations of Cd and Pb were both restricted.

## 1. Introduction

Currently, large areas of agricultural soil have been contaminated with heavy metals as a result of mining and smelting activities, wastewater irrigation, electroplating, and fertilization [1,2]. In China, about one fifth of farmland is contaminated by cadmium (Cd), arsenic (As), and lead (Pb) [3]. It is further known that heavy metal may pose a risk to human health through the food chain [4].

In spite of various technologies for remediation of soils contaminated with toxic heavy metals, it is difficult to put them into practice in polluted farmland in many developing countries because of the high demand for food and the high cost and slow process of remediation [5–7]. Therefore, some researchers tried to find a way to reduce the risk of pollutants entering the human diet from soils via agricultural products. Selection or breeding of crop cultivars like pollution-safe cultivars (PSCs), that is, the cultivars whose edible parts accumulate specific pollutants at a low enough level for safe consumption when grown in contaminated soil, has been investigated as a practical method of minimizing the concentrations of heavy metals in crops [4,8,9]. The PSC strategy is based on the fact that genotypic variation of edible parts accumulating pollutants is large enough at cultivar level. Furthermore, the stability of PSC accumulating pollutants decides the feasibility of PSC strategy.

Differences in the accumulation of Cd or Pb among cultivars have been investigated in rice (*Oryza sativa* L.) [8,10,11], wheat (*Triticum aestivum* L.) [12], peanut (*Arachis hypogaea* L.) [13], asparagus bean (*Vigna unguiculata* subsp. *sesquipedalis* L.) [9], leaf lettuce (*Lactuca sativa* L. var. *crispa*) [14], potato (*Solanum tuberosum* L.) [15], maize (*Zea mays* L.) [16], sunflower (*Helianthus annuus* L.) [17] and so on. However, in reality, Cd and Pb contaminants often co-exist in the fields. For example, a vegetable farm near a smelter in Nanning, Guangxi, China, was contaminated with Cd and Pb [18]. Therefore, an applicable PSC should be able to simultaneously minimize the accumulation of Cd and Pb, i.e. Cd + Pb-PSC, to effectively fight against Cd and Pb-contaminated soils. There was a report that Cd-PSCs of asparagus bean were simultaneously Pb-PSCs or Zn-PSCs when exposed in co-existence of Cd, Pb, and Zn contamination [9].

Genotype dependence of Cd or Pb accumulation at cultivar level has been reported in rice [8,11], wheat [12], maize [16], legume crops [9,19], leafy vegetables [14,19], and sunflower [17]. However, the hereditary patterns of heavy metal accumulation in most of the species are not yet investigated, and relevant information is quite limited.

Water spinach (*Ipomoea aquatica* Forsk.) is an important leafy vegetable in Southeast Asia, India, and southern China, but it is easily polluted by Hg, Cd, and Pb [20,21]. Some typical Cd-PSCs, non-Cd-PSC (Cd accumulative cultivar) [21], and Cd + Pb-PSCs were obtained in our previous studies by using local water spinach cul-

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**Table 1**
Properties of seven soils (dry weight basis).

| | Control | Middle Cd | High Cd | Middle Pb | High Pb | MCd + MPb | MCd + HPb |
|---|---|---|---|---|---|---|---|
| pH | 5.66 | 5.65 | 5.43 | 5.30 | 5.35 | 5.37 | 5.51 |
| Organic matter (%) | 1.18 | 1.29 | 1.15 | 1.07 | 1.26 | 1.08 | 1.28 |
| Total N ($\mathrm{g\,kg^{-1}}$) | 1.18 | 0.96 | 1.07 | 1.24 | 1.12 | 0.98 | 1.04 |
| Available P ($\mathrm{mg\,kg^{-1}}$) | 121.0 | 124.7 | 121.9 | 133.3 | 116.7 | 123.2 | 130.3 |
| Available K ($\mathrm{mg\,kg^{-1}}$) | 67.2 | 66.6 | 69.2 | 69.5 | 67.6 | 64.7 | 72.1 |
| Total Pb ($\mathrm{mg\,kg^{-1}}$) | 31.7 | 30.6 | 31.6 | 85.4 | 287.0 | 92.8 | 327.6 |
| DTPA Pb ($\mathrm{mg\,kg^{-1}}$) | 4.5 | 4.1 | 4.6 | 25.2 | 83.6 | 27.6 | 87.8 |
| Total Cd ($\mathrm{mg\,kg^{-1}}$) | 0.17 | 0.61 | 1.26 | 0.18 | 0.17 | 0.58 | 0.67 |
| DTPA Cd ($\mathrm{mg\,kg^{-1}}$) | 0.08 | 0.34 | 0.86 | 0.10 | 0.10 | 0.32 | 0.39 |

*Notes*: MCd + MPb and MCd + HPb represent the treatment abbreviations of middle Cd + middle Pb and middle Cd + high Pb, respectively.

tivars of southern China, and a hybrid was created by crossing a typical Cd-PSC and a typical non-Cd-PSC.

In this study, four typical water spinach cultivars, including a Cd-PSC, a non-Cd-PSC, and two Cd + Pb-PSCs, and a hybrid were grown in seven soils with different concentrations of Cd and Pb. The aims were to compare the Cd and Pb accumulations among the tested genotypes, and to investigate the hereditary stability in shoot Cd accumulation of water spinach. We hypothesized that the previously identified non-Cd-PSC would accumulate more Cd and Pb than the previously identified Cd-PSC and Cd + Pb-PSCs under Cd–Pb combined exposures. It is also expected that the results will help to increase understanding of the hereditary pattern of shoot Cd accumulation in water spinach.

## 2. Materials and methods

### 2.1. Cultivars of water spinach

Four cultivars (cv) and a hybrid of water spinach were used in the present study. They were cv. Qiangkunluyeqinggu (cv. QLQ), cv. Taiwan 308 (cv. T308), cv. Youqing (cv. YQ), cv. Baigengjisi (cv. BGJ), and hybrid QLQ × T308 from cv. QLQ (female parent) × cv. T308 (male parent). In our previous studies, cv. QLQ, YQ, BGJ, and T308 accumulated Cd 0.08, 0.11, 0.12, and 0.27 $\mathrm{mg\,kg^{-1}}$ (fresh weight, FW), respectively, when grown in Cd-contaminated soil (0.59 $\mathrm{mg\,kg^{-1}}$ dry weight, DW). Cv. YQ and BGJ accumulated Pb 0.19 and 0.25 $\mathrm{mg\,kg^{-1}}$ (FW), respectively, when grown in Pb-contaminated soil (111.4 $\mathrm{mg\,kg^{-1}}$, DW). Therefore, cv. QLQ, YQ, BGJ, and T308 were, respectively, identified typical Cd-PSC, Cd + Pb-PSC, Cd + Pb-PSC, and non-Cd-PSC according the definition of PSC.

### 2.2. Experimental site and soil

The experiment was conducted in an experimental garden of Heshan Institute of Agricultural Science (112°59′E, 22°42′N), Guangdong province, China.

The experimental soil was collected from a vegetable farm of the institute, and was air-dried, ground to pass through a 5 mm sieve. The soil was used as the control. Six treatments, including middle Cd, high Cd, middle Pb, high Pb, middle Cd + middle Pb (MCd + MPb), and middle Cd + high Pb (MCd + HPb), were conducted. The Cd concentrations of the soils were adjusted by mixing the control soil and a Cd-contaminated soil containing Cd for 69.8 $\mathrm{mg\,kg^{-1}}$ (DW) [8]. The Pb concentrations were adjusted by adding lead acetate solution (3.0 $\mathrm{g\,Pb\,L^{-1}}$) to the control soil. Cd and Pb concentrations in the control soil are shown in Table 1. The proportions of the control soil and the contaminated soil were 1000: 6.4 for the middle Cd soils and 1000: 15.0 for the high Cd soil, respectively. The middle Pb and the high Pb soils were obtained by adding the lead acetate solution into the control soil for 20 and 90 $\mathrm{mL\,kg^{-1}}$, respectively. Each of the six soils were thoroughly mixed in a large basin and then watered and left to balance outdoors under a waterproof tarpaulin for two months before the soil properties were analyzed (Table 1).

Soil pH was determined by a pH meter (PHS-3C, Shanghai, China) in a soil to water ratio of 1:2.5 [22]. Organic matter content was determined by wet digestion following the method of Nelson and Sommers [23]. Total N was determined by titration of distillates after Kjeldahl sample preparation and analysis [24]. Available P was determined by molybdenum blue colorimetry [25]. Available K [22] was determined using an flame atomic absorption spectrophotometer (Hitachi Z-2300, Japan). Total and extractable concentrations of Cd and Pb were determined by flame atomic absorption spectrophotometry following mixed acid digestion ($HNO_3$–$HClO_4$–HF) [26] and DTPA extraction, respectively.

According to the Farmland Environmental Quality Evaluation Standards for Edible Agricultural Products (HJ 332-2006), the maximum levels (MLs) for Cd and Pb are 0.3 and 50 $\mathrm{mg\,kg^{-1}}$ (DW), respectively, all the soils except the control were contaminated by Cd and/or Pb in different degrees. Other properties, including pH value, organic matter content, total N, available P, and available K, were similar among all the tested soils.

### 2.3. Experimental design

The pot experiment was conducted in a greenhouse with air temperature of 28–35 °C. Plastic pots, with 18 cm (top) and 13 cm (bottom) diameters and 15 cm height, were each filled with 2.5 kg (DW) of the prepared soil. For each treatment, three pots ($n = 3$) were planted for each of the five cultivars. Eight seeds per pot were sown into the soil on March 20, 2008. The experiment was laid out as a completely randomized design. After germination, seedlings were thinned to 4 plants $\mathrm{pot^{-1}}$ left in a week and watered with tap water daily. The solid compound fertilizer (N:P:K = 15:15:15) was applied into the soils for 3.0 g $\mathrm{pot^{-1}}$ in the 15th day after germination.

### 2.4. Sampling and chemical analysis

Only the shoots (including stems and leaves) were sampled as the work focused on the soil–plant–human pathway of heavy metals. The first and the second harvest were carried out in the 30th and 60th day after germination, respectively. All the samples were thoroughly washed with de-ionized water with the fresh weights measured, dried at 105 °C for 20 min and then at 70 °C to the constant weigh. The dried plant samples were crushed to pass through a 0.149 mm sieve for chemical analysis after their dry weights were weighed.

The Cd and Pb concentrations of the dry samples were determined by flame atomic absorption spectrophotometry after digestion with $HNO_3$:$H_2O_2$ (10:3) 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,

**Table 2**

Shoot biomasses (dry weight, g) of different water spinach cultivars (mean ± SE, $n = 3$).

| Treatment | QLQ | T308 | QLQ × T308 | YQ | BGJ |
|---|---|---|---|---|---|
| Control | 2.02 ± 0.09 | 2.87 ± 0.38 | 3.42 ± 0.21 | 1.97 ± 0.13 | 2.35 ± 0.18 |
| Middle Cd | 1.62 ± 0.11 | 3.13 ± 0.12 | 2.86 ± 0.21 | 2.30 ± 0.13 | 3.05 ± 0.08 |
| High Cd | 1.14 ± 0.12 | 1.69 ± 0.21 | 2.23 ± 0.06 | 1.64 ± 0.25 | 1.81 ± 0.07 |
| Middle Pb | 1.69 ± 0.18 | 2.67 ± 0.18 | 2.60 ± 0.10 | 2.11 ± 0.21 | 3.06 ± 0.10 |
| High Pb | 1.98 ± 0.07 | 2.65 ± 0.05 | 2.52 ± 0.21 | 2.44 ± 0.15 | 2.51 ± 0.14 |
| MCd + MPb | 2.37 ± 0.05 | 2.61 ± 0.17 | 3.59 ± 0.16 | 1.95 ± 0.09 | 2.44 ± 0.10 |
| MCd + HPb | 1.58 ± 0.07 | 3.53 ± 0.12 | 2.76 ± 0.18 | 1.91 ± 0.15 | 2.92 ± 0.11 |

*Notes*: QLQ, T308, QLQ × T308, YQ, and BGJ represent the abbreviations of cv. Qiangkunluyeqinggu, cv. Taiwan 308, hybrid QLQ × T308, cv. Youqing, and cv. Baigengjisi, respectively. MCd + MPb and MCd + HPb represent the treatment abbreviations of middle Cd + middle Pb and middle Cd + high Pb, respectively.

China) was used for quality control (QC) in Cd and Pb analytical procedures of water spinach tissues.

### 2.5. *Safety standard and statistical methods*

According to the General Standard for Contaminants and Toxins in Foods (Codex Standard 193-1995, Revision 4, 2008, http://www.codexalimentarius.net/web/more_info.jsp?id_sta=17), the Codex MLs in leafy vegetables for Cd and Pb were 0.2 and 0.3 mg kg$^{-1}$ (FW), respectively. The standard was used to evaluate the safety of consuming the tested water spinach cultivars.

To compare the relative response of cultivars to heavy metal exposures, we calculated the index of biomass response to stress (BRS) [9] as follows:

$$\text{BRS}\ (\%) = \frac{B_{\text{exposure}} - B_{\text{control}}}{B_{\text{control}}} \times 100$$

where $B_{\text{exposure}}$ and $B_{\text{control}}$ are the shoot biomass (DW) (including the first and second harvests) under the heavy metal exposures and the control, respectively.

To evaluate the transfer potential of Cd and Pb from soil to plant, the transfer factor (TF) [27] was calculated as follows:

$$\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{soil}}}$$

where $C_{\text{shoot}}$ is the average Cd or Pb concentration (DW) of the first and the second harvest of each cultivar, and $C_{\text{soil}}$ is the total Cd or Pb concentration in corresponding soil.

Data were statistically processed on a computer using the Excel 2003 and SPSS11.0. Data were analyzed by independent samples $t$ test and two-way ANOVA with the least significant difference (LSD) test. All the correlations were assessed using Pearson product–moment correlation.

## 3. Results

### 3.1. *Biomass response to heavy metal stress*

Shoot biomass (dry weight) under heavy metal exposures varied with the cultivars and treatments (Table 2). According to the results of biomass response to stress (BRS) (Fig. 1), cv. QLQ and hybrid QLQ × T308 had negative BRSs under middle Cd, but only the biomass difference of cv. QLQ between middle Cd and control was significant ($p < 0.05$). Biomass of cv. BGJ was significantly higher under middle Cd than under control ($p < 0.05$). However, all the cultivars had negative BRSs under high Cd, and the differences of biomasses between high Cd and control were significant ($p < 0.05$) except cv. YQ, indicating that cv. YQ was more tolerant to single Cd stress than other cultivars. The tolerance to Cd of hybrid QLQ × T308 was overall between its parents. Only biomasses of hybrid QLQ × T308 were always significantly lower under middle Pb and high Pb than under control ($p < 0.05$), indicating that hybrid QLQ × T308 had the lowest Pb tolerance. Under Cd and Pb co-exposures, only the biomass of cv. QLQ significantly increased ($p < 0.05$) under MCd + MPb (middle Cd + middle Pb treatment), but significantly decreased ($p < 0.05$) under MCd + HPb (middle Cd + high Pb treatment), while biomasses of other cultivars had no significant change, suggesting that cv. QLQ was sensitive, and other cultivars were not sensitive to the co-exposures.

### 3.2. *Cd and Pb accumulation*

For the two harvests, shoot Cd concentrations (fresh weight, FW) of all the cultivars under control were lower than the Codex ML for Cd (0.2 mg kg$^{-1}$). While under all the Cd exposures, including the co-exposures, the Cd concentrations were higher than the Codex ML for Cd (Fig. 2). In terms of the first harvest, the Cd concentrations (dry weight, DW) under high Cd were 1.4–2.2 and 8.4–12.7 times higher than those under middle Cd and control ($p < 0.05$), respectively. The differences of shoot Cd concentrations were much greater than the differences of soil total Cd concentrations between high Cd and middle Cd, high Cd and control (1.0 and 6.4 times higher, respectively) but close to the differences of DTPA-extractable Cd (1.5 and 9.8 times higher, respectively). By comparing the Cd concentrations (DW) in all the cultivars among the three treatments (middle Cd, MCd + MPb, and MCd + HPb), it was found that they were significantly enhanced ($p < 0.05$) by the presence of middle Pb but were significantly impeded ($p < 0.05$) by high Pb.

The Cd concentrations of cv. QLQ, the previously identified Cd-PSC, were always significantly lower than those of cv. T308, the previously identified non-Cd-PSC, under all Cd exposures ($p < 0.05$). The Cd concentrations of hybrid QLQ × T308 were always between its parents but closer to cv. T308 except under control, indicating that heterosis might be absent in Cd accumulation, that is the hybrid cannot accumulate more (or less) Cd than its parents, and shoot Cd concentration might be controlled by a single or plural gene(s) with high Cd dominance. The Cd concentrations of the two previously identified Cd + Pb-PSCs were similar in most cases and those of cv. YQ under high Cd and MCd + HPb were even significantly lower than those of cv. QLQ and BGJ ($p < 0.05$). The characteristic of low Cd accumulation in previously identified Cd-PSC and Cd + Pb-PSCs was thus proved to be stable.

Shoot Pb concentrations (FW) of all the cultivars under control were lower than the Codex ML for Pb (0.3 mg kg$^{-1}$) in both harvests (Fig. 3). While the Pb concentrations under high Pb and MCd + HPb were higher than the Codex ML for Pb. Under middle Pb and MCd + MPb, only the Pb concentrations of the previously identified Cd-PSC and Cd + Pb-PSCs measured up to the standard (Fig. 3), indicating that cv. QLQ could also be treated as a Pb-PSC. The shoot Pb concentrations (DW) under high Pb were 8.0–10.7 and 3.0–4.2 times higher than those under control and middle Pb ($p < 0.05$), respectively. The differences were similar to those of soil total Pb concentrations (8.1 and 2.4 times higher, respectively) and also similar to the difference of soil DTPA-extractable Pb between high Pb and middle Pb (2.3 times higher) but much

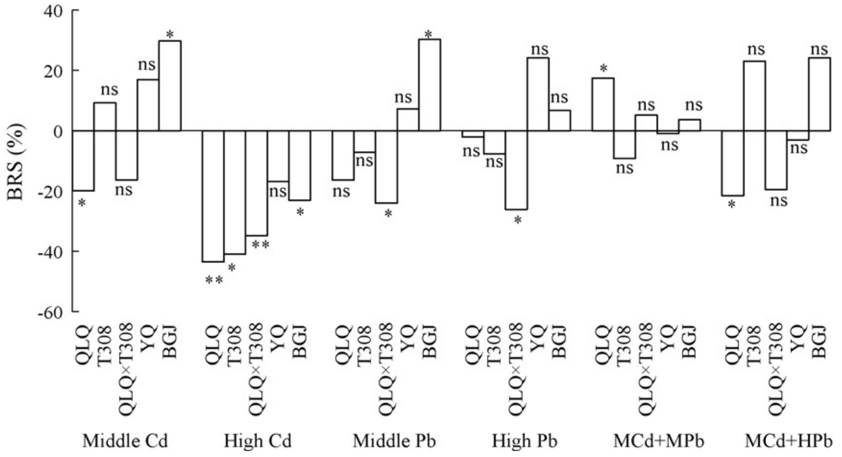

**Fig. 1.** The biomass response to stress (BRS) of five cultivars. BRS (%) = $(B_{\text{exposure}} - B_{\text{control}})/B_{\text{control}} \times 100$, where $B_{\text{exposure}}$ and $B_{\text{control}}$ are the shoot biomass (DW) (including the first and second harvests) under the heavy metal exposures and the control, respectively. ns, * and ** mean that the differences of the shoot biomass between the control and the heavy metal exposure were not significant, significant at $p < 0.05$ level, and significant at $p < 0.01$ level, respectively, as determined by the independent samples t test. QLQ, T308, QLQ × T308, YQ, and BGJ represent the abbreviations of cv. Qiangkunluyeqinggu, cv. Taiwan 308, hybrid QLQ × T308, cv. Youqing, and cv. Baigengjisi, respectively. MCd + MPb and MCd + HPb represent the treatment abbreviations of middle Cd + middle Pb and middle Cd + high Pb, respectively. The abbreviations are the same as below.

**Table 3**
Two-way ANOVA results of cultivar, treatment and cultivar × treatment effects for shoot Cd and Pb concentrations (dry weight) and transfer factors of water spinach.

| Source of variance | Cd | | | Pb | | |
|---|---|---|---|---|---|---|
| | SS | MS | F | SS | MS | F |
| First harvest | | | | | | |
| Cultivar | 148.6 | 37.12 | 58.8* | 62.3 | 15.57 | 38.8* |
| Treatment | 2224.6 | 556.15 | 880.5* | 1575.1 | 393.78 | 980.6* |
| Cultivar × treatment | 115.2 | 7.20 | 11.4* | 51.9 | 3.24 | 8.1* |
| Error | 31.6 | 0.63 | | 20.1 | 0.40 | |
| Total | 7078.6 | | | 4344.1 | | |
| Second harvest | | | | | | |
| Cultivar | 183.4 | 45.84 | 147.5* | 81.7 | 20.42 | 37.7* |
| Treatment | 2126.7 | 531.69 | 1710.3* | 1384.7 | 346.17 | 639.8* |
| Cultivar × treatment | 206.8 | 12.92 | 41.6* | 82.9 | 5.18 | 9.6* |
| Error | 15.5 | 0.31 | | 27.1 | 0.54 | |
| Total | 6286.4 | | | 3850.1 | | |
| Transfer factor | | | | | | |
| Cultivar | 283.3 | 70.82 | 143.3* | 2.1E−03 | 5.27E−04 | 56.6* |
| Treatment | 686.9 | 171.71 | 347.4* | 4.0E−03 | 9.99E−04 | 107.4* |
| Cultivar × treatment | 94.0 | 5.87 | 11.9* | 4.8E−04 | 3.00E−05 | 3.2* |
| Error | 24.7 | 0.49 | | 4.7E−04 | 9.30E−06 | |
| Total | 9479.9 | | | 0.1 | | |

* Significant at $p < 0.01$ level.

smaller than that of soil DTPA-extractable Pb between high Pb and control (17.6 times higher). Shoot Pb concentrations of all the cultivars under MCd + MPb were overall similar to those under middle Pb ($p > 0.05$), indicating that the presence of middle Cd did not markedly affect Pb accumulation in shoot under middle Pb exposures. However, under high Pb exposures, the presence of middle Cd significantly decreased Pb accumulations in all the cultivars ($p < 0.05$), suggesting that the interaction between middle Cd and high Pb reduced not only Cd but also Pb accumulation in water spinach.

The Pb concentrations of cv. QLQ, YQ and BGJ were significantly lower than those of cv. T308 ($p < 0.05$) in almost all cases, except cv. QLQ under middle Pb in the first harvest and cv. BGJ under control in the second harvest ($p > 0.05$). It suggested that the characteristic of low Pb accumulation in previously identified Cd + Pb-PSCs was stable and the non-Cd-PSC might also be a non-Pb-PSC (Pb accumulative cultivar). The differences of Pb concentrations between cv. T308 and hybrid QLQ × T308 in most cases were not significant ($p > 0.05$), implying that hybridization between cv. QLQ and cv. T308 did not fundamentally alter Pb accumulation in the hybrid, which was probably because the parents were not the cultivars typical enough in Pb accumulation.

The results of two-way ANOVA showed that shoot Cd and Pb concentrations (dry weight, DW) were significantly affected by cultivar, treatment (exposure level), and interaction between the two factors ($p < 0.01$) (Table 3), indicating that accumulation of Cd and Pb in shoots of water spinach was determined not only by the exposure levels, but also by genetic factors. The contribution of each factor or interaction between factors to total variation (sum of square, SS) varied greatly with heavy metal. Thus, for shoot Cd and Pb concentrations, effect of exposure level was the largest, and effect of cultivar and interactive effect of cultivar and exposure level are similar.

Correlations of Cd and Pb (Fig. 4A and B) concentrations (DW) of all the cultivars between the first and second harvests were significant at $p < 0.001$ level. The results implied a certain stability

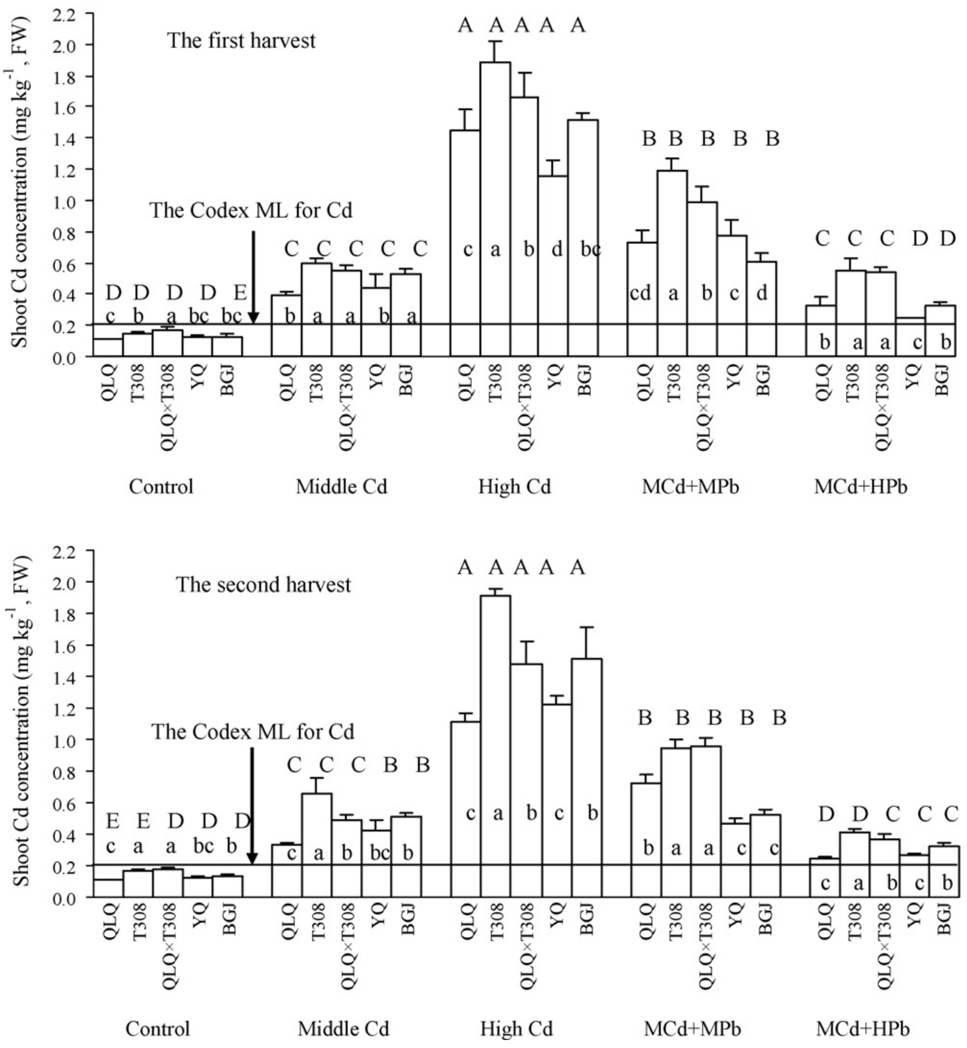

**Fig. 2.** Shoot Cd concentrations of the tested cultivars in the first and the second harvest in different soils. According to the least significant difference (LSD) test, different small letters indicate significant difference at $p < 0.05$ level between different cultivars in the same soil; different capital letters indicate significant difference at $p < 0.05$ level between different soils in the same cultivar; error bars represent SD ($n = 3$). The Codex ML for Cd, the Codex maximum level for Cd according to the Codex General Standard for Contaminants and Toxins in Foods.

and consistency of the genotypic response to different Cd and Pb exposures in terms of shoot Cd and Pb accumulations.

### *3.3. Transfer factors of Cd and Pb*

Overall, transfer factors (TFs) of Cd were 202–474 times higher than those of Pb (Fig. 5(A) and (B)), indicating that it is more difficult for Pb to transfer from soil to shoots of water spinach. In addition, transfer factors all significantly differed among cultivars ($p < 0.05$) and among heavy metal treatments ($p < 0.05$), and the TFs of the hybrid were between its parents (Fig. 5(A) and (B)).

The highest TFs of Cd were observed under MCd + MPb and high Cd, followed by middle Cd, control and MCd + HPb, and the TFs of Cd for each cultivar significantly differed among MCd + MPb, middle Cd, and MCd + HPb ($p < 0.05$) (Fig. 5(A)), suggesting that transfer potential of Cd increased with the increase of soil Cd concentration under single Cd exposures and TFs of Cd were elevated by the presence of middle Pb but depressed by the presence of high Pb.

High Pb treatment led to the highest TFs of Pb, followed by control, MCd + MPb, middle Pb and MCd + HPb, and the differences of TFs of Pb between high Pb and MCd + HPb were significant ($p < 0.05$) for all the cultivars (Fig. 5(B)), implying that transfer potential of Pb first decreased and then increased with the increase of soil Pb concentration under single Pb exposures and the presence of middle Cd lowered markedly transfer potential of Pb under high Pb exposures.

The results of two-way ANOVA showed that TFs of Cd and Pb were significantly affected by cultivar, treatment, and interaction between the two factors ($p < 0.01$) (Table 3), indicating that both genes and exposure levels decided Cd and Pb transfer potential. The contribution of each factor or interaction between factors to total variation changed greatly with heavy metal. Thus, for transfer potential of Cd and Pb, effect of exposure level was the largest, followed by genetic effect.

The TFs of Cd or Pb of all the cultivars were, respectively, correlated with their average shoot metal concentrations (FW) of the first and second harvests under similar exposures of the same metal (control, middle, and high exposures) (Fig. 6). The slopes of the linear regression equations are considered to be able to indicate the sensitivity of shoot metal accumulation responding to TF of the metal. The highest slopes were always together with high metal exposures, and the lowest ones always were in the control, demonstrating that the sensitivity increased with the elevation of the metal exposure levels.

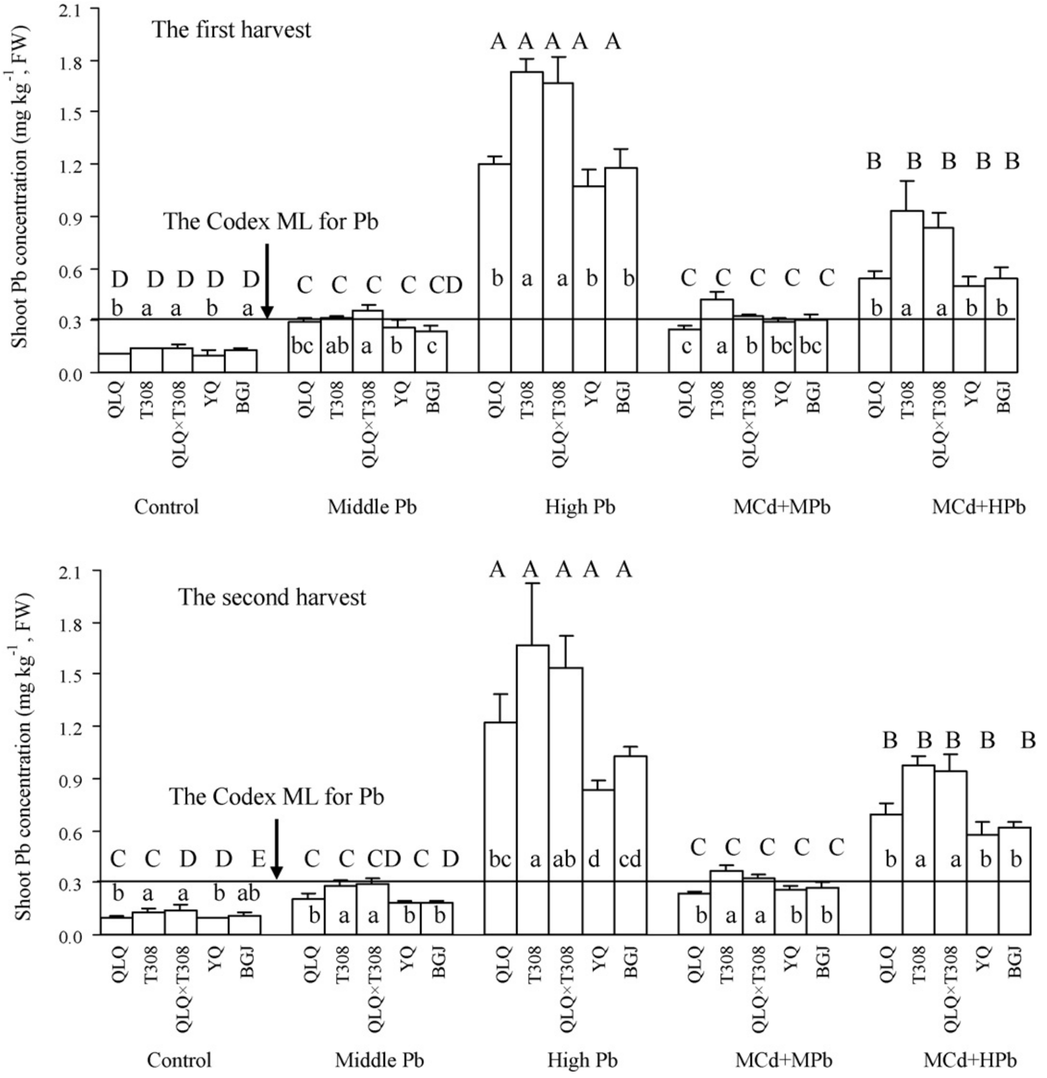

**Fig. 3.** Shoot Pb concentrations of the tested cultivars in the first and the second harvest in different soils. According to the LSD test, different small letters indicate significant difference at $p < 0.05$ level between different cultivars in the same soil; different capital letters indicate significant difference at $p < 0.05$ level between different soils in the same cultivar; error bars represent SD ($n = 3$). The Codex ML for Pb, the Codex maximum level for Pb according to the Codex General Standard for Contaminants and Toxins in Foods.

## 4. Discussion

The biomasses of all the cultivars were depressed under high Cd (1.26 mg kg$^{-1}$), but they were not significantly decreased under middle Cd (0.61 mg kg$^{-1}$) except cv. QLQ, which was treated as an adaptive response of plants to low heavy metal exposure [28,29]. The results suggest that water spinach has a middle Cd tolerance when compared to some other leafy vegetables [14]. However, growth of most water spinach cultivars responded insensitively to single Pb as well as Cd–Pb combined exposures even under

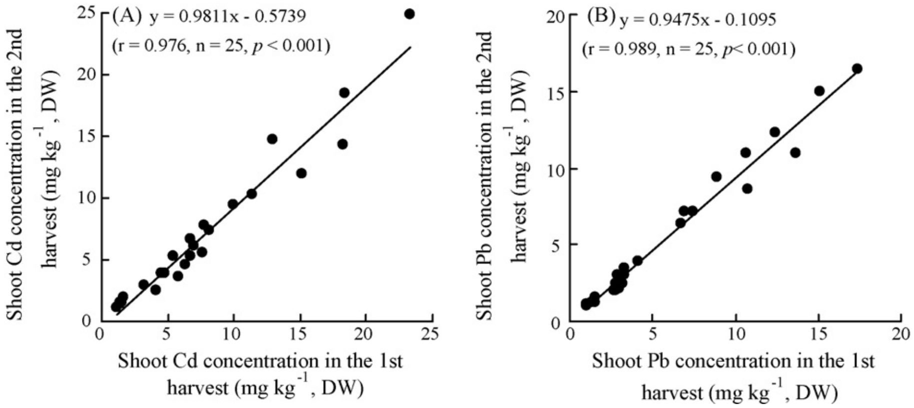

**Fig. 4.** Correlations of shoot Cd (A) and Pb (B) concentrations between the first and the second harvest.

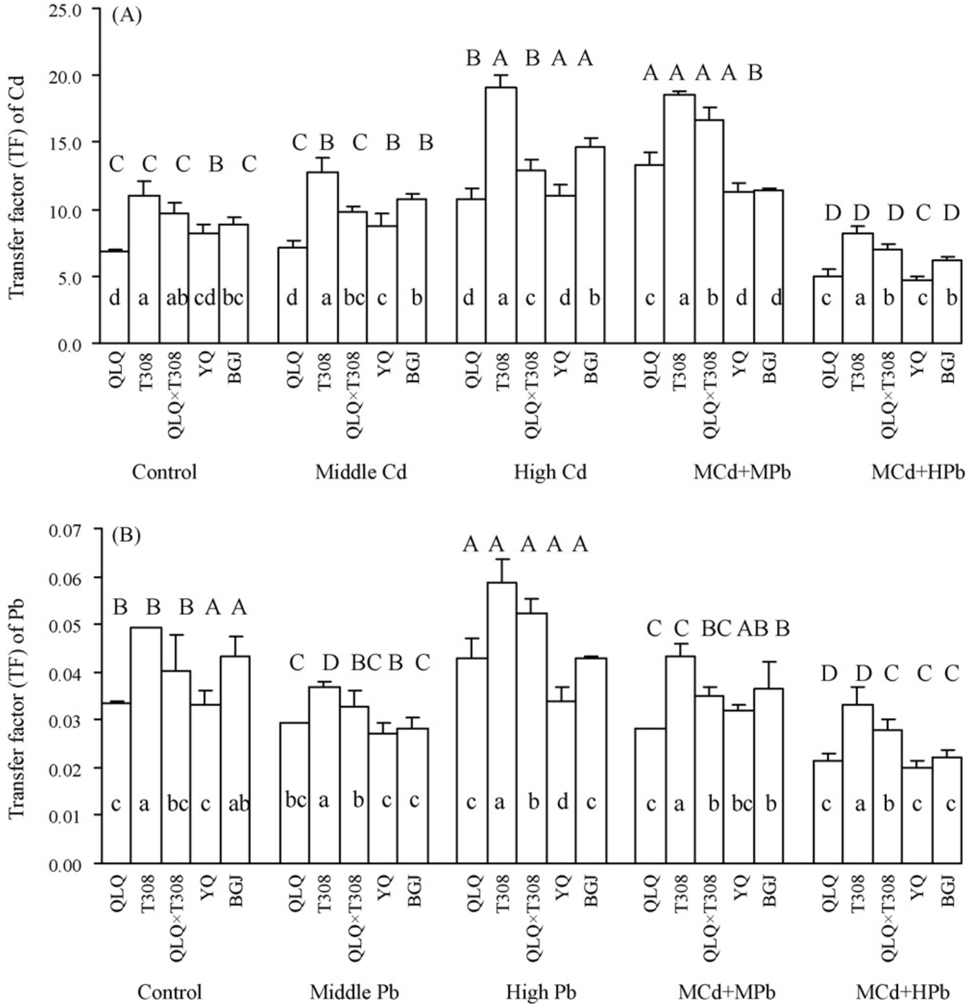

**Fig. 5.** Cd (A) and Pb (B) transfer factors of the tested cultivars in different soils. According to the LSD test, different small letters indicate significant difference at $p < 0.05$ level between different cultivars in the same soil; different capital letters indicate significant difference at $p < 0.05$ level between different soils in the same cultivar; error bars represent SD ($n = 3$).

high Pb exposures (287.0–327.6 mg kg$^{-1}$). Accordingly, farmers may not recognize the poisoned appearance when they are cropping water spinach in Cd and Pb-contaminated soils, which will increase the health risk from water spinach contaminated by Cd and Pb. Hybridization of the two typical cultivars in Cd accumulation did not obviously alter Cd tolerance of the hybrid, suggesting that no heterosis was available for Cd tolerance of water spinach. It was also found that the accumulation and tolerance of water spinach to Cd and Pb varied with cultivars but there was no necessary connection between them. For example, cv. YQ and cv. QLQ both had low Cd accumulation ability, but the former had the highest Cd tolerance and the latter had the lowest.

The present study verified the characteristics of low Cd and Pb accumulations of the previously identified Cd + Pb-PSCs, cv. YQ and BGJ, in Cd, Pb, and Cd–Pb-contaminated soils. The results were very valuable for selecting Cd + Pb-PSCs of leafy vegetables, but it was

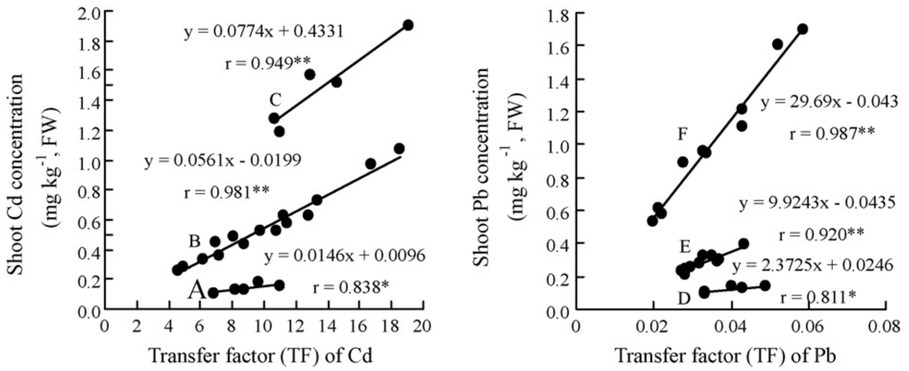

**Fig. 6.** Correlations between shoot Cd concentration and transfer factor of Cd (A–C), shoot Pb concentration and transfer factor of Pb (D–F). (A) and (D) Under control; (B) under middle Cd treatments (middle Cd, middle Cd + middle Pb, and middle Cd + high Pb); (C) under high Cd; (E) under middle Pb treatments (middle Pb and middle Cd + middle Pb); (F) under high Pb treatments (high Pb and middle Cd + high Pb); * and **, significant at $p < 0.05$ and at $p < 0.01$ levels.

lacking of investigation up to now. In addition, based on our experiment, the previously identified Cd-PSC (cv. QLQ) was also identified as a Pb-PSC, and low Cd accumulation in water spinach was probably together with low Pb accumulation at cultivar level. Thus, the hypothesis of this study is acceptable. Cv. YQ was the most impressive among the five cultivars, which could accumulate 1.4–2.3-fold less Cd and 1.4–2.0-fold less Pb than cv. T308 in the contaminated soils. The significant correlations of shoot Cd and Pb concentrations between the first and the second harvest illustrated the repeatability of metal accumulation patterns in these cultivars. Therefore, cv. YQ's use will contribute to reduce Cd and Pb entering food chain via water spinach products. However, water spinach is easily polluted by Cd and Pb. The two previously identified Cd + Pb-PSCs produced safe vegetable only under control and middle Pb. But no safe product was obtained for cv. T308 and hybrid QLQ × T308 except under control. The results were quite different from those reported by Wang [21] who found that 26 of 30 tested cultivars of water spinach measured up to the Codex standard when grown in a soil with Cd concentration as high as 0.59 mg $kg^{-1}$, similar to our middle Cd treatments (0.58–0.67 mg $kg^{-1}$). This can be explained by the differences of soil pH values between Wang's case (pH 7.05) and our case (pH 5.37–5.65), because it has been frequently reported that plant uptake of Cd is usually depressed by high soil pH [30,31]. Therefore, it is considered that the practice of PSC strategy is conditioned by soil conditions, not only quantities of contaminants, but also factors affecting plant uptake of contaminants. Strict monitoring is indispensable to ensure food safety, and soil improvement, such as pH regulation by liming, is recommended together with the use of PSCs. It was worth noting that the differences of shoot Pb concentrations between high Pb and control were much smaller than the difference of soil DTPA-extractable Pb. This may be explained by the previously reported "uptake plateau" phenomenon [32,33], that is, when soil DTPA-extractable Pb increases to a certain degree, the increase of Pb concentration in plant will be smaller with increasing soil DTPA-extractable Pb.

Interaction between Cd and Pb in soils influenced obviously accumulations of the metals in water spinach. The presence of middle Pb greatly increased Cd accumulation. A similar finding was reported by Lin et al. [34] that the presence of Pb enhanced the activity of Cd in soil and increased Cd accumulation in rice plant as a result. But the interaction of Cd and Pb in soil decreased accumulations of Cd and Pb in water spinach under MCd + HPb, which might be attributed to competitive absorption. It was reported that the decline of Cd uptake in lettuce was due to Pb antagonism and the increase of soil Cd concentration reduced Pb availability for binding and uptake at the root surface [35]. Interestingly, the great changes of Cd and Pb accumulations in water spinach depend on soil Pb level, which is worth further investigating.

There was no consistent trend between soil metal concentration and transfer factor (TF) of the metal. The variations of TFs of Cd and Pb in water spinach were related to cultivars and concentration combinations of Cd and Pb. The relationship between shoot metal concentration and TF of the metal was found to be in a very regular way under certain exposure level of the metal, and the correlative pattern was not affected by the presence of the alternative metal. It was also a valuable finding that metal (including Cd and Pb) exposure level decided the sensitivity of response of shoot metal concentration to TF of the metal.

Comparisons of Cd accumulations in hybrid QLQ × T308 and its parents assist us to understand preliminarily the hereditary pattern of Cd accumulation character in water spinach. Because shoot Cd concentrations of the hybrid are between its parents in most cases, it can conclude that there is no heterosis in Cd accumulation. There are no relevant reports about Cd accumulation heterosis in other leafy vegetables. It is considered that the phenomenon may be helpful for finding the hereditary law of Cd accumulation in leafy vegetables and further investigations for more species are thus valuable.

Shoot Cd concentration of the hybrid was closer to cv. T308 (Cd accumulative cultivar), so it was considered that the Cd concentration was probably controlled by a single or plural gene(s) with high Cd dominance. The result differed from what was reported in tomato by Kilchevsky et al. [36] who found that a general type of inheritance of Cd and Pb accumulations was an overdominance (negative heterosis) to the decrease of heavy metal accumulation in tomato fruits and the variation was controlled by one or two loci. In addition, there was also a report that grain Cd concentration of durum wheat was largely controlled by a single gene with low Cd dominance [37]. Maybe, water spinach shoots performed differently from tomato fruits and durum wheat grain. Therefore, it might not be a feasible way to minimize Cd accumulation in shoot of water spinach by the crossbreeding.

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