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Heavy Metal Accumulations of 24 Asparagus Bean Cultivars Grown in Soil Contaminated with Cd Alone and with Multiple Metals (Cd, Pb, and Zn)

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Crops grown in heavy metal contaminated soils are an important avenue for these toxic pollutants entering the human food chain. Information on how crops respond to soil contaminations of single versus multiple metals is scarce and much needed. This study investigated the accumulation of Cd by 24 cultivars of asparagus bean (*Vigna unguiculata* subsp. *Sesquipedalis* L., family Fabaceae) under a low level (0.8 mg kg^{-1}) and a high level (11.8 mg kg^{-1}) of Cd exposure in a garden experiment, and that in a field experiment with Cd, Pb, and Zn (1.2, 486, and 1114 mg kg^{-1}, respectively) contaminated soil. Both experiments showed that there were highly significant variations among the tested cultivars in Cd accumulation by roots, stems, leaves, and fruits of asparagus bean. In the garden experiment, all cultivars under the low Cd exposure and 41.7% of the tested cultivars under the high Cd exposure bore fruits (pods) whose Cd concentrations were lower than 0.05 mg kg^{-1} fw and therefore were safe for consumption. In addition, the fruit Cd concentrations of cultivars with black seed coats were significantly lower than those with red or spotted seed coats. These results suggest that asparagus bean is a hypo-accumulator to Cd pollutant and the trait of Cd accumulation is genetic-dependent among cultivars. In the field experiment, correlation between fruit Cd and Pb concentrations was significantly positive ($p < 0.05$). Additional correlation analyses between two experiments showed that fruit Cd concentrations in the field experiment were significantly correlated with those exposed to the high level of Cd stress, instead of to the low level of Cd stress in the garden experiment. This suggests that the presence of other toxic heavy metals in the soil might have facilitated the accumulation of Cd in fruits, and the selection of pollution-safe-cultivars (PSC) in multi-metal polluted condition could refer to the PSCs selected under a high level exposure of a single heavy metal.

KEYWORDS: **Heavy metals; *Vigna unguiculata*; cultivars; type; accumulation**

1. INTRODUCTION

Substantial increase in heavy metal contamination of agricultural soils is becoming a global problem accompanied with rapid industrial development, population expansion, and insufficiency of pollution controls. The area of contaminated farmland in China is estimated to reach up to 20 million hm^2 (*1*), and there is reportedly 314 750 hm^2 of farmland that has been contaminated by Cd in Japan (*2*). Cadmium is a toxic heavy metal that is readily taken up by growing plants from the contaminated soil (*3*−*5*). Consequently, it can easily enter the human food chain and cause acute and chronic illness (*6*). Although there are many studies on the uptake and accumulation of Cd pollutants by a number of crop species (e.g., *7*−*11*), most experiments were conducted with single-metal treatments. However, in the field, heavy metal contaminations are often compounded with multiple metals (*12*−*15*). We know very little about how toxic metals such as Cd affect plants in single- versus multi-metal pollution situations.

On the other hand, in recent years, great stride has been made over various techniques of remediation of contaminated soils. However, in many developing countries, it is difficult to practice them in farmland because the high cost and slow process of remediation often succumb to the high demand to produce foodstuff. In a recent study, a novel alternative strategy to reduce the risk of soil contaminants entering the human food chain without fallowing the land has been proposed: Pollution-safe cultivars (PSCs), that is, the cultivars in which edible parts

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accumulate certain pollutant at low enough level for safe consumption when grown in contaminated soil, were screened and explored among the cultivars of a major staple crop-paddy rice (*Oryza sativa* L.) when they were grown experimentally in Cd contaminated soil (*16*).

The concept of PSC is grounded on the basis of prior studies, which have shown that the uptake and accumulation of metal pollutants by plants not only differ among species, but also among cultivars. Intraspecific variation in Cd accumulation has been investigated in rice (*17*−*25*), wheat (*Triticum aestivum* L.) (*5*, *25*−*27*), maize (*Zea mays* L.) (*8*, *10*), soybean (*Glycine max* Merr.) (*7*, *11*), barley (*Hordeum vulgare* L.) (*28*), and potato (*Solanum tuberosum* L.) (*9*). In Australia, potato varieties with low or medium ability to uptake Cd are recommended for quality production (*29*). These studies laid the foundation of the PSC strategy for controlling Cd contamination in agricultural products.

Vegetables, among all food groups, are most vulnerable to soil Cd pollution. It is estimated that vegetables contributed to 83% of the total intake of Cd in human bodies (*3*). However, information on PSCs of vegetables is much less available than on staple crops. The only few attempts were made on Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *communis*) (*30*), pea (*Pisum sativum*) (*31*), and flowering Chinese cabbage (*Brassica parachinensis*) (*32*), none of which had explored the differential reactions of vegetable crops to single- versus multi-metal pollutions.

Asparagus bean (*Vigna unguiculata* subsp. *sesquipedalis* L.) is an important legume vegetable in the world, especially in China, Southeast Asia, and the West Indies (*33*). In China, the annual productions of asparagus bean in a regular year such as 2003 were 7.25 million tons, and its growth area in 2003 was 342 000 hm^2 based on government statistics.

In this study, we use asparagus bean as a model vegetable species to explore their differential reactions to single- (Cd) versus multi-metal (Cd, Pb, and Zn) pollutions under the light of screening potential PSCs. We hypothesize that the patterns of Cd uptake and accumulation by asparagus bean among the 24 tested cultivars are consistent between single- and multi-metal contaminations given the soil Cd concentrations are similar. In another word, it is hypothesized that plants react to one toxic heavy metal, Cd in this case, regardless of the presence of other toxic heavy metals.

2. MATERIALS AND METHODS

2.1. Culitvars of Asparagus Bean. There were 24 cultivars of asparagus bean used in this study, all of which were currently planted by the farmers of Guangdong province, China. These cultivars were grouped into three types based on the color patterns of their seed coat: 11 red, 9 black, and 4 striped. Seeds of the cultivars were acquired from a local seed market at Guangzhou, China.

2.2. Experimental Design and Treatments of the Garden Experiment (Single-Metal Effect). The garden experiment was conducted in an experimental garden in the suburb of QingYuan city (111°55′E, 23°30′N), Guangdong Province, China, during the summer (July−October) of 2003. Two levels of Cd exposure (0.8 and 11.0 mg kg^{-1}) were implemented to evaluate the differential responses of asparagus bean plants to different levels of soil pollution by a single metal, Cd. For each treatment, three pots ($n = 3$) were planted for each of the 24 cultivars.

Five seeds per pot were initially sown into the soil in the pots. Within 15−25 days after germination, seedlings were gradually thinned until 1−2 seedling pot^{-1} was left. Daily irrigation and monthly fertilization with compound fertilizer (N:P:K = 26:6:13) for 2.0 g pot^{-1} were carried out. By the end of the growing season, only one plant per pot was harvested and thoroughly measured.

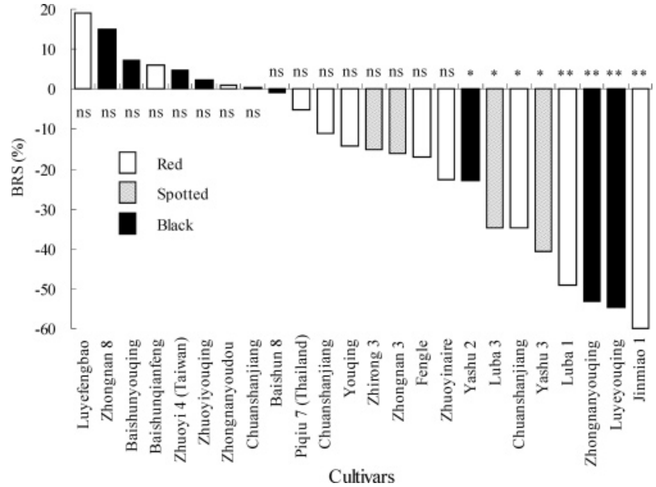

Figure 1. Biomass response to stress (BRS) of the tested cultivars in garden experiment. Note: ns not significant between low and high Cd; * significant between low and high Cd at $p < 0.05$ level; ** significant between low and high Cd at $p < 0.01$ level.

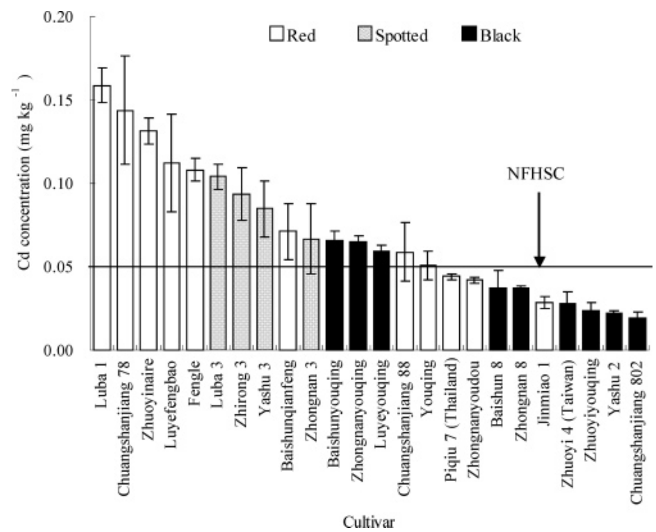

Figure 2. Cd concentrations in fruit of the tested cultivars under Cd heavily exposed in the garden experiment. Note: NFHSC, the maximum limitation of Cd according to the National Food Hygiene Standard of China (NFHSC).

The experimental soil was collected from farmland adjacent to the garden. It was air-dried and sifted through a 2 mm sieve. For each experimental pot (18 cm in upper diameter and 16 cm in height), 1.5 kg of the air-dried soil was used and fertilized with 2.0 g of compound fertilizer (N:P:K = 26:6:13) 2 weeks before sowing the seeds. Cd concentration of the original soil was 0.8 mg kg^{-1}, which exceeded the Cd limit of the second grade soil (0.3 mg kg^{-1}, the highest allowable soil Cd concentration for vegetable production) of the National Soil Environmental Quality Standard of China (NSEQSC, GB15618-1995), and therefore it served as the low Cd exposure with no additional Cd added. The high Cd exposure was implemented by adding $Cd(NO_3)_2 \cdot 4H_2O$ solution, and the final Cd concentration in the soil was 11.8 mg kg^{-1}.

Prior to the experiment, the soil pH was 5.2, as determined by a pH meter (PHS-3C, Shanghai, China) in a soil to water ratio of 1:2.5 (*34*). Contents of organic matter, NH_4^+−N, available P, available K, and total Cd, in the soil were 1.8%, 24.8 mg kg^{-1}, 7.3 mg kg^{-1}, and 31.5 mg kg^{-1}, respectively. Organic matter content was determined by wet digestion following the method of Nelson and Sommers (*35*). Ammonia−N was determined with colorimetry using Nessler's reagent (*36*). Available P was measured by molybdenum blue colorimetry (*37*). Available K (*34*) was measured using atomic absorption spectrophotometry (Perkin-Elmer AA 100, Norwalk, CT). Total soil Cd was

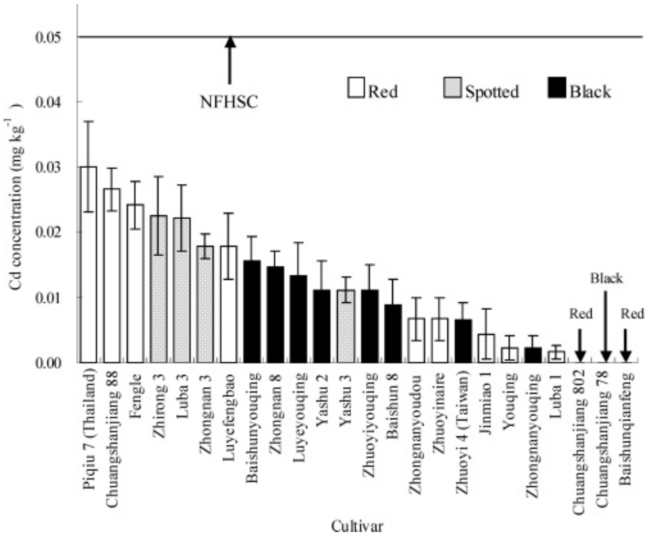

Figure 3. Cd concentrations in fruit of the tested cultivars under Cd lightly exposed in the garden experiment. Note: NFHSC, the same as in **Figure 2**.

determined by atomic absorption spectrophotometry following mixed acid digestion (HNO_3-HClO_4-HF) (*38*).

2.3. Experimental Design and Treatments of the Field Experiment (Multi-metal Effect). The field experiment was conducted in a farmland located at Lechang county (113°21′E, 23°09′N), Guangdong Province, China. This area was about 1500 m away from a Pb/Zn mining factory and had been irrigated with wastewater from the Pb/Zn mining for more than 50 years during the farming practice. The soil concentrations of Cd, Pb, and Zn were 1.2, 486, and 1114 mg kg^{-1}, respectively. It exceeded the limits of the second grade soil (for safe vegetable production) for Cd, Pb, and Zn and the third grade soil (for common plant growth) for Cd and Zn of the NSEQSC. Over the years, lime had been applied to amend the acidic soil by the local farmers. Therefore, the soil Ca^{2+} concentration was 13.94 mg kg^{-1} (Ca was detected by flame atomic absorption spectrometry; refer to ref *39*), and pH value was as high as 7.34. Contents of organic matter, total N, P, and K were 6.31%, 3.49 g kg^{-1}, 1.01 g kg^{-1}, and 9.9 g kg^{-1}, respectively.

The same cultivars from the garden experiment except cv. Fengle, which failed to germinate, were used in the field experiment. For each of the 23 cultivars, five seeds were sown to each of the 16 holes evenly distributed within a 1.0 m × 1.0 m plot designated to this particular cultivar. After the seeds germinated, seedlings were thinned to two seedlings per hole, and bamboo sticks were used to support the growth and climbing of the tendrils. The seeds of the 23 cultivars of asparagus bean were sown on April 4, 2005. Compound fertilizer (N:P:K = 16:16:16) was applied for 50 g per plot before the sowing, and an additional 20 g per plot was applied during the climbing and blossoming stages.

Although the physical layout of the experiment was not a complete randomized block (CRB) design, all 23 1.0 m × 1.0 m plots were located tightly close to each other. The experimental site was very flat due to many years of plowing. Because of the intensive disturbance during the farming practice, contents of the heavy metals in the soil were rather homogeneous. Coefficients of variation (CV) for Cd, Pb, and Zn among the 3−8 soil samples picked randomly from the experimental site were only 7.5%, 3.7%, and 6.4%, respectively, and the variations were all not significant. Therefore, we consider our experimental design equivocal to a CRB design.

2.4. Tissue Sampling and Chemical Analyses of the Garden Experiment. Plants of the garden experiment were harvested in October, 2003, after the asparagus beans reached maturity. Roots, stems, leaves, and fruits of the 24 tested cultivars were sampled separately (n = 3). To remove the heavy metal on the root surface, all root samples were submerged in 0.01 mol L^{-1} EDTA for 10 min twice (*40*, *41*) after they were washed with distilled water. All tissue samples were then washed with deionized water three times, dried under 70 °C to the constant weight, crushed, and passed through a 100-mesh sieve. The dry biomass of all tissue samples was measured before the chemical analyses. Cd concentrations of the samples were determined with an atomic absorption spectrophotometer (AAS, AA100 of Perkin-Elmer) following HNO_3:H_2O_2 (4:1) microwave digestion (Microwave Digester 7295, O.I. Corp., USA). The veracity of the AAS was checked by analyzing the standard Cd solution (GSB G62040-90, NACIS, China) 10 times. The relative standard deviation (RSD) of the measurement was 1.36%, and the average recovery was 98.2% (±2.4%). We also used a plant CRM (GBW-07603, National Research Center for Certified Reference Materials, China, the certified Cd concentration is 0.057 mg kg^{-1}) to ensure precision of the analytical procedure, and the results averaged 0.059 mg kg^{-1} with 0.099% RSD (n = 6).

2.5. Tissue Sampling and Chemical Analyses of the Field Experiment. The experiment ended on July 10, 2005. Tissue samples were prepared as in section 2.4. Heavy metal concentrations (Cd, Pb, and Zn) of the tissue samples were determined.

2.6. Safety Standard and Statistical Methods. The National Food Hygiene Standard of China (NFHSC) was employed to measure the safety of consuming the fruits (pods) of asparagus bean grown in heavy metal contaminated soil. The maximum limits of Cd (GB15201-94), Pb (GB14935-94), and Zn (GB13106-91) concentrations in the vegetables for safe consumption are 0.05, 0.2, and 20 mg kg^{-1} fresh weight, respectively.

Mixed model nested ANOVAs on fruit biomass and fruit Cd concentration were conducted using SPSS 11.0 after normality and homogeneity of variance were both confirmed in the data. The ANOVA model included cultivar type (red, black vs striped seeds) as a fixed effect, with cultivar nested within cultivar type as a random factor. Correlation analyses (using Pearson product−moment correlation) were conducted using SPSS 11.0. To compare the relative response of cultivars to different Cd exposures, we calculated the index of biomass response to stress (BRS) as follows:

$$\text{BRS}\ (\%) = (B_{\text{high}} - B_{\text{low}})/B_{\text{low}} \times 100$$

where B_{high} and B_{low} are the fruit dry biomass (g) under the high Cd and low Cd exposures, respectively.

To estimate Cd translocation to the fruits, the edible part, we calculated the translocation rate (TR) (*42*) as follows:

$$\text{TR}\ (\%) = (\text{Cd accumulation in fruits})/(\text{total Cd in the whole plant}) \times 100$$

3. RESULTS

3.1. Biomass Response to Cd Stress in the Garden Experiment. Overall, the root (0.356 g ± 0.002 vs 0.225 g ± 0.001), stem (2.056 g ± 0.195 vs 1.676 g ± 0.148), leaf (1.388 g ± 0.129 vs 1.135 g ± 0.071), and fruit (1.375 g ± 0.061 vs 1.102 g ± 0.057) biomass of asparagus beans all decreased when exposed to a higher level of soil Cd exposure. However, only 8 of the 24 tested cultivars had significantly lower fruit biomass produced when exposed to a higher level of Cd contamination (**Figure 1**). Out of the 24 tested cultivars, 8 cultivars had positive BRSs (**Figure 1**), but the differences of the biomasses between low and high Cd treatments were not significant ($p > 0.05$). In another word, for each of the aboveground tissues, about one-third of the cultivars did not have biomass decrease in response to the soil Cd exposure that was 14-fold higher (i.e., from 0.8 to 11.8 mg kg^{-1}), while almost all cultivars responded negatively to the higher Cd exposure in their belowground biomass.

3.2. Cadmium Accumulation and Translocation in the Garden Experiment. Cd concentrations in root, stem, leaf, and fruit under both levels of Cd exposure were all significantly different among the 24 tested cultivars (7 at $p < 0.01$ level) except stem Cd concentration under the low Cd treatment ($p > 0.05$). Under the low Cd exposure, the Cd concentrations ranged from 0.068 to 2.942 mg kg^{-1} and averaged 1.365 mg kg^{-1} in

Table 1. Average Cd Concentrations (mg kg^{-1}) in Fruit of the Cultivars of Different Types Divided by Color of Seed Coat[a]

type	root	stem	leaf	fruit
High Cd Treatment				
spotted	13.78 ± 2.70 a	8.83 ± 2.47 a	3.81 ± 0.51 ab	0.087 ± 0.009 a
red	15.55 ± 1.05 a	9.46 ± 1.55 a	3.914 ± 0.33 a	0.086 ± 0.015 a
black	12.77 ± 1.52 a	4.88 ± 0.59 b	3.03 ± 0.14 b	0.040 ± 0.007 b
Low Cd Treatment				
spotted	1.69 ± 0.49 a	1.76 ± 0.46 a	1.33 ± 0.18 a	0.018 ± 0.003 a
red	1.46 ± 0.17 a	1.32 ± 0.17 a	1.01 ± 0.06 ab	0.011 ± 0.004 a
black	1.10 ± 0.12 a	1.19 ± 0.07 a	0.96 ± 0.12 b	0.009 ± 0.002 a

[a] Different letters within the same column indicate significant difference at $p < 0.05$ level. Samples size $n = 4$ for spotted, $n = 11$ for red, and $n = 9$ for black.

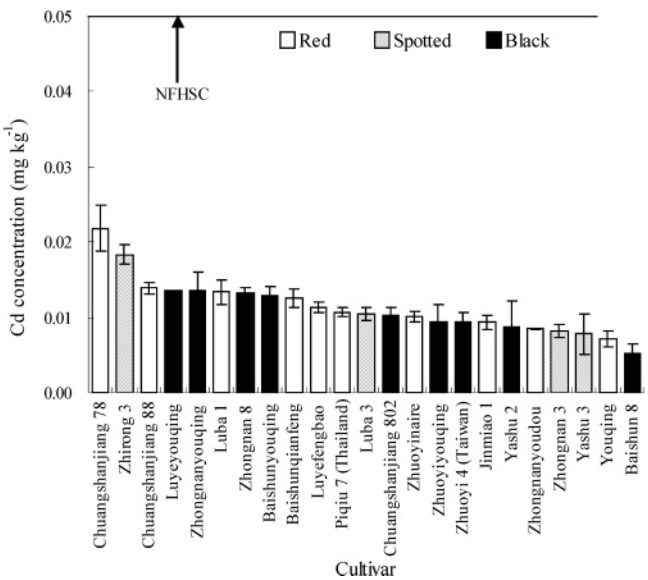

Figure 4. Cd concentrations in fruit of the tested cultivars under multi-metal contamination in the field experiment. Note: NFHSC, the same as in **Figure 2**.

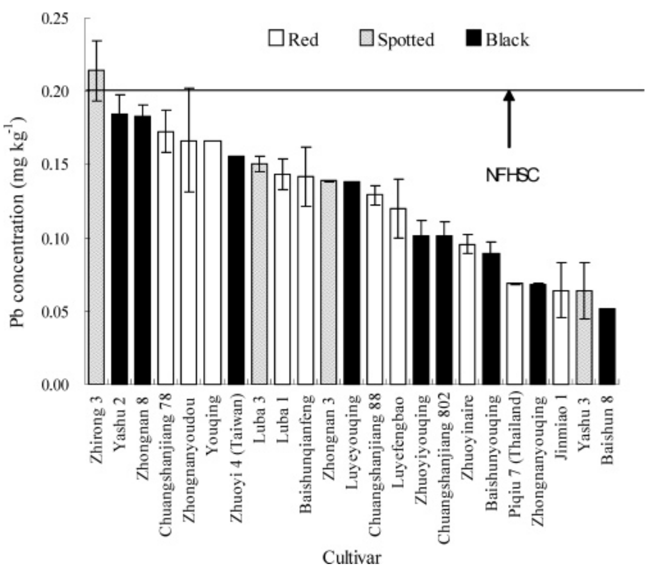

Figure 5. Pb concentrations in fruit of the tested cultivars under multi-metal contamination in the field experiment. Note: NFHSC, the same as in **Figure 2**.

root, and they were 0.667−2.765 mg kg^{-1} and 1.364 mg kg^{-1} in stem, 0.294−1.783 mg kg^{-1} and 1.045 mg kg^{-1} in leaf, and 0.000−0.029 mg kg^{-1} and 0.012 mg kg^{-1} in fruit. The average

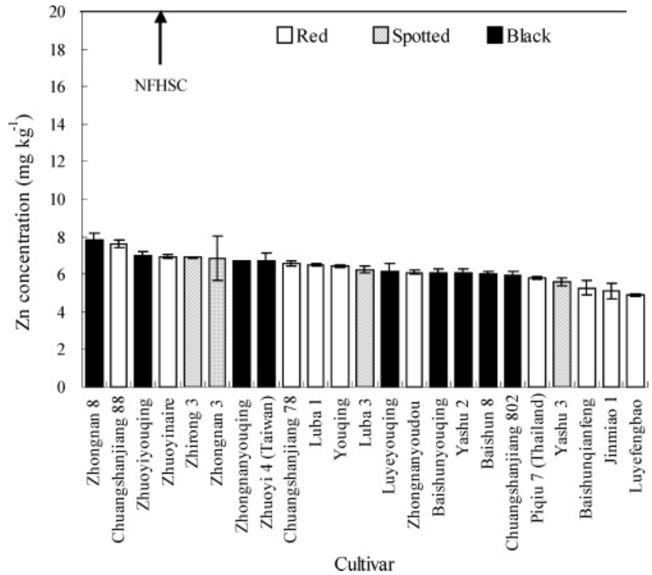

Figure 6. Zn concentrations in fruit of the tested cultivars under multi-metal contamination in the field experiment. Note: NFHSC, the same as in **Figure 2**.

Cd concentrations in the vegetative tissues were similar to each other, but were 86−113-fold higher than that in fruit. While under the high Cd exposure, the Cd concentrations were greatly increased and ranged from 8.849 to 22.855 mg kg^{-1} in root, 2.465−17.328 mg kg^{-1} in stem, 2.471−6.770 mg kg^{-1} in leaf, and 0.020−0.159 mg kg^{-1} in fruit. The averages were 9.4-, 4.7-, 2.4-, and 5.1-fold higher than those under the low Cd exposure for root, stem, leaf, and fruit, respectively. Again, the concentrations in the vegetative tissue were 48−194-fold higher than that in fruit. Therefore, only a small fraction of the Cd absorbed by asparagus bean was translocated to its fruit. The TRs under the low and high levels of Cd exposures were 0−1.09% and 0.13−0.87%, respectively.

3.3. Selection of Cd-PSCs of Asparagus Bean in the Garden Experiment. Fruit Cd concentrations in fruit of the tested cultivars of asparagus bean under the two Cd exposure levels are shown in **Figures 2** and **3**, respectively. Under the low Cd exposure, all tested cultivars had fruit Cd concentrations lower than the NFHSC (GB 15201−94, Cd ≤ 0.05 mg kg^{-1} FW for vegetables). Under the high Cd exposure, 9 out of 24 tested cultivars (37.5%) still contained Cd in their fruits less than 0.05 mg kg^{-1} while grown in the soil containing Cd as high as 11.8 mg kg^{-1}. In another word, all of the tested cultivars could be treated as Cd-PSCs under the low Cd treatment of 0.8 mg kg^{-1}, and 9 cultivars could be treated as Cd-PSCs under the high treatment of 11.8 mg kg^{-1}.

Furthermore, although 80.8% and 87.8% of total Cd absorbed were translocated to aboveground tissues under Cd heavily and lightly exposed, respectively, only 0.40% and 0.28% of them were translocated to fruit.

Under the high level of Cd exposure, fruit Cd concentrations of cultivars with black seed coat (0.040 mg kg^{-1} ± 0.007) were significantly lower than those with red (0.086 mg kg^{-1} ± 0.015) or spotted seeds (0.087 mg kg^{-1} ± 0.009) (**Table 1**). The stem and leaf Cd concentrations of cultivars with black coat were also significantly lower than those with red or spotted seed coat (**Table 1**). Under the low level of Cd exposure, a similar trend existed, but the differences were not statistically significant except partially in leaves. Because all three seed types produced comparable fruit biomass ($p > 0.05$), the average total Cd accumulation in fruits per plant of cultivars with black seeds

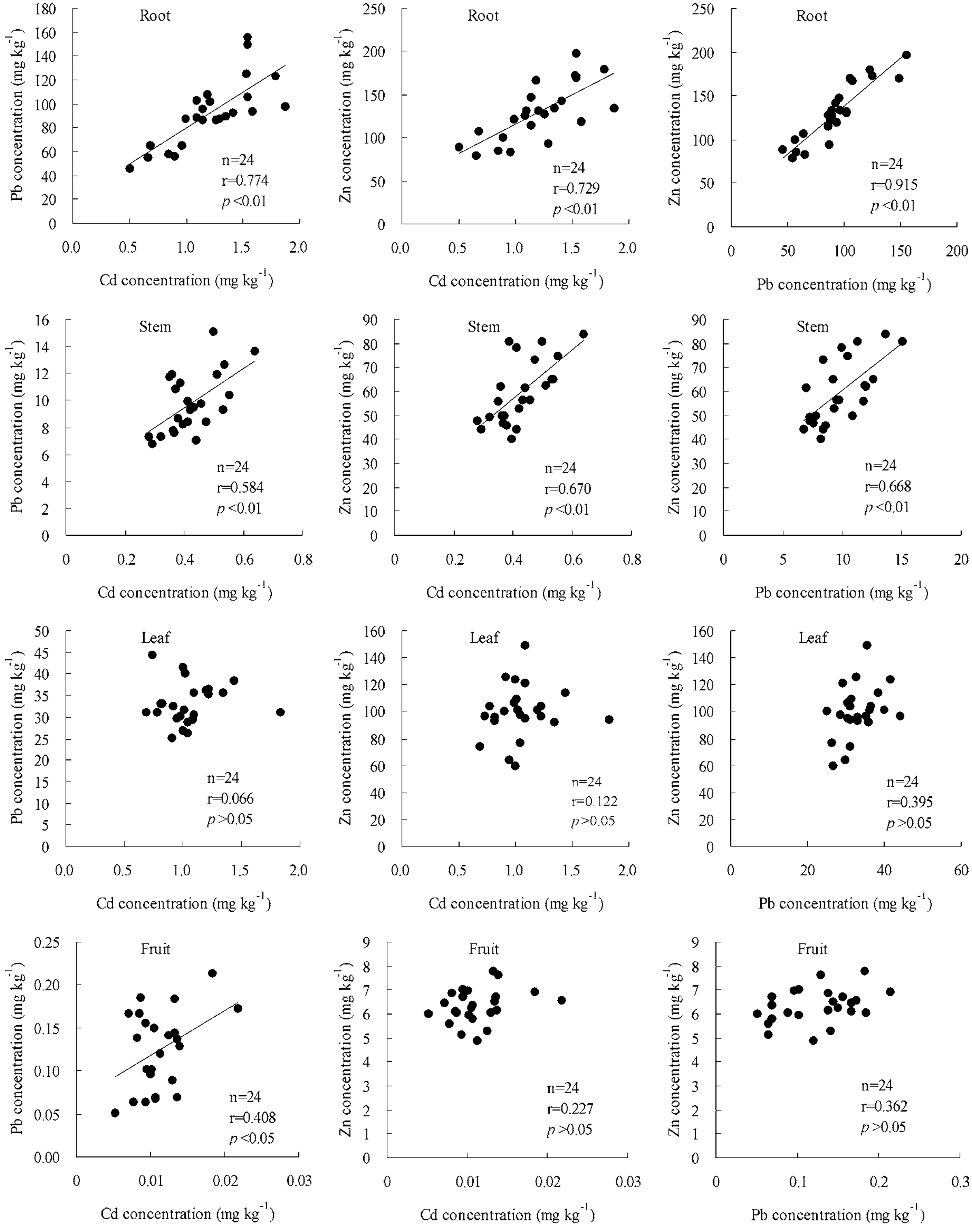

Figure 7. Correlations of Cd, Pb, and Zn concentrations in the root, stem, leaf, and fruit of asparagus bean in the field experiment.

(5.88 μg $\pm$ 0.07) was also significantly lower than those of cultivars with red (10.66 μg $\pm$ 0.11) or spotted seeds (9.92 μg $\pm$ 0.14). Overall, two-thirds of the Cd-PSCs under the high Cd exposure was black-seeded cultivars, and the other one-third was red-seeded. None of the cultivars with spotted seeds were found to be Cd-PSCs under the high Cd exposure of this garden experiment.

3.4. Cadmium, Lead, and Zinc Accumulation in the Field Experiment. Fruit Cd, Pb, and Zn concentrations of the 23 tested cultivars in the field experiment are shown in **Figures 4−6**, respectively. Although the concentrations of Cd, Pb, and Zn in soil were as high as 1.2, 486, and 1114 mg kg^{-1}, respectively, their concentrations in fruit of the 23 tested cultivars of asparagus bean were almost all below the maximum limits of the NFHSC (0.05, 0.2, and 20 mg kg^{-1} for Cd, Pb, and Zn, respectively), except fruit Pb concentration in cv. Zhirong 3 (0.214 mg kg^{-1}). The fruit Cd concentrations varied from 0.005 to 0.022 mg kg^{-1}, with an average of 0.011 mg kg^{-1}, similar to the average fruit Cd concentration (0.012 mg kg^{-1}) under the low Cd exposure in the garden experiment. Except cv. Zhirong 3, the fruit Pb concentrations ranged from 0.051 to 0.185 mg kg^{-1} with an average of 0.120 mg kg^{-1}. For Zn, it ranged from 4.886 to 7.805 mg kg^{-1}, with an average of 6.320 mg kg^{-1}.

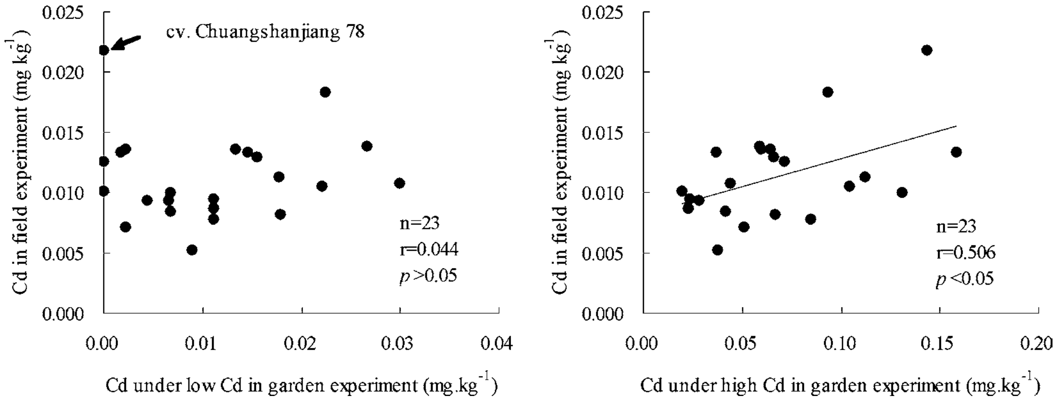

Figure 8. Correlations of Cd concentrations in fruit between the garden and the field experiments.

3.5. Correlations of Cd, Pb, and Zn Accumulation in the Field Experiment. Correlations of Cd, Pb, and Zn concentrations in four tissue types (root, stem, leaf, and fruit) of the field experiment are shown in **Figure 7**. All of the correlation coefficients were positive, which suggests that uptake and accumulation of the three metals were positively correlated. The correlation between Pb and Zn concentration in root was the highest ($r = 0.915$, $p < 0.01$), and it was also very high between Cd and Pb ($r = 0.774$, $p < 0.01$), and Cd and Zn ($r = 0.729$, $p < 0.01$) in root. All three correlations in stem were also significant at $p < 0.01$ level, while they were not significant ($p > 0.05$) in leaf. In fruit, only Cd and Pb concentrations were significantly correlated ($p < 0.05$).

3.6. Correlation of Fruit Cd Concentrations between the Garden and Field Experiments. Correlation of fruit Cd concentrations between the field experiment and the garden experiment under the low Cd exposure was surprisingly not significant ($r = 0.044$, $n = 23$, $p > 0.05$) (**Figure 8**). One exception was cv. Chuangshanjiang 78, which had the highest fruit Cd concentration (0.0218 mg kg^{-1}) among all tested cultivars in the field experiment and the lowest one (nondetectable) in the garden experiment under the low Cd exposure. However, the correlation was still not significant even after excluding this outlier cultivar ($r = 0.288$, $n = 22$, $p > 0.05$). On the contrary, correlation of fruit Cd concentrations between the garden experiment under the high level of Cd exposure and the field experiment was significantly positive ($r = 0.506$, $n = 23$, $p < 0.05$).

4. DISCUSSION

4.1. Some Cultivars of Asparagus Bean Have High Tolerance to Soil Cd Toxicity. Although the average root, stem, leaf, and fruit biomass decreased significantly when exposed to a higher level of soil Cd contamination, only one-third of the 24 tested cultivars yielded significantly less fruit biomass (**Figure 1**). Among the two-thirds of the tested cultivars that did not produce significantly less fruit biomass when exposed to a Cd stress that was 14-fold higher, one-half of them (8 cultivars) even had positive BRSs (i.e., biomass increase), and some BRS was as high as 20%. Similar positive and neutral responses of biomass to heavy metal stress have also been observed in paddy rice (*16*), tomato (*43*), and other crop species. Consequently, farmers may not get sufficient warning about the uptake of toxic heavy metals based on the apparent growth of these crops. The selection and breeding of PSCs would reduce the potential risk of heavy metal influx to the human food chain more directly and effectively.

4.2. Asparagus Bean Is an Example of "Cd Hypo-accumulator Species". The ability of accumulating certain heavy metals has been shown to be species-dependent (*44*−*46*). Hyper-accumulator plants can accumulate certain metal in aboveground tissues at a level of up to 1000-fold higher than normal plant species (*13*, *47*). They have been used in phytoremediation to absorb and remove toxic metals from the contaminated soil. On the other hand, for the purpose of agricultural production, it is desirable to select crop species and cultivars that tend to accumulate less toxic metals in their edible tissues when grown in polluted soils, so that less toxic metals would enter the food chain. In this paper, we propose to call this type of plant species "hypo-accumulator species".

In the garden (under the low level of Cd exposure) and field experiments of our study, the average fruit Cd concentrations of all tested cultivars of asparagus bean were 0.012 and 0.011 mg kg^{-1} while grown in Cd polluted soils with Cd concentrations of 0.8 and 1.2 mg kg^{-1}, respectively. None of the 24 tested species had fruit Cd concentrations higher than 0.05 mg kg^{-1} (the NFHSC of vegetables for safe consumption) (**Figures 3** and **4**). The translocation rate of Cd from the root to the fruit is considerably lower than many other crop species. For example, the Cd concentrations in brown rice of 10 tested cultivars of paddy rice ranged from 0.48 to 1.17 mg kg^{-1} while grown in the soil with Cd concentration of 1.1 mg kg^{-1} (*19*). A similarly high translocation rate of Cd in paddy rice was also found by Yu et al. (*16*). Tomato (*Lycopersicon esculentum*) also has a relatively higher translocation rate of Cd than asparagus bean. Seven out of the 36 tested cultivars (19.4%) bore fruit with Cd concentration higher than 0.05 mg kg^{-1} (one of them was as high as 0.09 mg kg^{-1}), while the soil Cd concentration was 1.1 mg kg^{-1} Cd (*43*). Engqvist and Martensson (*31*) found that, for 24 cultivars of pea grown in soil containing Cd from 0.09 to 0.55 mg kg^{-1} in 11 locations throughout France and Sweden, Cd concentration in the pea kernels reached up to 0.066 mg kg^{-1} (cv. Laser). These data suggest that asparagus bean tends to accumulate less Cd at the edible tissue, and therefore is qualified to be called a hypo-accumulator species.

4.3. Cd Accumulation of Asparagus Bean Is Correlated to Pb and Zn Accumulations under Multiple-Metal Contamination in the Soil. Heavy metal contaminations rarely occur with a single metal. For example, a vegetable farm at Naning, Guangxi Province, China, was polluted with Pb, Zn, Cu, and Cd (*48*). Vegetable products in Chongqing, China, were found be contaminated with Pb, Cd, and Hg (*49*). Chinese cabbage and cucumber (*Cucumis sativus*) produced in about 10 000 hm^2 farmland in the suburb of Shenyang, Liaoning Province, China, were found to be contaminated with Pb and Cd, and with Pb, Cd, and Hg, respectively (*50*). Vegetables grown along the Sinza and Msimbazi rivers in Dar es Salaam, Tanzania, contained relatively high concentrations of Cd, Pb, and Zn (*51*). Therefore, a feasible strategy of selecting PSCs under the situation of multi-metal contamination is much needed and has been neglected in most previous studies.

For asparagus bean in our experiment, Cd, Pb, and Zn accumulations were positively correlated with each other, which proved the rationality of our second hypothesis concerning that the Cd-PSCs of asparagus bean are simultaneously Pb-PSCs or Zn-PSCs when exposed in coexistence of Cd, Pb, and Zn contamination. Many similar results reported before demonstrated a profile that [Cd+Pb+Zn]-PSC would be existing for many crops, which settles a foundation for applying the PSC strategy to deal with the ubiquitous multiple-metal contamination in soil. Liu et al. (*52*) reported that correlations between Cd and Fe, Cd and Zn, and Cd and Cu were significant in root and leaf in 20 rice cultivars at both heading and ripening stages. Qin et al. (*53*) reported that Cd toxicity enhanced when Pb added in solution contains Cd. Pb present could enhance the activity of Cd and as a result could enhance the activity of rice and wheat to accumulate more Cd (*54*). Pb present also caused more Cd accumulation in tobacco (*Nicotiana tabacum* L.) (*55*). However, interactions between Zn and Cd and Zn and Pb were also indicated to be complex and dosage-dependent (*56*, *57*). Therefore, it is still requested to check the metal interactions for more species and more metals to confirm the feasibility of the PSC strategy.

In the present study, some scientific problems about Cd-PSC were investigated, and it was concluded that the PSC strategy is reasonable and practicable for asparagus bean. Furthermore, the needs for reducing risk of human exposure to heavy metal and the genotype-dependent accumulation of heavy metal in crops bring on a necessity and feasibility for the breeding of PSC, which will efficiently and easily enhance a safer food production for human. However, further studies, such as uptake, translocation, and accumulation mechanism of pollutants in PSC, as well as genetic principles and genetic stability of PSC, etc., are needed for establishing the breeding of PSC as a new crop breeding direction.

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