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Inter- and Intraspecific Variations of Cadmium Accumulation of 13 Leafy Vegetable Species in a Greenhouse Experiment

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Leafy vegetables are among the crop species that are most vulnerable to heavy metal pollution. This study investigated inter- and intraspecific variations of cadmium accumulation in 13 species with a total of 39 cultivars of leafy vegetables under two levels of soil Cd stress (1.5 and 7.7 mg kg^{-1}). Intraspecific variations of shoot biomass and Cd concentration of the tested leafy vegetables were significantly larger than interspecific variations under both Cd treatments and were also more significantly correlated between two Cd stress levels when grouped by cultivar than grouped by species. These results indicate that cultivar is a more reliable taxa level for screening pollutant-safe leafy vegetables than species. Any screening for pollutant hypoaccumulator species, or other similar species-based concepts, without considering intraspecific variation should be avoided.

KEYWORDS: Cadmium (Cd); leafy vegetable; pollutant hypoaccumulative species (HAS); pollution-safe cultivar (PSC); soil contamination

INTRODUCTION

Accumulation of heavy metals and metalloids in agricultural soils is of increasing concern due to its detrimental effects on human health and soil ecosystems (*1, 2*). Soil-to-plant transfer of heavy metals is the major pathway of human exposure to soil contamination. Among all of the heavy metals, Cd is one of the most important to consider regarding contamination via the human food chain, because it is readily taken up from soil and translocated to different plant parts (*3*). Health risks related to Cd contamination, such as renal dysfunction, have been widely studied.

Vegetables, especially leafy vegetables, absorb and accumulate heavy metals more easily than any other kinds of crops (*1, 4, 5*), and it has been estimated that vegetables contribute 83% of the total Cd intake in human bodies (*6*). One way to reduce the influx of Cd to the human food chain is to ameliorate the contaminated soil physically, chemically, or biologically. However, the remediation may not take effect soon enough or be economic enough to be widely applied (*7, 8*). Another choice is to grow plant species or cultivars with low heavy metal uptake and accumulation. It is well-known that the uptake of Cd can vary greatly among plant species and among cultivars within a species (*9–11*). Kurz et al. (*12*) reported that Cd concentrations in shoots and kernels of 50 inbred lines of maize (*Zea mays* L.) differed by a factor of about 20. Consequently, the annual Cd intake by humans could be lowered by a factor of 2–3 through suitable selection of cultivars.

In recent years, the concepts of both hypoaccumulative species (HAS) (*13*) and pollution-safe cultivar (PSC) (*14*) have been proposed and applied in searching for crops with low levels of heavy metal uptake and accumulation, in order to cut down pollutant flow to the human food chain. However, screening at the cultivar level was mostly conducted within one particular crop species (*13–15*) and mostly on staple crops and least on leafy vegetables. On the other hand, studies on hypoaccumulative species mostly compared large species groups (such as leafy vegetables, roots and bulbs, vegetable fruits, seed vegetables, etc.) (*16, 17*) rather than individual species and are mostly field surveys rather than well-designed experiments (*17–19*). There is a gap of knowledge about the relative importance of interspecific and intraspecific variation of metal uptake and accumulation among closely related crop species.

In this study, we filled this gap by examining both the interspecific and intraspecific variations of plant growth and Cd accumulation of 13 species (with 39 cultivars) of leafy vegetables, grown in Cd-contaminated soils, with a split-block nested experiment in a greenhouse. We also examined how soil condition would influence plant performance at the species and cultivar levels. It is one of the first attempts to identify the optimum level of taxa for screening pollutant-safe crops among closely related species.

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Table 1. Species and Cultivars of the Tested Leafy Vegetables

family	species and variety	cultivar	code	seed provider[a]
Amaranthaceae	*Amaranthus mangostanus* L.	Hongye	AMT-am-ma-1	E
		Luye	AMT-am-ma-2	E
Chenopodiacea	*Spinacia oleracea* L.	unknown	CH-sp-ol-1	B
		Riben	CH-sp-ol-2	B
Compositae	*Lactuca sativa* L.	Jianyewosun	CO-la-sa-1	E
		Meiguosiji	CO-la-sa-2	F
	Lactuca sativa L. var. *crispa*	Luoma	CO-la-sa-v.cr-1	F
		Yidali	CO-la-sa-v.cr-2	F
		Quannian	CO-la-sa-v.cr-3	F
		Ziye	CO-la-sa-v.cr-4	F
		Meiguonaiyou	CO-la-sa-v.cr-5	G
	Cichorium endivia L.	Shuangkougaochan	CO-ci-en-1	G
		Liujiang	CO-ci-en-2	G
Convolvulaceae	*Ipomoea aquatica* Forsk.	Baigeng	CON-ip-aq-1	G
		Qinggeng	CON-ip-aq-2	G
Cruciferae	*Brassica alboglabra* Bail.	Zaohua	CR-br-al-1	H
		Zhongchi	CR-br-al-2	H
	Brassica oleracea L. var. *botrytis*	Youlu 6	CR-br-ol-v.bo-1	A
		Youlu 5	CR-br-ol-v.bo-2	A
		Sijiyouqingtian	CR-br-ol-v.bo-3	A
		Guoshuyinyouqing	CR-br-ol-v.bo-4	A
		Tianyouxin 45	CR-br-ol-v.bo-5	A
		Tianbai	CR-br-ol-v.bo-6	B
		Honggeng	CR-br-ol-v.bo-7	B
	Brassica oleracea L. var. *capitata*	Qiangliqiushi	CR-br-ol-v.ca-1	D
		Sugan 11	CR-br-ol-v.ca-2	D
	Brassica chinensis	Tangshibai	CR-br-ch-1	C
		Kuishanheiye	CR-br-ch-2	C
		Guilinxiao	CR-br-ch-3	C
		Huangyefengshan	CR-br-ch-4	C
		Datouqingjiang	CR-br-ch-5	I
	Brassica juncea L.	Texuankejiajie	CR-br-ju-1	I
		Dayechuncai	CR-br-ju-2	I
		Qianbaocai	CR-br-ju-3	J
		Datoujiecai	CR-br-ju-4	J
	Brassica pekinensis (Loureiro) Rupr.	Jinlu 70	CR-br-pe-1	D
		Fengkang 90	CR-br-pe-2	D
Umbelliferae	*Apium graveolens* L.	Cuihuangfuqin	UM-ap-gr-1	E
		Jinnanshiqingwang	UM-ap-gr-2	E
6 families	13 species or varieties	39 cultivars		

[a] Seed providers: A, Yinong Garden Seeds Co., Ltd.; B, Guanglian Seedling & Seed Agency; C, Yashu Garden Seeds Co., Ltd.; D, Nongxin Agriculture Co., Ltd.; E, Changhe Seeds Co., Ltd.; F, Honghai Seedlings and Seeds Co., Ltd.; G, Guangzhou Vegetable Research Institute; H, Vegetable Research Institute of Guangdong Academy of Agricultural Sciences; I, Daliminan Seeds Shop; J, Dalixingnong Seeds Shop. A–H are locaed in Guangzhou, Ghangdong, China, and I and J are in Nanhai, Guangdong, China.

MATERIALS AND METHODS

Soil Preparation and Cd Treatments. The experimental soil was collected from the Zhaotian Farm of Qingyuan, Guangdong Province, in southern China, and was air-dried, ground, and sifted through a 5 mm sieve before the experiment. Prior to the experiments, the soil pH was 6.3, as determined by pH-meter (PHS-3C, Shanghai, China) in a soil-to-water ratio of 1:2.5 (*20*). Organic matter content, total N, available P, available K, and total Cd concentrations of the soil were 3.1%, 1.1 g kg^{-1}, 31.56 mg kg^{-1}, 107.5 mg kg^{-1}, and 1.5 mg kg^{-1}, respectively. Organic matter content was determined by wet digestion following the method of Nelson and Sommers (*21*). Total N was measured by titration of distillates after Kjeldahl sample preparation and analysis (*22*). Available P was measured by molybdenum blue colorimetry (*23*). Available K (*20*) was measured using atomic absorption spectrophotometry (Perkin-Elmer AA 100, Norwalk, CT). Total soil Cd was determined by atomic absorption spectrophotometry following mixed acid digestion (HNO_3–$HClO_4$–HF) (*24*).

Cd concentration in the original soil (1.5 mg kg^{-1}) already exceeded the maximal limitation for Edible Agricultural Products required by the Environmental Quality Evaluation Standard of China for Farmland (HJ332-2006) and therefore was used as the low-level Cd treatment in the experiment without further addition of Cd. The high level of Cd exposure (7.7 mg kg^{-1}) was implemented by adding $Cd(NO_3)_2 \cdot 4H_2O$ solution to the prepared farm soil.

Tested Species and Cultivars. The tested leafy vegetables included a total of 39 cultivars within 13 species or varieties (hereafter referred to as species) of 6 families (**Table 1**). Experimental seeds were obtained from various research institutes, seed suppliers, and gardening shops in Guangdong Province, China. The number of cultivars within each species varied from two to seven due to their availability and relative commercial importance.

Experimental Design. A split-block nested experiment was conducted in the greenhouse (temperature varying from 26 to 32 °C) of Sun Yat-sen University, Guangdong Province, China. Experimental pots, with 24 cm (top) and 18 cm (bottom) diameters and 20 cm height, were each filled with 2.5 kg (dry weight) of prepared soil 2 weeks before the experiment, with either a low-Cd (1.5 mg kg^{-1}) or a high-Cd treatment (7.7 mg kg^{-1}) as a block factor. Thirty-nine cultivars were nested within 13 species with uneven sample sizes (*2–7*), with cultivar and species both being random factors. Three pot replications ($n = 3$) were used for each of the 39 tested cultivars with each of the two soil Cd treatments, with a total of 234 pots. For each pot, 10 seeds of the tested cultivar were sown into the soil on

Table 2. Split-Block Nested ANOVAs of Cd Concentrations of Different Plant Parts or Shoot Biomass[a]

		shoot Cd DW		root Cd DW		shoot Cd FW		shoot biomass	
source	DF	*F*	Pr	*F*	Pr	*F*	Pr	*F*	Pr
Cd treat	1	32.37	***	77.61	***	36.62	***	2.77	ns
species	12	1.04	ns	0.95	ns	1.04	ns	2.70	ns
Cd treat × species	12	4.42	***	1.88	ns	3.83	**	2.02	ns
cultivar (species)	26	1.08	ns	0.97	ns	1.10	ns	2.20	*
Cd treat × cultivar (species)	26	21.37	***	32.70	***	19.16	***	129.03	***
error	156								

[a] Cd treat, Cd treatment levels (low or high), the block factor; species and cultivar are random factors; each column indicates a separate ANOVA; shoot Cd DW, shoot Cd concentration (mg kg^{-1} dry weight); root Cd DW, root Cd concentration (mg kg^{-1} dry weight); shoot Cd FW, shoot Cd concentration (mg kg^{-1} fresh weight); *, significant at the level $0.01 < Pr \leq 0.05$; **, significant at the level $0.001 < Pr \leq 0.01$; ***, significant at the level $Pr \leq 0.001$; ns, not significant, $Pr > 0.05$.

Table 3. Nested ANOVAs of Cd Concentrations of Different Plants Parts under Low or High Levels of Experimental Cd Treatment[a]

		shoot Cd DW		root Cd DW		shoot Cd FW		shoot biomass	
source	DF	*F*	Pr	*F*	Pr	*F*	Pr	*F*	Pr
				Low Cd Treatment					
species	12	1.93	ns	1.52	ns	2.96	**	2.86	*
cultivar (species)	26	10.46	***	20.48	***	9.36	***	110.38	***
error	78								
				High Cd Treatment					
species	12	4.39	***	1.85	ns	3.76	**	2.67	*
cultivar (species)	26	22.28	***	32.30	***	20.22	***	262.97	***
error	78								

[a] Species and cultivar are random factors; each column indicates two separate nested ANOVAs. Shoot Cd DW, shoot Cd concentration (mg kg^{-1} dry weight); root Cd DW, root Cd concentration (mg kg^{-1} dry weight); shoot Cd FW, shoot Cd concentration (mg kg^{-1} fresh weight); *, significant at the level $0.01 < Pr \leq 0.05$; **, significant at the level $0.001 < Pr \leq 0.01$; ***, significant at the level $Pr \leq 0.001$; ns, not significant, $Pr > 0.05$.

May 14, 2004. After germination, seedlings were thinned until three plants were left per pot. The plants were watered daily, and the excess water was collected and added back to the pot. Compound fertilizer (N/P/K = 26:6:13) was applied (3.3 g per pot) once on June 3, 2004 (halfway into the experiment).

Sampling and Chemical Analysis. All plants were harvested on June 24, 2004, after they reached maturity at 40 days of growth. Shoot and root samples of the 234 experimental pots were washed three times with deionized water before being oven-dried at 70 °C to constant weight. The dry biomass of shoot and root were recorded as the averages of three plants grown in the same pot. Fresh weights of shoots (edible parts) per plant were also measured before the drying procedure. Oven-dried samples were then ground to pass through a 100 mesh sieve for chemical analysis.

Shoot or root Cd concentrations of the samples for each experimental pot were determined with an atomic absorption spectrophotometer (Perkin-Elmer AA 100) after digestion with HNO_3:H_2O_2 (5:2) in a microwave oven (Microwave Digester 7295, O. I. Corp.) of 0.2 g of plant tissue. A Certified Reference Material (CRM) of plant (GBW-07603, National Research Center for CRM, China, the certified Cd concentration is 0.057 mg kg^{-1}) was used to ensure the precision of the analytical procedures, and the results averaged 0.059 mg kg^{-1} with 0.099% relative standard deviation (RSD).

Data Analysis. Split-block mixed model nested ANOVAs on shoot Cd concentrations (by fresh weight and dry weight), root Cd concentration, and shoot dry biomass were conducted using SAS-PC 9.1 (SAS Institute, Cary, NC) after normality and homogeneity of variance were both confirmed in the data. The ANOVA model included Cd treatment level (low vs high) as a fixed block factor, with cultivar nested within species, both being random factors. Because there were significant interactions between Cd treatment and species and between Cd treatment and cultivar (**Table 2**), separate nested ANOVAs were conducted for each of the four variables under either low or high level of soil Cd treatment (**Table 3**).

Correlation analyses (using Pearson product-moment correlation) were conducted using SAS-PC 9.1 (SAS Institute, Cary, NC) on shoot Cd concentrations (by dry weight) between low- and high-Cd treatments.

To compare the relative response of tested species or cultivars to different levels of Cd exposure, the index of biomass response to stress (BRS) was calculated as

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

where B_{high} and B_{low} are the shoot (edible part) biomass (g) under the high- and low-Cd exposures, respectively. A positive (or negative) BRS value indicates that the shoot biomass was higher (or lower) in the high-Cd treatment than in the low-Cd treatment, and the significance of the biomass variation between the low- and high-Cd treatments was tested directly on shoot biomass.

To estimate Cd uptake from soil to shoot, the edible part, the bioaccumulation factor (AF) (*25*) was calculated as

$$AF\ (\%) = \text{shoot Cd concn (mg kg}^{-1}\text{ of FW)} \times 100 / \text{soil Cd concn (mg kg}^{-1}\text{ of DW)}$$

where FW is fresh weight and DW is dry weight.

To estimate Cd translocation to shoot, the edible part, the translocation rate (TR) was calculated as follows:

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

RESULTS

Biomass Response to Soil Cd Treatment. Split-block nested ANOVAs showed that shoot biomass was not significantly different under two soil Cd treatments (**Table 2**). Most leafy vegetable cultivars (27 of 39) actually grew better under high Cd than under low Cd exposure (with positive BRS, **Figure 1**). They mostly belong to the families of Cruciferae, Compositae, and Convolvulaceae. Cv. Zaohua of *Brassica alboglabra*, cvs. Fengkang 90 and Jinlu 70 of *Brassica pekinensis*, and cvs. Luoma, Quannian, and Ziye of *Lactuca sativa* var. *crispa* appeared to have especially high Cd tolerance, and their BRS values exceeded 100%.

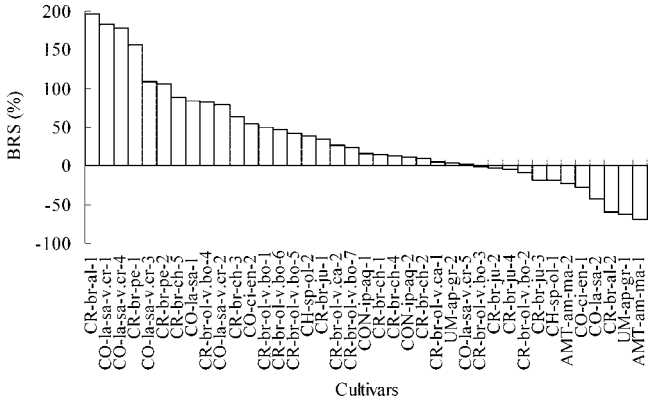

Figure 1. Biomass response to stress (BRS) of the tested leafy vegetable cultivars (refer to **Table 1** for the details of each cultivar code).

Most species also had positive BRS. Species of *B. pekinensis*, *B. alboglabra*, *L. sativa*, and *L. sativa* var. *crispa* under high Cd produced especially higher biomass than under low Cd, and their biomasses increased by 131.2, 68.4, 85.3, and 101.5%, respectively. Only *Apium graveolens* and *Amaranthus mangostanus* had negative BRS values; that is, their biomasses were lower under high Cd than under low Cd.

Inter- and Intraspecific Variation of Shoot Biomass. Split-block nested ANOVA showed that shoot biomass was not significantly different among species and had no significant interaction between species and Cd treatment level, whereas shoot biomass was significantly different among cultivars ($p < 0.05$) and had highly significant interaction between cultivar and Cd treatment. Therefore, the intraspecific variation was much larger than interspecific variation in shoot biomass.

Inter- and Intraspecific Variation of Shoot and Root Cd Concentrations. Split-block nested ANOVAs showed that shoot and root Cd concentrations responded to different soil Cd levels significantly differently among different species or cultivars (interactions highly significant, **Table 2**); therefore, separate nested ANOVAs were conducted for low- and high-Cd treatments. In both split-block nested ANOVA and separate nested ANOVAs, shoot Cd concentrations in dry weight (DW) or fresh weight (FW) showed very similar patterns.

Under low-Cd treatment (1.5 mg kg^{-1}), both shoot and root Cd concentrations (in DW) were highly significantly different among cultivars within the same species ($p < 0.001$) and were not significantly different among species ($p > 0.05$) (**Table 3**). Under high-Cd treatment (7.7 mg kg^{-1}), root Cd concentration showed the same pattern (highly significant among cultivars nested within species, but not significant among species). Shoot Cd concentrations (in DW) had a highly significant effect at both cultivar and species levels; however, the relative *F* values were still much larger for the cultivar effect than for the species effect (4.39 vs 22.28). Under both Cd exposures, the cultivar effect was larger and more significant than the species effect ($p < 0.01$ vs $p < 0.001$) in shoot Cd concentrations (in FW).

To summarize, the intraspecific variations were much larger than the interspecific variations in shoot and root Cd concentrations under both levels of Cd exposure.

Correlation between Two Cd Treatments. Correlation of shoot Cd concentration (in DW) between two Cd treatments was not significant when grouped by species ($p = 0.138$, **Figure 2A**) and was highly significant when grouped by cultivar ($p = 0.009$, **Figure 2C**). Correlation of shoot biomass between two Cd treatments was also less significant when grouped by species ($p = 0.011$, **Figure 2B**) than when it was grouped by cultivar ($p = 0.0001$, **Figure 2D**).

Shoot Cd concentration (in FW) and accumulation factor are equivalent for correlations between two Cd treatments. They were not significant when grouped by species ($p = 0.334$, **Table 4**), but were significantly different when grouped by cultivar ($p = 0.031$, **Table 4**).

Cd Translocation Rates (TRs) to Edible Parts and Bioaccumulation Factors of Different Cultivars. Cd TRs to edible parts of the tested cultivars were mostly near 100%, especially under low-Cd treatment. The average TRs under low and high Cd were 93.6% (80.8–98.5%) and 87.8% (56.0–97.4%), respectively. Therefore, most of the Cd absorbed by the vegetables was transferred to edible parts, especially for the species belonging to the family Cruciferae. Compared to the Codex Alimentarius Commission (CAC) standard, shoot Cd concentrations (in FW) of all the tested cultivars exceeded the maximum CAC limit (0.2 mg kg^{-1} of FW, CODEX STAN 248-2005) under both levels of Cd exposure.

DISCUSSION

Biomass Response to Soil Cd Treatment. With a 5-fold difference in soil Cd concentrations (1.5 and 7.7 mg kg^{-1}, respectively), the lack of negative biomass response in tested leafy vegetables was particularly alarming. Among the tested 13 species and 39 cultivars, 11 species (84.6%) and 27 cultivars (69.2%) accumulated more shoot biomass under high-Cd than under low-Cd treatment, especially when all of the tested cultivars exceeded the maximum CAC limit in their shoot Cd concentration (in FW). The results suggest that soil Cd level as high as 7.7 mg kg^{-1} may still promote, instead of prohibit, the growth of most tested leafy vegetable crops. Similar growth responses to heavy metal exposures had been reported before (*26–29*). Consequently, Cd contaminations in soil could be easily ignored because farmers cannot be warned by the growth responses to Cd stress, especially for the tested species from the genera *Brassica* and *Lactuca*. Education and precaution are much needed to control the influx of heavy metal pollutants to the human food chain (*30, 31*).

Practical Implications of the Experimental Treatments and Screening for PSCs. The soil Cd concentration in the high-Cd treatment of this experiment is consistent with habitats surrounding metal-mining sites or other seriously polluted sites. It is clear that leafy vegetables produced at this level of Cd exposure can never be safe for consumers, the shoot Cd concentrations (in FW) being 7–67-fold the CAC standard. The soil Cd concentration in the low -Cd treatment is within the range that is commonly found in lightly polluted agricultural land; therefore, the results obtained from the low-Cd treatment would help us to identify an effective screening strategy for Cd-HAS and/or Cd-PSC. Although HAS or similar concepts have been proposed before (*13, 17, 19*), it seems to be questionable for leafy vegetable because the interspecies variation of Cd concentration in their edible parts was far smaller and less important than intraspecies variation under low-Cd treatment. It seems Cd-HAS is more likely to be identified among fruit or legume vegetables (*13, 16*).

Prior studies showed that Cd accumulation in crops is genetically controlled (*32–35*). Andersson and Pettersson (*36*) reported that the wheat (*Triticum aestivum* L.) cultivar 'Holme' had consistently lower grain Cd than four other wheat cultivars grown at five locations in Sweden. Li et al. (*34*) proposed that estimates of genetic variation and effects could provide useful guidelines to determine the value of some populations and appropriate procedures to use in a breeding program. Penner et al. (*9*) identified a gene governing cadmium uptake in durum wheat (*Triticum turgidum*var. *durum*),

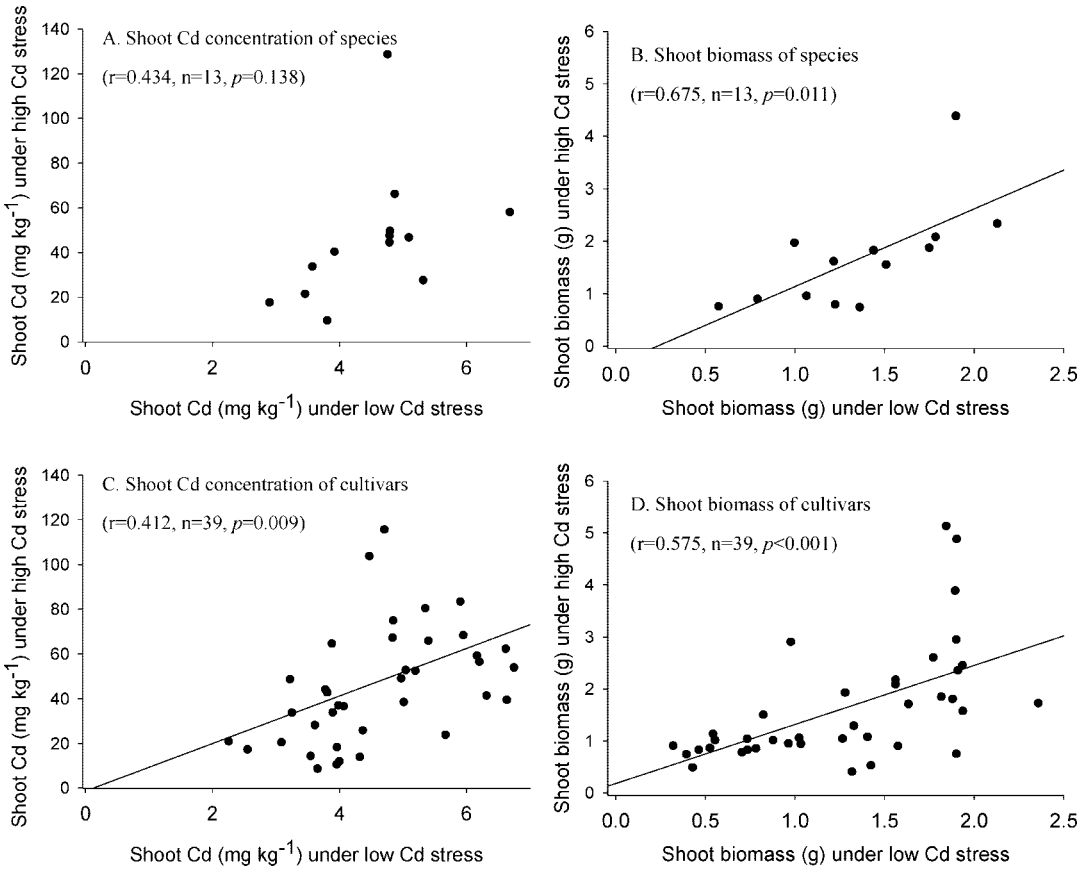

Figure 2. Correlations of shoot Cd concentrations (**A**, grouped by species; **C**, grouped by cultivar) and shoot biomass (**B**, grouped by species; **D**, grouped by cultivar) between two levels of soil Cd treatments.

Table 4. Correlations of Cd Concentrations of Different Plant Parts, Shoot Biomass, Translocation Rate, or Accumulation Factor between Two Levels of Cd Treatment When Grouped by Species or Cultivar[a]

	shoot Cd concn (mg kg^{-1} DW)	root Cd concn (mg kg^{-1} DW)	shoot Cd concn (mg kg^{-1} FW)	shoot biomass	translocation rate	accumulation factor
			Grouped by Species			
correlation coefficient	0.4343	−0.2618	0.2916	0.6746	0.7987	0.2916
p value	0.1381	0.3875	0.3336	0.0114	0.0013	0.3336
			Grouped by Cultivar			
correlation coefficient	0.4127	−0.2354	0.3452	0.5754	0.6955	0.3452
p value	0.009	0.1492	0.0314	0.0001	<0.0001	0.0314

[a] Each column indicates two separate correlation analyses.

whereas Miller et al. (*37*) registered three low-Cd sunflower (*Helianthus annuus* L.) genetic stocks. These studies and the finding of this study suggest that it is feasible to search for Cd-PSCs as a new crop-breeding strategy.

One caveate is that the purpose of searching for PSC is to cut down potential Cd influx to the human food chain given everything else being equal. It is not to justify or encourage the use of contaminated soil for crop production, but to minimize the food contamination risks and to establish a cleaner crop production system, similar to that in Australia where cultivars like Cd-PSC have been used for Cd management in potato (*38*). Due to the widespread pollution problem in agricultural land throughout the world, the potential implications and impact of the PSC concept to agricultural practice and human health are substantial and far-reaching.

Correlation between Two Cd treatments. Correlation between two Cd treatments measures the consistency of a particular measure of plant performance for plants grown in different soil conditions. When we screen for pollutant-safe crops, we prefer indices that have higher consistency across different contamination conditions, and we prefer the taxa level, by which these measures were grouped, that shows higher consistency. Results in **Table 4** suggest that translocation rate can be a highly consistent index, whereas root Cd concentration is a relatively inconsistent measure for leafy vegetables. Shoot biomass and shoot Cd concentration are indices with moderate levels of consistency. When we compared the correlations of these measures between being grouped by cultivar and being grouped by species, cultivar seemed always to be the taxa level that led to a higher consistency.

Because these measures also had higher intraspecific variation than inter-specific variation, it is highly desirable to screen for pollutant-safe leafy vegetables at the cultivar level rather than the species level. Studies and practices that use only one cultivar

to represent particular leafy vegetable species, in searching for hypoaccumulative species, could lead to false conclusions and therefore should be avoided.

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