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Genotypic variations in the accumulation of Cd exhibited by different vegetables

Junxing Yang[1], Haitao Guo[1,2], Yibing Ma[1,*], Liqun Wang[1,2],
Dongpu Wei[1], Luo Hua[2]

1. Ministry of Agriculture Key Laboratory of Plant Nutrition and Nutrient Cycling, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China. E-mail: yangajx@126.com
2. The Key Laboratory of Resource Environment and GIS, College of Resource Environment and Tourism, Capital Normal University, Beijing 100037, China

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Abstract

It is an important approach to use the Cd-contaminated soils properly by growing low accumulator or excluder plants for Cd to produce safe foods. To find the suitable vegetable species for growing in Cd-contaminated soils, in the present study the variations in the Cd accumulation for twenty eight vegetable species and several cultivars of five common vegetables (cowpea, kidney pea, bitter gourd, cucumber and squash) were investigated in two soil Cd levels (1 and 2 mg/kg Cd). Experimental results showed that highly significant differences in Cd concentration were evident among 28 vegetables. For example, spinach Cd concentrations were 110-fold and 175-fold higher than that of sweet pea under the 1 and 2 mg/kg Cd exposures, respectively. For Cd accumulation, the order of vegetable species was: leafy vegetables > solanaceous vegetables > kale vegetables > root vegetables > allimus > melon vegetables > legumes. Distinctive differences were also identified when comparing different cultivars of the five common vegetables with an average range of 0.003–0.094 mg/kg Cd. Our results indicated that a large genotypic variation existed among vegetable species or cultivars when subjected to Cd exposure. Therefore, it is important and feasible to elect/breed vegetable species/cultivars with low accumulation of Cd, especially in mildly Cd-contaminated soils.

Key words: vegetables; cadmium; accumulation; genotypic variations

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Introduction

Anthropogenic activities, such as pesticide and herbicide application, mining, or irrigation with wastewater have significantly enhanced heavy metal levels in soils in many areas in the world, which have imposed adverse environmental problems (Wong, 2003; Demirezen and Aksoy, 2004; Tüzen, 2009). Among all the toxic heavy metals, cadmium (Cd) is one of the most mobile elements and easily taken up by plants and translocated to aerial organs where it can accumulate to high levels. Thereby it can readily enter food chain (Sesli and Tüzen, 1999; Satarug et al., 2003; Soylak et al., 2007) and become detrimental to human and animal health.

Vegetable is the staple food for much of the world and plays an important role in human diets. In many regions, vegetable is heavily exposed to Cd, causing a health hazard (Tüzen, 2003; Yusuf et al., 2003; Demirezen and Aksoy, 2006; Li et al., 2006). In sewage irrigated region of Lagos City, Nigeria, vegetable Cd concentrations ranged from 0.09 to 0.62 mg/kg, exceeding the maximum allowable limit (0.2 mg/kg) of National Food Hygiene Standard of China (NFHSC, GB 15201-94; Yusuf et al., 2003). In the sewage irrigated area in the Middle Anatalia, Turkey, the vegetable Cd concentrations ranged from 0.24 to 0.97 mg/kg, which also exceeded the NFHSC limit (Demirezen and Aksoy, 2006). In the area adjacent to Cd contaminated districts of Shaoxing, Hangzhou, Zhejiang Province, China, vegetable Cd concentrations were 30-fold higher than the "normal" level of about 0.2 mg/kg Cd (Li et al., 2006). Besides Cd contamination, vegetable yield was also decreased when vegetable was grown in soils contaminated by Cd (Panwar et al., 1999; Brennan et al., 2005). In many Cd-contaminated agricultural soils in China, the demand and pressure to produce to foodstuff are so high that farmers can not afford to fallow agricultural soils for remediation. It is therefore urgent to select/breed vegetable species/cultivars which are low accumulators or excluders to Cd.

Differences between among vegetable species in the accumulation of Cd have been recognized when grown in medium containing high levels of Cd under both greenhouse and field conditions (Fleming and Parle, 1977; Kuboi et al., 1986; Lehoczky et al., 1998). Fleming and

* Corresponding author. E-mail: ybma@caas.ac.cn

Parle (1977) reported that lettuce was high Cd accumulator while beans were low Cd accumulator. Kuboi et al. (1986) classified plant families into three groups according to the extent to which they accumulated Cd, which were as follows: (1) low Cd accumulators (Leguminosae), (2) moderate accumulators (Gramineae, Liliaceae, Cucurbitaceae and Umbelliferae), and (3) high accumulators (Chenopodiaceae, Cruciferae, Solanaceae and Compositae). Lehoczky et al. (1998) identified lettuce, beetroot, radish and carrot as being high Cd accumulators and cauliflower, red cabbage and savoy cabbage were moderate accumulators, which were followed exactly the classification by Kuboi et al. (1986).

Some vegetable cultivars accumulated significantly low Cd levels in their shoots (including edible parts) than others (Crews and Davies, 1985; McLaughlin et al., 1994; Alexander et al., 2006). Crews and Davies (1985) reported that significant differences in Cd accumulation among six cultivars of lettuce. McLaughlin et al. (1994) found significant differences between the 14 common potato cultivars with an average range of 30–50 μg/kg Cd (fresh weight). Alexander et al. (2006) reported that carrot and pea cultivars exhibited significant differences in accumulated Cd concentrations.

However, there is still less information available on varietal differences in Cd accumulation within vegetable species, especially cultivars. Therefore, the objectives of this study were (1) to investigate variations in Cd accumulation within a wider range of different vegetable species to advise farmers selecting suitable vegetable species to grow in a Cd-polluted soil; and (2) to investigate variations of Cd accumulation by cultivars of five common vegetables grown in the Cd-polluted soil.

1 Materials and methods

1.1 Experimental design and treatments

Two experiments were conducted in an experimental greenhouse, one in the spring (Exp. 1) in April and one in the summer (Exp. 2) in August of 2007. In each experiment, two added Cd exposures (1 and 2 mg/kg) were implemented to evaluate the differential responses of vegetable species or cultivars. For each Cd treatment in Exp. 1, there were 4 plants (n = 4) each of 28 vegetable species. For each Cd treatment in Exp. 2, there were 4 plants (n = 4) each of the cultivars of 5 vegetable species.

Soil samples used for the experiment (0.03 mg/kg Cd) were collected (0–20 cm depth) at the Beijing Fluvo–aquic Soil Fertility and Fertilizer Efficiency Long Term Monitoring Base (116°15′23″E, 40°13′12″N), and was spiked with Cd (1 and 2 mg/kg as $Cd(NO_3)_2\cdot4H_2O$), then mixed thoroughly and allowed to equilibrate for 2 months. During the equilibration, the soil water was maintained 70% of maximum water holding capacity by weighing method. After 2 months, the soil sample was air-dried and passed through a 2-mm sieve, and then 700 g air-dried soil was used in each pot. The soil properties used for the experiment were 15.9 mg/kg organic matter, 1.40 g/kg total N and 1.17 g/kg total P, respectively, with a pH value of 8.11. The chemical agents used in the two experiments were all of guarantee reagent grade from Beijing Chemical Works, China.

1.2 Vegetable species and planting

Seeds of 28 vegetable species were acquired from Chinese Academy of Agricultural Science (Table 1). Vegetable seeds were disinfected in 30% H_2O_2 (*W/V*) solution for 15 min, followed by thorough washing with deionized water, and then germinated in moist perlites. After one week, uniform seedlings were selected and transplanted to PVC pots (12 cm diameter and 10 cm height). One seedling from each of the vegetable species or cultivars was transplanted to each pot.

There were four replicates for each treatment/plant. To ensure normal growth and development of vegetable plants, potassium sulfate (K_2SO_4) solution (in deionized water) was applied after transplantation of the seedlings to give 28.7 mg/kg, and nitrogen was supplied as a solution of urea ($CO(NH_2)_2$) (in deionized water) in four equal splits to a total of 46.6 mg N/kg soil during the plant growth period. The plant pots were placed in a greenhouse and arranged in a randomized completed block design during the vegetable-growing season.

After 40 days, the aboveground portion of each vegetable species or cultivars was harvested by cutting at 2 cm above the soil. The samples were washed thoroughly with tap water following with deionized water, and then

Table 1 Twenty-eight species of vegetables used in experiment

Species of vegetables	Type
Garlic (*Allium sativum* L.)	Alliaceae allium vegetables
Onion (*Allium cepa Linn.*)	Liliaceae allium vegetables
Scallion (*Allium fistulosum* L.)	liliaceae allium vegetables
Bitter gourd (*Momordica charantia* Linn.)	Cucurbitaceae, melon vegetables
Cucumber (*Cucumis sativus* L.)	Cucurbitaceae, melon vegetables
Pumpkin (*Cucurbita moschata* Duch.)	Cucurbitaceae, melon vegetables
Squash (*Cucurbita pepo* Linn.)	Cucurbitaceae, melon vegetables
Towel gourd (*Luffa cylindrica* L.)	Cucurbitaceae, melon vegetables
White-flowered gourd (*Lagenaria siceraria* (Molina) Standl.)	Cucurbitaceae, melon vegetables
Cauliflower (*Brassica oleracea* Linn.)	Cruciferae, kale vegetables
Kale (*Brassica olerace*a L.)	Cruciferae, kale vegetables
Celery (*Apium graveolens* Linn.)	Umbelliferae, leafy vegetables
Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.)	Cruciferae, leafy vegetables
Lettuce (*Lactuca satiua* L.)	Compositae, leafy vegetables
Spinach (*Spinacia oleracea* L.)	Chenopodiaceae, leafy vegetables
Cowpea (*Vigna sesquipedalis* Koern.)	Leguminosae, legumes
Kidney bean (*Phaseolus vulgaris* L.)	Leguminosae, legumes
Sweet pea (*Lathyrus odoratus* L.)	Leguminosae, legumes
Carrot (*Daucus carota* L. var. sativa Dc.)	Umbelliferae, root vegetables
Cherry radish (*Raphanus sativus* L. var. radculus pers)	Cruciferae, root vegetables
Fennel (*Foeniculum dulce* D.C.)	Umbelliferae, root vegetables
Long red radish (*Raphanus sativus* L.)	Cruciferae root vegetables
Mustard (*Brassica juncea* (Linnaeus) Czernajew)	Cruciferae, root vegetables
Radish (*Raphanus sativus* Linn.)	Cruciferae, root vegetables
Eggplant (*Solanum melongena* L.)	Solanaceae, solanaceous vegetables
Red pepper (*Capsicum annuum* Linn.)	Solanaceae, solanaceous vegetables
Sweet pepper (*Capsicum frutescens* Linn.)	Solanaceae, solanaceous vegetables
Tomato (*Lycopersicum esculentum* Mill.)	Solanaceae, solanaceous vegetables

oven-dried at 70°C to constant weight.

1.3 Analytical methods

Oven-dried shoot samples were ground using a Retsch-grinder (2 mm) made in Germany, digested with HNO_3 (Alexander et al., 2006). Samples of plant (0.5 g) were weighed into 200 mL block digestion tubes, concentrated nitric acid (10 mL) added and allowed to stand overnight. They were then heated for 3 hr) at 60°C, followed by 6 hr at 110°C. After cooling, the digests were passed through a pre-washed Whatman 540 filter paper (Whatman Company, UK). The digestion tubes were rinsed four times, passing through the filter and the filtrates made up to 50 mL volume using ultrapure water. Cadmium concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS, Elan 5000, PerkinElmer, USA). Blank and bush leaf material (BGW-07603) (China Standard Materials Research Center, China) were used for quality control. The Cd recovery rates were (90 ± 10)%.

To compare the relative response of vegetables tested to different Cd exposures, the index of yield response to stress (YRS, %) was calculated as follows (Yu et al., 2006):

$$YRS = (Y_{high} - Y_{low})/Y_{low} \times 100\%$$

where, Y_{high} and Y_{low} are the shoot yields (biomass) under the high Cd (2 mg/kg Cd) and low Cd (1 mg/kg Cd) exposures, respectively.

The results are presented as arithmetic means with standard errors. A statistical comparison of means of plant data was examined with one-way ANOVA followed by LSD test as available in the SPSS statistical package. Correlation coefficient analyses were conducted using Origin 7.0.

2 Results

2.1 Shoot yield response to stress (YRS) vegetables

Under either level of Cd exposure, the variation of shoot yield among vegetables was highly significantly ($P < 0.01$). Shoot yield was 0.79–6.67 g (average of 3.41 g) for 1 mg/kg Cd and 0.9–7.23 g (average of 3.46 g) per plant for 2 mg/kg Cd. The yield response to Cd stress (YRS) was significantly different among vegetables. Some vegetables, such as lettuce and cowpea increased shoot yields with higher soil Cd and other vegetables, such as radish and cucumber, decreased yields with higher soil Cd (Fig. 1). This suggests that yield variation due to species differences among vegetables was much greater than yield variation due to different of soil Cd. Therefore, the effects of Cd on vegetable growth and development appear to be species dependent.

2.2 Shoot Cd concentrations of 28 vegetables under Cd exposures

The differences in mean Cd accumulation among the 28 vegetable species under two Cd levels are shown in Fig. 2. Marked differences were exhibited among the 28 vegetables with regard to the mean concentrations of Cd accumulated in all the vegetables grown in the two Cd exposures. Under 1 mg/kg Cd exposure, shoot Cd concentration ranged from 0.01 (sweet pea) to 1.12 (spinach) mg/kg and averaged 0.35 mg/kg. Under 2 mg/kg Cd exposure, shoot Cd concentration ranged from 0.02 (sweet pea) to 2.86 (spinach) mg/kg, and averaged 0.43 mg/kg, which was 1.2 fold higher than with 1 mg/kg Cd. The correlation between shoot Cd concentrations under 1 and 2 mg/kg Cd exposure was significant ($R^2 = 0.85$, $n = 28$, $P < 0.001$, data not shown).

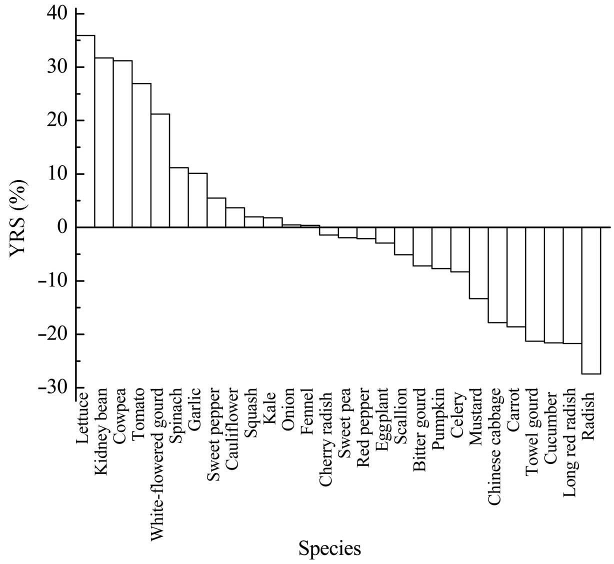

Fig. 1 Shoot yield response to stress (YRS) of the 28 vegetable species.

2.3 Shoot Cd concentrations of the cultivars of vegetables under Cd exposures

The differences of Cd accumulation between cultivars of the five vegetables under two Cd exposures are shown in Table 2. In cowpea, kidney bean, bitter gourd, cucumber and squash, the cultivars showed significant difference under the two Cd exposures ($P < 0.05$).

3 Discussion

The results in the present study clearly showed that the decrease in dry matter of shoots of vegetables grown in soil contaminated by Cd varied greatly. In this study, about 50% of the tested vegetables produced greater or similar shoot yield under 2 mg/kg Cd exposure than under 1 mg/kg Cd exposure. Also, the effect of Cd on vegetable species yield depended on species or cultivar specifically. This is consistent with previous findings showing effects of Cd pollution on vegetable species yield (Panwar et al., 1999; Belimov et al., 2003; Brennan and Mann, 2005). Panwar et al. (1999) reported that high Cd exposure (20 mg Cd/kg soil, pH 8.6) markedly decreased the dry matter of shoot of cowpea (*Vigna unguiculata* L. Wlap) and mungbean (*Vigna radiata* L.). Belimov et al. (2003) reported that the differential dry matter of shoots of pea cultivars grown in Cd exposures (5, 7 and 13 mg Cd/kg quartz sand, pH 5.5) was not related to uptake or accumulation of Cd in plants.

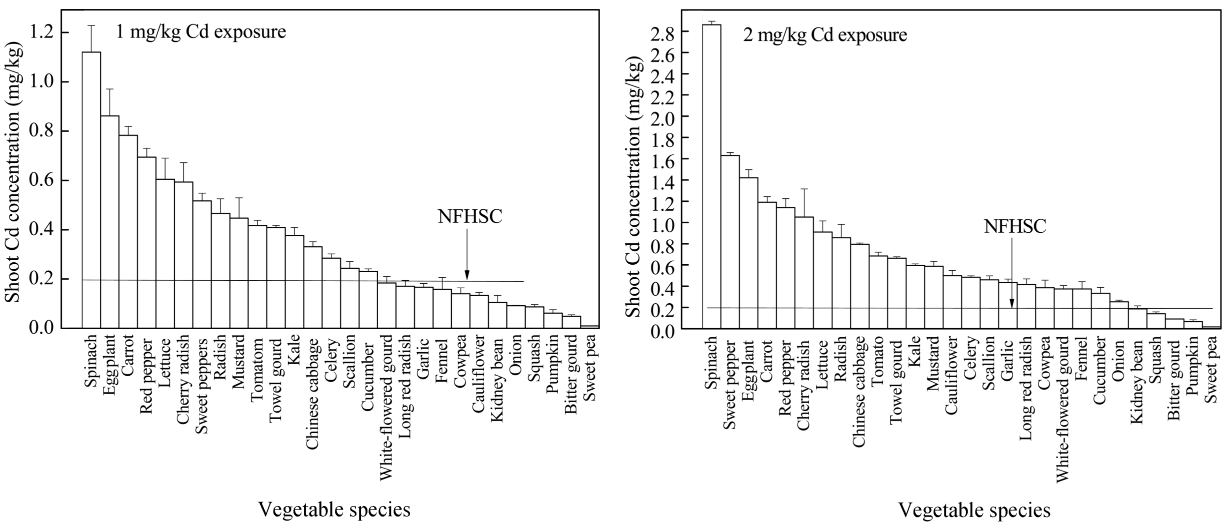

Fig. 2 Shoot Cd concentrations of the tested vegetable species under Cd exposures (mean ± SD, n = 4). NFHSC: The NATIONAL Food Hygiene Standard of China (NFHSC, GB 15201-94).

Table 2 Shoot Cd concentrations of the tested vegetable cultivars under 1 and 2 mg/kg Cd exposures

Species	Cultivars	Shoot Cd (mg/kg)	
		1 mg/kg Cd exposure	2 mg/kg Cd exposure
Cowpea	Jindijingdian	0.062 ± 0.015 b	0.127 ± 0.017 b
	Chaojihuangmei	0.080 ± 0.011 a	0.140 ± 0.021 a
	Hanfenglvwang	0.088 ± 0.009 a	0.143 ± 0.020 a
	Jingxuan No. 28	0.050 ± 0.003 b	0.108 ± 0.020 b
Kidney bean	Xinlvlong No. 2	0.078 ± 0.014 a	0.087 ± 0.007 b
	Huabijia	0.027 ± 0.004 b	0.049 ± 0.010 d
	Chaojisiji	0.038 ± 0.004 b	0.074 ± 0.005 c
	Baifeng	0.070 ± 0.013 a	0.099 ± 0.004 a
Bitter gourd	Dabai	0.027 ± 0.007 b	0.085 ± 0.013 c
	Zhongdu No. 4	0.046 ± 0.003 a	0.128 ± 0.010 ab
	Teyuchangbai	0.051 ± 0.007 a	0.110 ± 0.008 b
	Changlv	0.031 ± 0.005 b	0.068 ± 0.003 c
	Hanyudarou	0.051 ± 0.007 a	0.146 ± 0.034 a
Cucumber	Sijicuibai	0.101 ± 0.011 c	0.118 ± 0.007 c
	Qianchun No. 7	0.173 ± 0.022 a	0.205 ± 0.016 a
	Zhongnong No. 106	0.133 ± 0.013 b	0.154 ± 0.016 b
	Helanmini	0.137 ± 0.003 b	0.154 ± 0.013 b
	Baiyesan	0.149 ± 0.011 b	0.164 ± 0.009 b
Squash	Heimeili	0.131 ± 0.010 b	0.257 ± 0.017 a
	Zhonglu No. 1	0.127 ± 0.017 b	0.202 ± 0.008 b
	Zhonglu No. 2	0.144 ± 0.020 ab	0.253 ± 0.013 a
	Zhonglu No. 3	0.096 ± 0.005 c	0.163 ± 0.009 c
	Lvbaoshi	0.158 ± 0.010 a	0.253 ± 0.009 a

Mean values (± standard deviations) with different letters indicate a significant difference among cultivars of the same vegetable at $P < 0.05$ according to LSD test.

Brennan et al. (2005) reported that significant difference in dry matter of shoots were found between yellow lupin (*Lupinus leteus* L.) and narrow-leafed lupin (*Lupinus angustifolius* L.) grown in soil polluted by Cd (3.35 mg Cd/kg soil, pH 4.5). And they also reported that the decrease in dry matter of shoot of yellow lupin varied significantly among cultivars. According to our results, spinach, which had the highest Cd concentration in shoot, also had a higher shoot yield under 2 mg/kg Cd exposure than under 1 mg/kg Cd exposure. Consequently, farmers may not get sufficient warning about the toxic concentration of Cd in vegetable species based on yield alone. Therefore, the toxic concentration of Cd in vegetables can not be predicted only based on overall yield or biomass allocation.

The uptake and accumulation of Cd in vegetable species varied greatly under both greenhouse and field conditions (Pettersson et al., 1977; Lehoczky et al., 1998; Demirezen Aksoy, 2006). Based on our experiments, significant differences ($P < 0.05$) in the accumulation of Cd were found between the 28 vegetables species. At the 1 mg/kg Cd exposure 11 out of the 28 tested vegetables and at the 2 mg/kg Cd exposure 23 out of the 28 tested vegetables, both edible and non-edible portions, were found to below the NFHSC limit (⩽ 0.2 mg/kg Cd) (Fig. 2). Interestingly, the relative order Cd accumulation by the 28 vegetable species investigated follows exactly the classification by Kuboi et al. (1986) mentioned earlier. Sweet pea, kidney pea and cowpea, which had the lower Cd concentrations, are members of the Leguminosae, which were classed by Kuboi et al. (1986) as "low accumulators". Carrot, celery and fennel (Umbelliferae), and onion and scallion (Liliaceae), and cucumber, squash, pumpkin, white-flowered gourd, towel gourd and bitter gourd (Cucurbitaceae), had intermediate concentrations of Cd, which were classed as "moderate accumulators". The highest Cd concentrations found in this study were in lettuce (Compositae), spinach (Chenopodiaceae), red pepper, sweet pepper, tomato and eggplant (Solanaceae), and Chinese cabbage, mustard, kale, cauliflower, long red radish, radish and cherry radish (Cruciferae), which were classed as "high accumulators". Garlic, also had intermediate concentrations of Cd and is a member of the Amaryllidaceae family, which should be classed as "moderate accumulators". The results in the present study indicated that distinctive differences in Cd accumulation when comparing one vegetable to another, following the order: legumes (Leguminosae) < melon vegetables (Cucurbitaceae) < alliums (Amaryllidaceae and Liliaceae) < root vegetables (Umbelliferae and Cruciferae) < kail vegetables (Cruciferae) < solanaceous vegetables (Solanaceae) < leafy vegetables (Cruciferae, Compositae and Chenopodiaceae). There were also several studies that reported high level of metal accumulation in leafy vegetables than non-leafy vegetables (Bingham et al., 1979; Kuboi et al., 1986; Alexander et al., 2006; Wang et al., 2006). The difference in level of heavy metal accumulation between leafy vegetables and non-leafy vegetables might be due to their morpho-physiological differences in terms of heavy metal uptake, exclusion, accumulation, foliage deposition and retention efficiency (Carlton-Smith and Davis, 1986).

A lot of studies showed that the uptake, translocation and accumulation of Cd in vegetable cultivars also varied greatly (John and Van Laerhoven, 1976; Garate et al., 1993; McLaughlin et al., 1994; Belimov et al., 2003; Alexander et al., 2006). The present study also clearly showed that significant differences in Cd concentrations were found between cultivars of the 5 vegetables (cowpea, kidney pea, bitter gourd, cucumber and squash) grown in 1 and 2 mg/kg Cd exposures. Cowpea and kidney pea, which were classed as "low accumulators", had significant difference ($P < 0.05$) between cultivars with an average range of 0.003–0.05 mg/kg Cd (dry weight). Bitter gourd, cucumber and squash, which were classed as "moderate accumulators", had highly significant difference ($P < 0.01$) between cultivars with an average range of 0.003–0.094 mg/kg Cd (dry weight). Similar results have been reported by Alexander et al. (2006) when five cultivars of the six common vegetables (spinach, carrot, French bean, pea, onion and lettuce) were grown in soil contaminated with Cd.

Many studies revealed that a large variation existed among vegetables species or cultivars when subjected to Cd stress, suggesting that some importance of internal mechanisms in expression of the differential Cd tolerance in different vegetable species or cultivars (Li et al., 1995; Clarke, 1995; Penner et al., 1995; Belimov et al., 2003; Tiryakioglu et al., 2006). Li et al. (1995) reported that the expression of kernel Cd accumulation in sunflower hybrids was predominantly controlled by additive genetic effects. Clarke (1995) also reported that the differences in grain Cd concentrations among durum wheat cultivars were controlled by a single gene and genetic control over Cd concentrations was stronger than environmental influence. Penner et al. (1995) reported that modifying a gene governing Cd uptake has enhanced the possibility to select and breed crop cultivars with low Cd concentration in grain. Belimov et al. (2003) screened ninety-nine varieties of garden pea (*Pisum sativum* L.) for tolerance to Cd and found that the relationship between Cd tolerance, Cd accumulation and biomass production of pea plants was complicated and depended on the plant genotype, which indicating that, at least partial, the existence of an independent genetic control of these traits. Tiryakioglu et al. (2006) reported that some internal mechanisms, such as antioxidant defense system, may play important roles in expressing differential Cd tolerance in barley cultivars. The present study showed that significant positive correlation were found between concentration factor (CF) of vegetable species grown in 1 and 2 mg/kg Cd exposures, which was based on Cd in the plant divided by Cd in the soil (CF = M_{plant}/M_{soil}, where, M is the Cd concentration) ($R^2 = 0.85$, $n = 28$, $P < 0.001$, Fig. 3). The CF value is a function of the properties of the metal itself, especially the degree to which it is adsorbed in an unavailable form, soil properties, especially pH and the genotype of the plant (Alexander

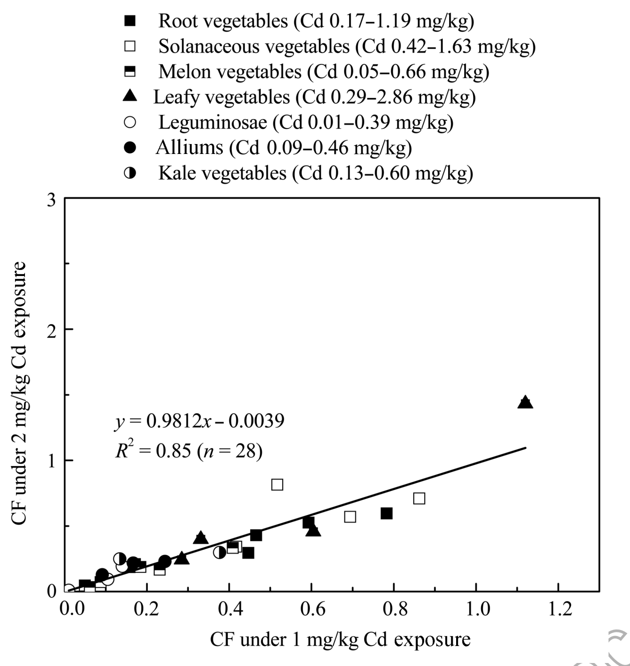

Fig. 3 Correlation between shoot Cd concentration under 1 mg/kg and 2 mg/kg Cd exposures.

et al., 2006). Therefore, our results indicated that the differential Cd uptake, accumulation and tolerance among the vegetable species studied may be strongly related to genetics.

4 Conclusions

This study clearly demonstrated that highly significant differences were evident among the selected 28 vegetables species when subjected to Cd exposures. The order of Cd accumulation by vegetable species was: leafy vegetables > solanaceous vegetables > kale vegetables > root vegetables > allimus > melon vegetables > legumes. Significant differences were also identified when comparing different cultivars of the five common vegetables (cowpea, kidney pea, bitter gourd, cucumber and squash) with an average range of 0.003–0.094 mg/kg Cd.

These results suggested that the uptake, translocation and accumulation of Cd in vegetable species, even at the cultivar level, might be strongly related to genetics. To fully understand the differences in Cd accumulation of different vegetable species or cultivars, further investigations are needed to examine the mechanisms of Cd uptake, translocation and accumulation, and on the genetic principles and genetic stability.

In addition, our results indicated that the toxic concentrations of Cd in vegetables can not be predicted only based on overall yield or biomass allocation.

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