Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Accumulation of mercury in rice grain and cabbage grown on representative Chinese soils*

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Received Jan. 4, 2013; Revision accepted Aug. 15, 2013; Crosschecked Nov. 15, 2013

Abstract: A pot culture experiment was carried out to investigate the accumulation properties of mercury (Hg) in rice grain and cabbage grown in seven soil types (Udic Ferrisols, Mollisol, Periudic Argosols, Latosol, Ustic Cambosols, Calcaric Regosols, and Stagnic Anthrosols) spiked with different concentrations of Hg (CK, 0.25, 0.50, 1.00, 2.00, and 4.00 mg/kg). The results of this study showed that Hg accumulation of plants was significantly affected by soil types. Hg concentration in both rice grain and cabbage increased with soil Hg concentrations, but this increase differed among the seven soils. The stepwise multiple regression analysis showed that pH, Mn(II), particle size distribution, and cation exchange capacity have a close relationship with Hg accumulation in plants, which suggested that physico-chemical characteristics of soils can affect the Hg accumulation in rice grain and cabbage. Critical Hg concentrations in seven soils were identified for rice grain and cabbage based on the maximum safe level for daily intake of Hg, dietary habits of the population, and Hg accumulation in plants grown in different soil types. Soil Hg limits for rice grain in Udic Ferrisols, Mollisol, Periudic Argosols, Latosol, Ustic Cambosols, Calcaric Regosols, and Stagnic Anthrosols were 1.10, 2.00, 2.60, 2.78, 1.53, 0.63, and 2.17 mg/kg, respectively, and critical soil Hg levels for cabbage are 0.27, 1.35, 1.80, 1.70, 0.69, 1.68, and 2.60 mg/kg, respectively.

Key words: Mercury accumulation, Soil safety, Soil types, Rice grain, Cabbage, Intake
doi:10.1631/jzus.B1300004 **Document code:** A **CLC number:** X53

1 Introduction

Mercury (Hg) is known as the only liquid metal found in many common products and processes that make use of its unique characteristics. Hg contamination sources include solid waste incineration, such as municipal and medical waste, coal and oil combustion, pyrometallurgical processes, and production of gold (Wang *et al*., 2004). Hg is a hazardous and nonessential element. It can enter into the human body by various means like inhalation, food intake, drinking water, and skin contact, and can damage human organs, including the developing nervous system, cardiovascular system, immune system, kidneys, and liver (Harada, 1995; Eto *et al*., 2010). Hg should be controlled strictly by any feasible strategy. National initiatives for control of Hg releases, limiting use and exposure were carried out in different countries (OECD, 1994). Environmental media standards, specifying a maximum acceptable Hg concentration for different media, were established. The World Health Organization (WHO) has been providing guidelines for drinking water and air quality with Hg limits since 1993 (WHO, 2004; 2005). However, soil environmental quality guidelines

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* Project supported by the Ministry of Environmental Protection of China (No. 2011467057) and the Fundamental Research Funds for the Central Universities of China
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regarding Hg contamination have still not been applied as a global, legally binding agreement until now.

Hg contamination in soil is a key concern because of its strong tendency for bioaccumulation in the food chain. Despite the lower concentration factors and bioaccumulation factors calculated for terrestrial food chains compared to aquatic and marine food chains, it is essential to monitor the fate of Hg in polluted terrestrial ecosystems to prevent possible harmful effects on wildlife and humans (Gnamuš *et al*., 2000). Agricultural products like rice, corn, and vegetables in Hg-contaminated areas (e.g., Guizhou China) were found to have high Hg concentrations, which has already affected the health of local people (Zhang *et al*., 2010). Hg concentration in different parts of various plant species demonstrated that the Hg accumulation threat between plant species is different (Rop *et al*., 2008). Currently, there is a lack of knowledge regarding Hg accumulation in plants growing on different soil types. Approximately 30 different types of soils have been classified across China (Gong *et al*., 2007). However, the Chinese standard of soils was not perfect. Chinese officials and researchers are following the Environmental Quality Standard of Soils established in 1995 (GB 15618-1995) to monitor food safety. These standards provide a basis for preventing Hg pollution, considering that pH of soil can impact Hg uptake by plants. Likewise, physicochemical characteristics of soils like organic matter (OM), particle size distribution (PSD), cation exchange capacity (CEC), Fe(II), Mn(II), and pH may also have an effect on the uptake of Hg (Yin *et al*., 1996; Morel *et al*., 1998; Fernández-Martínez *et al*., 2006; Khwaja *et al*., 2006). Therefore, it is much better that soil types and plant species are considered for establishing the Environmental Quality Standard of Soils (John, 1972; Rop *et al*., 2008).

The present study was conducted to identify a method to regulate the soil Hg limit to assure the safety of agricultural products. To achieve this goal, we investigated differences in this relationship among different soil types collected from different areas of China using pot experiments. This study provides new information about Hg accumulation in rice grain and cabbage that can be incorporated into revisions of the Environmental Quality Standard of Soils on the basis of soil types from different areas.

2 Materials and methods

2.1 Experimental soil collection and preparation

Seven soils, Udic Ferrisols (UF), Mollisol (M), Periudic Argosols (PA), Latosol (L), Ustic Cambosols (UC), Calcaric Regosols (CR), and Stagnic Anthrosols (SA), were collected respectively from Nanning (107°45′–108°52′ E, 22°13′–23°33′ N), Harbin (125°42′–130°10′ E, 44°04′–46°40′ N), Huzhou (119°14′–120°29′ E, 30°22′–31°11′ N), Guangzhou (113°14′–113°34′ E, 22°45′–23°05′ N), Jinan (116°54′–117°02′ E, 36°35′–36°40′ N), Chengdu (102°54′–104°53′ E, 30°05′–31°26′ N), and Ningbo (120°55′–122°16′ E, 28°51′–30°33′ N) for this study.

Soil samples were collected from the surface layer (0–20 cm). The soil samples were air-dried, ground to pass through a 2-mm sieve, and stored in plastic bags until analyses. Physicochemical characteristics of the soils were measured in the Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, China. Nitrogen, phosphate, and potassium contents of soils were measured by the colorimetric method (Onduru and Du Preez, 2007), pH was measured using a 1:2.5 (w/w) soil to water ratio (Chaturvedi and Sankar, 2006), redox potential (Eh) was measured by the potentiometric method (Munichandraiah *et al*., 2003), CEC was measured by an ammonium acetate method (Hendershot and Duquette, 1986), OM was measured by the Walkley Black method (Ryan *et al*., 2007), PSD was measured by the hydrometer method (Gee and Baunder, 1986), and Fe(II) and Mn(II) were measured by a chromatographic method (Schnell *et al*., 1998). Physicochemical characteristics of the experimental soils are listed in Table 1.

2.2 Pot experiments

Pot experiments were carried out for this research in a greenhouse. Two plant species, rice (*Oryza sativa*) and cabbage (*Brassica chinensis* L.), were evaluated in the experiments. Beyond investigating differences among background Hg levels in different soil types, we also spiked each soil with additional Hg(II) as $HgCl_2$ to examine the relationship between increasing soil Hg concentration and Hg uptake in the plant. Soils were spiked with different concentrations of Hg (background level, 0.25, 0.50, 1.00, 2.00, and 4.00 mg/kg), which were signed by

Hg-CK, Hg-0.25, Hg-0.5, Hg-1, Hg-2, and Hg-4 in data tables, respectively. All spiked soil samples were aged for six months at a moisture content of 70% of water holding capacity prior to pot experiments, which made the soils homogenized after the addition. Each treatment had 3 replications (i.e., 3 pots per treatment) and each pot contained 6 plants. Pots were filled with 8 kg soils. Greenhouse temperature was maintained at (20±2) °C during a daily 16-h photoperiod and (15±2) °C during darkness. Planting management practices like regular watering and deinsectization were carried out daily to maintain better plant growth. Rice grain and cabbage were planted in June and September, respectively, and harvested in the consuming stage of ripeness, rice after 135 d and cabbage after 70 d. After harvesting, the plants were moved to the laboratory, cleaned with deionized water, and dried with a lyophilizer. The dry weights of rice grain and cabbage were recorded and the samples were ground with an agate mill and passed through a 60-mesh sieve before analyses.

2.3 Chemical and statistical analyses

Samples of soils (0.2 g) and plants (1.0 g) were digested with HNO_3-$HClO_4$-HF (5:5:1) acid mixture and diluted up to 25 ml with deionized water (Shentu *et al*., 2008). Hg concentrations in plants and soils were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Agilent 7500a, USA). HNO_3-EDTA was used as a cleaning fluid to eliminate the residual effect of Hg using ICP-MS (Li *et al*., 2006). Standard reference materials (SRMs) for soils and plant samples were included in the digestion and analysis as a part of the quality control protocol, and 3 replications were conducted for each sample. The analytical results showed that the recoveries for SRMs were nearly 98% and that the precision and bias of the analysis were generally <5% for Hg. Statistical analyses, including one-way analysis of variance (ANOVA) and multiple regression analysis, were performed using Statistic Package for Social Sciences (SPSS 18.0).

3 Results

3.1 Physicochemical characteristics of the seven types of soil

The physicochemical characteristics of the seven soils are shown in Table 1. Udic Ferrisols, Periudic Argosols, Latosol, and Stagnic Anthrosols were the acid soils, and Mollisol, Ustic Cambosols, and Calcaric Regosols are alkaline. Udic Ferrisols showed the highest bulk density of the seven soils, while Latosol showed the lowest one. Latosol is also loaded with manganite, which is two or three times more than that of other soils. Mollisol had the highest OM and CEC of the seven soils.

Table 1 Physicochemical characteristics of the soils used in this study

Soil type	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	pH	OM (g/kg)	CEC (cmol/kg)	Eh (mV)
Udic Ferrisols	1.72±0.12	2.94±0.12	2.28±0.11	4.43±0.07	19.08±0.15	17.33±1.96	360±23
Mollisol	2.65±0.08	1.01±0.06	6.63±0.09	7.23±0.08	32.19±0.32	34.00±2.51	280±46
Periudic Argosols	1.14±0.07	1.72±0.08	7.27±0.26	4.85±0.06	11.56±0.17	12.63±1.52	356±28
Latosol	1.12±0.04	2.87±0.07	0.41±0.06	5.16±0.05	6.37±0.56	8.33±2.14	388±19
Ustic Cambosols	1.28±0.10	1.11±0.04	3.00±0.08	7.80±0.02	7.54±0.20	15.80±1.62	248±36
Calcaric Regosols	1.46±0.07	1.00±0.07	9.53±0.15	8.02±0.04	21.80±0.14	25.47±1.46	300±26
Stagnic Anthrosols	1.58±0.11	0.60±0.01	11.04±0.13	6.49±0.03	21.40±0.34	20.20±1.41	286±31
Soil type	**Fe(II) (mg/kg)**	**Mn(II) (mg/kg)**	**Sand (%)**	**Coarse silt (%)**	**Medium-fine silt (%)**	**Clay (%)**	**Background Hg (μg/kg)**
Udic Ferrisols	34.41±1.48	36.8±0.23	10.6±0.15	14.2±1.26	25.6±0.43	49.6±1.19	81.3±6.7
Mollisol	30.48±0.56	109.2±5.21	20.6±1.54	34.2±1.46	26.0±0.89	19.2±1.24	76.1±7.5
Periudic Argosols	34.33±0.78	133.7±6.45	24.8±0.65	26.2±1.16	32.0±0.87	17.0±0.34	62.0±0.4
Latosol	24.66±0.75	403.4±4.23	37.4±0.96	19.6±1.39	21.2±0.82	21.8±0.82	35.4±10.7
Ustic Cambosols	27.59±0.47	139.6±5.63	21.6±1.29	51.2±1.48	14.2±2.01	13.0±1.05	83.2±3.0
Calcaric Regosols	30.20±0.75	263.6±10.10	31.6±0.57	16.0±0.68	28.0±0.67	24.4±1.32	56.3±7.3
Stagnic Anthrosols	42.36±0.89	230.6±9.42	11.4±0.26	37.6±1.08	35.4±1.35	15.6±1.17	154.8±4.9

Data are expressed as mean±standard deviation (n=3)

3.2 Hg accumulation of plants

Hg concentrations in rice grain and cabbage varied with Hg levels and types of soils (Fig. 1). However, the variations of Hg concentration in rice grain and cabbage were not significant (P>0.05). The accumulation of Hg was lower in rice grain than in cabbage under the same soil and level of contamination with a mean (and range) of 27.4% (3.4%–71.7%).

Hg concentration in plants showed a highly significant difference (P<0.01) between soil Hg levels. The result showed a significant increase in the mean of Hg concentration in both rice grain and cabbage in all soil types with elevated Hg level in soils. Meanwhile, there was a significant difference of Hg accumulation in plants grown under different soil types (P<0.05). The highest Hg accumulation of grain was noticed in rice grown under Calcaric Regosols, with mean (range) of Hg concentration in rice grain of 94.19 (6.19–218.79) μg/kg, while the lowest Hg accumulation of rice grain was found in Latosol, with mean (range) of Hg concentration in rice grain of 39.09 (7.64–68.88) μg/kg. In cabbage, the highest Hg accumulation occurred in Ustic Cambosols, with mean (range) of Hg concentration of 92.81 (8.07–209.29) μg/kg, while the lowest Hg accumulation of rice grain was found in Stagnic Anthrosols, with mean (range) of Hg concentration of 43.96 (11.55–82.00) μg/kg.

3.3 Statistical analyses

A positive relationship was observed between Hg concentration in rice grain and cabbage plants and Hg concentration in soils for all soil types depending on the result of a two-way ANOVA with post-hoc multiple comparisons (Fig. 2). Significant differences

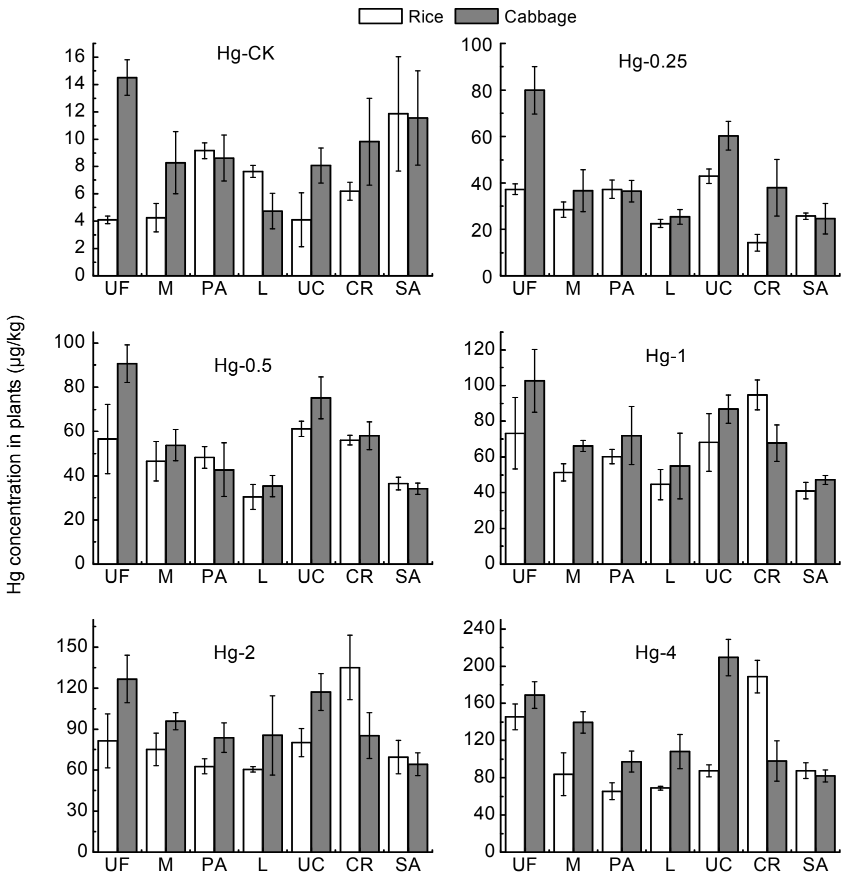

Fig. 1 Hg concentrations in rice and cabbage plants grown in different soils spiked with different concentrations of Hg
Data are expressed as mean±standard deviation (n=3). UF: Udic Ferrisols; M: Mollisol; PA: Periudic Argosols; L: Latosol; UC: Ustic Cambosols; CR: Calcaric Regosols; SA: Stagnic Anthrosols

in Hg concentrations in both types of plants among the different soil types were observed (P<0.05). To further understand the relationship between Hg concentration in plants and Hg concentration in soils, multiple regression analysis was used to examine the relative roles of soil Hg concentration and other physicochemical characteristics of the soil (McIntyre *et al*., 1983). Five soil variables (Hg concentration, pH, coarse silt content, Mn(II) concentration, and CEC) and four soil variables (Hg concentration, medium-fine silt content, Mn(II) concentration, and clay content) showed a significant correlation (P<0.01) and they were optioned by stepwise regression on the basis of significance with Hg concentration in rice grain (R^2=0.806) and cabbage (R^2=0.867), respectively. These significant studies showed that these soil variables may influence the Hg accumulation in plants. Multiple regression results for both rice grain and cabbage indicated a strong positive relationship between Hg concentration in the plant and Hg concentration in the soil, clay content of the soil, and soil pH. In contrast, silt content, Mn(II) concentration, and CEC of the soil showed a negative relationship to Hg concentration in both plants.

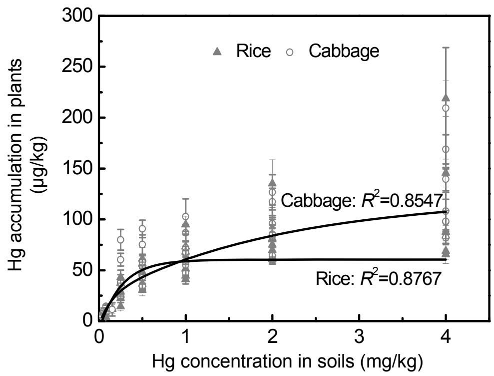

Fig. 2 Correlation of Hg concentration in rice grain and cabbage and Hg concentration in soil for all soil types

4 Discussion

4.1 Relationships between Hg accumulation in plants and soil Hg concentrations and soil types

The difference between Hg accumulations of rice grain and cabbage in seven types of soils is obvious according to Fig. 1. However, Fig. 2 showed that Hg accumulation of cabbage was basically higher than that of rice. This result confirmed that the Hg accumulation capacity of vegetables is higher than that of herbage (Huckabee *et al*., 1983), which may be relevant to the difference between self-defensive abilities of various plants (Yadav, 2010).

The effect of pH on ionic balance processes of Hg is quite different from other heavy metals like cadmium or zinc (Sims, 1986; Appel and Ma, 2002). The hydroxide form of Hg like $HgOH^+$, HgOHCl, and $Hg(OH)_2$ can be absorbed more easily than the Hg-Cl form, which will exponentially increase as pH rises (Yin *et al*., 1996). The highest Hg concentrations in rice grain and cabbage were observed from plants grown in soils with relatively high pH, for example Calcaric Regosols (pH=8.02) and Ustic Cambosols (pH=7.80), while Hg concentration in both rice grain and cabbage grown in Periudic Argosols (pH=4.85) was lower. This indicated the importance of soil pH in controlling the uptake of Hg by experimental plants. PSD was important for Hg adsorption in the soil; for example, silt weakly adsorbs Hg, while clay strongly adsorbs it (Loring and Rantala, 1992). In addition, as Hg is volatile, a soft structure like silt causes Hg to leave the soil much more easily than a compact structure like clay (Cunningham and Ow, 1996). In Udic Ferrisols, the percentage of clay (49.6%) was approximately three times more than that in other soils, and Hg accumulation in both rice grain and cabbage was recorded as higher, even pH of Udic Ferrisols was only 4.43. Manganese oxide is widely considered to be a good tool for remediation of heavy metal contamination in soils (Spark *et al*., 1995; Chou *et al*., 2001; Daniels *et al*., 2001). Specific adsorption of manganese oxide can prevent Hg from being absorbed by plants (Kooner, 1993). In Latosol, manganese oxide is much more prevalent than in other soil types, so Hg accumulations of rice grain and cabbage in Latosol were low. CEC was reported to have a relationship with the heavy metal uptake by plants, and high CEC blocked heavy metal to enter into plants (Mathur *et al*., 1979). However, CEC had no significant correlation with Hg accumulation of cabbage based on statistical analyses of this study.

4.2 Assessment of different soil limits of Hg in rice grain and cabbage

Based on the extent of Hg accumulation in the rice grain and cabbage plants grown in different representative soil types, acceptable soil Hg levels for agricultural soils based on WHO Food Safety Guidelines can be identified. The criteria for Hg pollution in different soils based on the limits of Hg in rice grain and cabbage were evaluated according to the guidelines of the WHO. Total Diet Studies recommended by WHO, assess the daily food intake of humans based on a survey of the total consumer diet sample. Accordingly, the quantities of rice grain and cabbage consumed were 83.3 and 75.5 g/d, respectively (Muñoz *et al*., 2005). According to WHO (2002), daily allowed intake of Hg was 5 μg/d. According to the information above, the maximum level of Hg was 60.0 and 66.2 μg/kg in rice grain and cabbage, respectively. The regression equations for estimating safety level of soil Hg contamination were established based on Hg accumulation of plants from soils. The results are shown in Table 2.

Based on the results of the above mentioned analysis, soil safety for Hg can be evaluated according to the types of soils. The Chinese environmental quality standard for Hg in soils established in 1995 (GB 15618-1995) is only based on soil pH, but from the results mentioned above it is much more comprehensive and practical to evaluate the environmental safety of soils based on soil types rather than only by soil pH. In addition, identification of soil types is easier than conducting an assessment of the physicochemical characteristics of soils. In terms of the standard established following the present results of this experiment, higher levels of Hg contamination do not pose a food safety threat in Periudic Argosols and Latosol, as the uptake of Hg in rice grain and cabbage grown in these soils remained very low. However, rice grown in Calcaric Regosols or cabbage grown in Udic Ferrisols should be of more concern for dietary toxicity due to high plant uptake of Hg in these soil types.

5 Conclusions

Hg concentration in both rice grain and cabbage increased with increased soil Hg concentrations, but this increase differed among the experimental soils. Soil Hg concentrations and soil types have a far greater impact on the Hg accumulation of plants rather than plant species. Physicochemical characteristics of soils like OM, PSD, CEC, Fe(II), and Mn(II) have a significant correlation with Hg accumulation in rice grain and cabbage. Limits of Hg in different soils for rice and cabbage cultivation were identified based on the diet habits of the population, daily allowed intake of Hg, and Hg accumulation of plants from different soil types. Hg limits for rice grain in Udic Ferrisols, Mollisol, Periudic Argosols, Latosol, Ustic Cambosols, Calcaric Regosols, and Stagnic Anthrosols were 1.10, 2.00, 2.60, 2.78, 1.53, 0.63, and 2.17 mg/kg, respectively, and for cabbage were 0.27, 1.35, 1.80, 1.70, 0.69, 1.68, and 2.60 mg/kg, respectively. The results indicated that accumulation of Hg that varies depending on soil type should be considered in standardization of soil safety against Hg contamination in rice grain and cabbage. The results of this study suggested that the selection of proper plant species with proper soil type can help us to avoid the toxicity of Hg in our daily diet.

Table 2 Regression equations based on Hg accumulation of plants from soils and soil Hg limits for potential dietary toxicity in edible parts of rice and cabbage calculated from the estimated safe and adequate daily dietary intake

Soil type	Rice			Cabbage		
	Regression equation	R^2	Limit of soil Hg (mg/kg)	Regression equation	R^2	Limit of soil Hg (mg/kg)
Udic Ferrisols	y=0.03x+0.027	0.8981	1.10	y=0.03x+0.058	0.7734	0.27
Mollisol	y=0.017x+0.026	0.7503	2.00	y=0.029x+0.027	0.9300	1.35
Periudic Argosols	y=0.01x+0.034	0.5011	2.60	y=0.019x+0.032	0.7574	1.80
Latosol	y=0.014x+0.021	0.8188	2.78	y=0.026x+0.022	0.8152	1.70
Ustic Cambosols	y=0.015x+0.037	0.5733	1.53	y=0.044x+0.036	0.9311	0.69
Calcaric Regosols	y=0.052x+0.027	0.8659	0.63	y=0.018x+0.036	0.7316	1.68
Stagnic Anthrosols	y=0.018x+0.021	0.9065	2.17	y=0.017x+0.022	0.8870	2.60

x represents Hg concentration in soils, y represents Hg accumulation of plants

Compliance with ethics guidelines

Chun-fa LIU, Cheng-xian WU, Muhammad T. RAFIQ, Rukhsanda AZIZ, Dan-di HOU, Zhe-li DING, Zi-wen LIN, Lin-jun LOU, Yuan-yuan FENG, Ting-qiang LI, and Xiao-e YANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Cadmium accumulation in different pakchoi cultivars and screening for pollution-safe cultivars
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doi:10.1631/jzus.B1100356
J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.), 2012 Vol.13 No.6 P.494-502

Abstract: The selection and breeding of pollution-safe cultivars (PSCs) is a practicable and cost-effective approach to minimize the influx of heavy metal to the human food chain. In this study, both pot-culture and field experiments were conducted to identify and screen out cadmium pollution-safe cultivars (Cd-PSCs) from 50 pakchoi (*Brassica rapa* L. ssp. *chinensis*) cultivars for food safety. When treated with 1.0 or 2.5 mg/kg Cd, most of the pakchoi cultivars (>70%) showed greater or similar shoot biomass when compared with the control. This result indicates that pakchoi has a considerable tolerance to soil Cd stress. Cd concentrations in the shoot varied significantly ($P<0.05$) between cultivars: in two Cd treatments (1.0 and 2.5 mg/kg), the average values were 0.074 and 0.175 mg/kg fresh weight (FW), respectively. Cd concentrations in the shoots of 14 pakchoi cultivars were lower than 0.05 mg/kg FW. In pot-culture experiments, both enrichment factors (EFs) and translocation factors (TFs) of six pakchoi cultivars were lower than 1.0. The field studies further confirmed that the Hangzhouyoudonger, Aijiaoheiye 333, and Zaoshenghuajing cultivars are Cd-PSCs, and are therefore suitable for growth in low Cd-contaminated soils (≤1.2 mg/kg) without any risk to food safety.