Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 168–172
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Variation of grain Cd and Zn concentrations of 110 hybrid rice cultivars grown in a low-Cd paddy soil

SHI Jing, LI Lianqing, PAN Genxing*

Institute of Resource, Ecosystem and Environment of Agriculture, Nanjing Agricultural University, Nanjing 210095, China.
E-mail: shjing1980@yahoo.com.cn

Received 01 March 2008; revised 23 April 2008; accepted 23 May 2008

Abstract

Enhanced Cd uptake and Zn depletion in rice grains and high potential for food Cd exposure by the high-yielding hybrid cultivars of China had been addressed. A field experiment was conducted in 2006 to determine the difference in grain Cd and Zn between cultivars. Total 110 cultivars including super rice and common hybrid rice cultivars were grown on a single paddy soil (Entic Haplaquept) with a neutral reaction and low total Cd content. Grain Cd and Zn concentrations were determined with graphite atomic adsorption spectrophotometer (GFAAS) and flame atomic adsorption spectrophotometer (AAS) respectively. Wide variation of Cd content in grain was found in a range of 0.004–0.057 mg/kg, while the Zn content in a range of 10.25–30.06 mg/kg among the cultivars. Higher Cd but lower Zn concentration in grains of super rice cultivars was observed compared to the common hybrid ones. A highly significant positive linear correlation of grain Cd/Zn with grain Cd was found for super rice and common hybrid cultivars, meanwhile much higher slope for these hybrid cultivars than the reported non-hybrid cultivars was also observed. Using the limit value of the Chinese chemical guidelines for foods (MOHC and SSC, 2005), calculated potential risk of food Cd exposure with "Zn hungry" through diet intake was prominent with all the studied 110 hybrid rice cultivars, possessing high potential health problems for rice production in South China using the super rice cultivars. Breeding of genotypes of rice cultivars with low grain Cd and low Cd/Zn ratio is needed for rice production in acidic red soils where Cd bioavailability is prevalently high.

Key words: rice cultivar; rice grain; cadmium; zinc; genotype difference

DOI: 10.1016/S1001-0742(08)62246-9

Introduction

Intake of heavy metals via the soil-crop-food chain has been considered as the predominant pathway of human exposure to potentially toxic heavy metals (Chang *et al*., 2002). Among the major staple crops, rice is the particular one with high Cd uptake and accumulation (Chaney *et al*., 2004). Recently, special attention has been paid to Zn-Cd relation in rice as Zn-deficient diets were evidenced to enhance the potential health risk of Cd for animal and even people (Reeves and Chaney, 2004; Liu *et al*., 2005; Gong and Pan, 2006a). Insufficiency of Zn in human diet may occur with monotonous vegetarian diets in areas with Zn-deficient soils (Chaney *et al*., 2005). In China, rice is consumed at about 200 kg per capita annually and is produced predominantly in South China where the soils are high in Cd mobility (Wang *et al*., 2003; Cao *et al*., 1999) and/or deficient in Zn. However, for meeting the demand by the increasing population and decreasing arable lands, high-yielding hybrid rice or super rice cultivars with high Cd affinity (Wang and Gong, 1996; Wu *et al*., 1999; Gong *et al*., 2006b) are under extension in China, which may raise the potential health problems due to increasing of Cd intake and deficiency of Zn.

There have been many studies on difference in abilities of crop species to uptake potentially toxic heavy metals in their edible parts (Yu *et al*., 2006). Even within one species, there are also great genotypic differences in grain concentration (Li *et al*., 1995; Wu and Zhang, 2002; Liu *et al*., 2003, 2005). To address the variation in Cd and Zn uptake with the cultivars and screen every cultivar with both low Cd and Cd to Zn ratio, 110 rice cultivars were selected to grow in a single rice paddy soil and the Cd and Zn concentrations in grain were determined for comparison.

1 Rice cultivars

Total 110 hybrid rice cultivars of different genotypes were used in the experiment, including 98 common hybrid rice cultivars and 12 super rice cultivars. The growing period of the cultivars was within (155 ± 20) d. The seeds of these cultivars were ordered from rice breeding agencies such as seed companies, breeding institutes of Sichuan, Yunnan, Guangxi of South China. Rice seeds

* Corresponding author. E-mail: gxpan@njau.edu.cn

were submerged in a water bath for about 48 h at room temperature and germinated under moisture condition (seeds were covered with two layers of moist gauze cloth) at 32°C for another 30 h. The germinated seeds were grown in a nursery paddy soil till 3 leaves appearing. Then seedlings were transplanted in the experiment plot.

1.1 Field experiments and soil condition

The field experiment was carried out at the Experiment Farm of Nanjing Agricultural University located in Jiangpu, Nanjing, China. Being neutral reaction (Table 1), the soil is classified as Entic Haplaquept (USDA, 1999). Fifty rice seedlings of a single cultivar were transplanted in an area of 2 m^2. The experiment was conducted in triplicate and arranged in a random completely block design (RCBD). A protection row at width of 0.5 m was set around the experimental plots, which had one opening as the inlet and another as the outlet for irrigation water. Fertilizer of N, P, K were applied 3 times during the rice growth: (1) as basal with N, P, and K on the 5th day in advance of seedlings planting, (2) when tillering, and (3) when spiking with only N. The total dressing of N, P, K was applied at 200 kg N/hm^2 as urea, 100 kg P_2O_5/hm^2 as calcium magnesium phosphate and 150 kg K_2O/hm^2 as potassium chloride. All the phosphorus and potassium fertilizer and 60% of N as urea were applied as basal dressing before transplantation.

1.2 Sample preparation, and Cd and Zn determination

Whole plant of rice was sampled in triplicate when ripening as removal from the soil randomly from each plot and separated into subsamples of root, shoot, and grain respectively, after washing with deionized water. The subsample was transferred to a paper bag and dried in an air-forced oven at 60°C for 48 h. Dry plant material was ground in a stainless steel blender to pass through a 0.4-mm sieve. All samples were stored at ambient temperature and humidity.

Three topsoil samples from the plot were subject to mixed acid digestion (HNO_3-$HClO_4$-HF). A certified reference material of sediment GBW 07406 with (0.13 ± 0.04) mg/kg Cd and (97 ± 9) mg/kg Zn was purchased from the National Centre for Certificate Reference Materials, China, and used with all patch of digestions. Dried plant samples were digested in a 4:1 (*V/V*) mixture of HNO_3-$HClO_4$ (Lu, 2000). Ten milliliter of mixed acids was added to 0.75 g of plant sample in a high-walled beaker and allowed to stand for 12 h at 25°C. The samples were then heated in a sand bath at 170°C until clear. After cooling, the solution was diluted to 25 mL with deionized water and filtered through a Xinhua No.11 filter paper (< 0.45 μm, China Xinhua Filter Company). Two certified plant reference materials GBW 07602 with (0.14 ± 0.01) mg/kg Cd and (20.6 ± 1.0) mg/kg Zn and GBW 07605 with (0.057 ± 0.008) mg/kg Cd, and (26.3 ± 0.9) mg/kg Zn were purchased from the National Centre for Certificate Reference Materials, China, and used respectively with all patches of digestions. The recovery of Cd and Zn were in the range of 81.76%–118.61% and 92.02%–109.92%, respectively.

The total Zn and Cd contents of the digested solutions were determined by flame atomic adsorption spectrophotometry (AAS) and graphite furnace AAS (TAS986, Puxi Analytical Instrument Company, China), respectively. A reagent blank was incorporated within each batch of analytical samples. The chemical reagents used in the analysis were in guaranteed reagent grade.

1.3 Statistical analysis

Statistical analysis was conducted using statistical software SPSS. The statistical significance level was defined at $P \leqslant 0.05$.

2 Results and discussion

2.1 Variation of Cd and Zn concentrations in grain

The Cd concentration of unpolished rice grain of the 110 cultivars ranged from 0.004 to 0.057 mg/kg with a mean of 0.022 mg/kg. In contrast, the grain Zn contents ranged from 10.25 to 30.06 mg/kg with a mean of 19.16 mg/kg. The variability coefficients of Cd, Zn, and Cd/Zn ratio among the 110 rice cultivars are 51.30%, 24.66%, and 61.08%, respectively (Table 2). Apparently, there is much higher variability with grain Cd than with Zn. Grain Zn concentration here is in higher consistency with the reported results (Pongsakul and Attajarusit, 1999; Marr *et al*., 1995), although the cultivars were from a diverse

Table 1 Physicochemical properties of the topsoil (0–20 cm)

pH (H_2O)	Org C (g/kg)	CEC (cmol/kg)	Available P (mg/kg)	Total Cd (mg/kg)	Total Zn (mg/kg)
6.23	10.54	16.63	18.09	0.15	19.00

CEC: cation exchange capacity of soil.

Table 2 Grain concentrations (mg/kg) of Cd and Zn, and the Cd/Zn ratio of 110 rice cultivars

Concentration	Cd	Zn	Cd/Zn
Maximum	0.057 (Zhongzheyou-1)	30.06 (R234)	0.0039 (Teyougui-3)
Minimum	0.004 (J314)	10.25 (45A/998)	0.00014 (J196)
Arithmetic mean	0.022	19.16	0.001
Middle value	0.020 (Jinyou-398, Teyou-559, TianART, P742)	19.34 (R287, J296)	0.0011 (IIyou-559, Jinyou-398)
Standard error	0.011	4.72	0.00078
Coefficient of variation (%)	51.30	24.66	61.08

The number or characters in the brackets are the name of the cultivars while breeded.

range of locations, rice varieties, soil conditions. Over half of the cultivars had grain Cd below the mean of 0.022 mg/kg, and about 10% of the cultivars (including 5 super rice cultivars) had grain Cd over 0.04 mg/kg, as double as the mean value (Fig. 1). Nevertheless, over 12% of the cultivars (14 cultivars) had a grain Cd under 0.0l mg/kg. This result indicates that low Cd cultivars may be screened from common hybrid cultivars but super rice ones. The Chinese State Standard Committee set up 0.2 mg Cd/kg as limit for rice while the Codex Committee draft of provisional maximum level (ML) for Cd in rice grains was 0.4 mg/kg (CCFAC, 2005). In this study, grain Cd concentrations of rice cultivars growing in this neutral paddy soil were all below these two guidelines. The low grain Cd concentrations could be attributed to low soil indigenous Cd and low availability with pH over 6.0. In contrast, Li *et al*. (2003) reported that Cd concentrations in the rice grain varied in the range of 0.026–0.199 mg/kg of the 57 rice cultivars grown in a paddy soil with pH of 6.54 with total soil Cd content of 0.43 mg/kg. As reported by Wang and Stuane (2003) and Wang *et al*. (2003), rice grain Cd level could increase a lot when grown in acidic soils such as the red-soil region. Li *et al*. (2005) reported that grain Cd concentration of common rice cultivars planted in a red soil with pH 4.95 and Cd-spiked treatment were as 14-fold as in a paddy soil with pH 6.54 and non-spiked Cd. Similarly, Gong *et al*. (2006b) have stated that a hybrid rice cultivar grown in a red earth paddy of pH 5.25 had grain Cd 13-fold as grown in a typical paddy of pH 5.84 and Cd-spiked treatment. In other words, Cd accumulation by 10 folds may be expected when grown in a soil with pH lower by 1 unit and soil Cd level increased by 3 folds. This would infer that such variation of pH and the increase of soil total Cd to the level of 0.4–0.5 mg/kg, is very common in red soil region of China. We assume these 110 hybrid rice cultivars planted in the red soil region as in the case of Li *et al*. (2005) and Gong *et al*. (2006b). This would infer that there would be about 10% of the cultivars (including 5 super rice cultivars) grain Cd concentrations over 0.4 mg/kg (ML for Cd by CCFAC). While over 12% of the cultivars (14 common hybrid cultivars, no super rice) grain Cd under 0.1 mg/kg (half of the limit value 0.2 mg/kg by CFCG).

About 50% cultivars and, 10 out of total 12 super rice cultivars, had grain Zn below the total mean of 19.16 mg/kg. A daily dietary intake of Zn could be estimated at 10.7 mg/d assuming the consumption of rice with the mean grain Zn here (19.16 mg/kg), which is close to the Chinese daily intake recommendation (RNI) of Zn at 15 mg/d for adults, but much under the maximum content of Zn (50 mg/kg) proposed by CFSG, suggesting a possible dietary intake deficiency for the monotonous vegetarian diets in China.

2.2 Relationship between Cd and Zn in rice grains

Table 3 shows the difference in grain Cd, Zn contents and the ratio of Cd/Zn between the studied two groups of hybrid rice. The super rice cultivars had a significantly higher grain Cd uptake and lower grain Zn uptake than the common hybrid rice cultivars. The calculated mean Cd/Zn ratio in grain was 0.0019 for super rice cultivars and 0.0012 for common hybrid rice cultivars.

Mean grain Cd content and Cd/Zn ratio of super rice cultivars were significantly higher than that of common hybrid by 67.86% and 72.43%, respectively. However, no significant difference in rice grain Zn content was observed. A comparison of grain Cd/Zn ratio between hybrid rice and super rice in our experiment revealed limited uptake of Zn by these rice cultivars of high Cd/Zn ratio. A highly significant positive linear correlations of grain Cd/Zn with grain Cd content for super rice and hybrid rice studied was observed (Fig. 2a) in comparison to that of hybrid and conventional rice (Fig. 2b) calculated by Li *et al*. (2003). The higher slope of the linear regression curve for all the hybrid rice compared to that for conventional rice indicated Zn deficiency is enhanced with super rice cultivars (Gong and Pan, 2006a).

Simmons *et al*. (2003) studied the Cd and Zn level of rice and soybean grains produced in a region of Thailand where soils were contaminated by Zn and Cd in mine area and concluded that rice was a particular crop without increase Zn content and with increased Cd in rice

Table 3 Grain Cd and Zn contents and Cd/Zn ratio of the two groups of hybrid rice

	Cd (mg/kg)	Zn (mg/kg)	Cd/Zn
Super rice	0.034 ± 0.015 a	16.87 ± 2.79 a	0.0019 ± 0.0018 a
Hybrid rice	0.020 ± 0.009 b	19.43 ± 4.84 a	0.0012 ± 0.00076 b

Different characters in the same column mean significant difference between the two cultivars at $P \leqslant 0.05$.

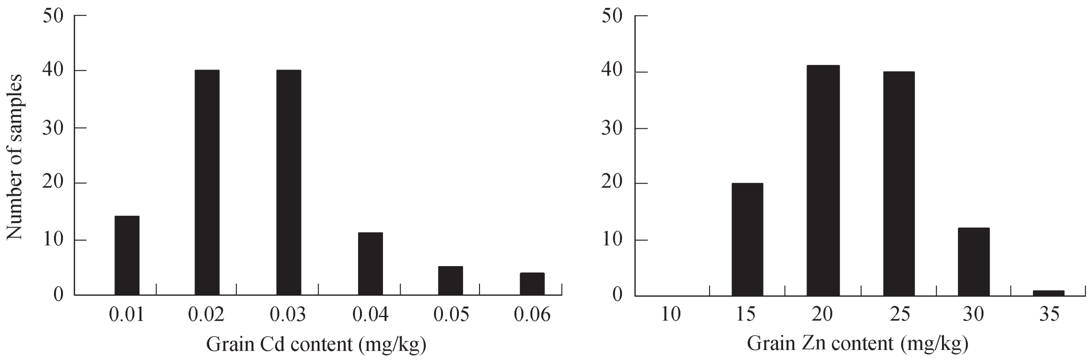

Fig. 1 Distribution of grain Cd and Zn contents of the 110 cultivars.

grain. Similarly, Kukier and Chaney (2002) found that Zn concentrations in rice plants decreased with increased Cd concentrations and the higher plant Zn contents could diminish Cd toxicity symptoms in shoot. While hybrid cultivars had greater uptake of Cd and lower uptake of Zn than the non-hybrid ones, the genotype effect on Zn depletion was considerably striking. The interaction between Cd and Zn on uptake and distribution in crops may impose a public concern.

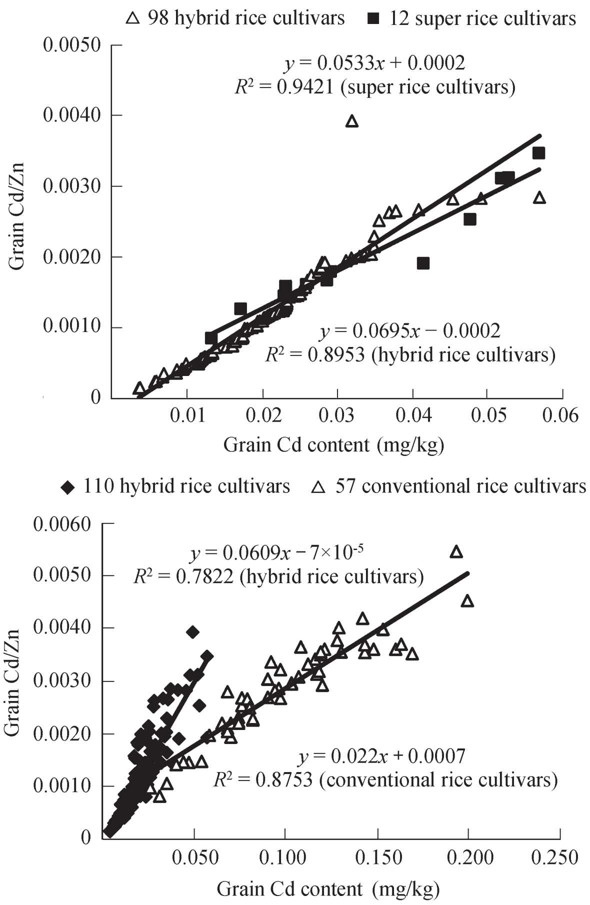

Fig. 2 Correlation of grain Cd/Zn ratio with grain Cd content in (a) 12 super rice cultivars and 98 hybrid rice cultivars in the same soil and (b) in 110 hybrid rice cultivars and 57 conventional rice cultivars (Li *et al*., 2003).

In addition, significant differences existed in Cd, Zn accumulation and Cd/Zn ratio for rice cultivars and genotypes. For example, the ratio of Cd/Zn varied in wide range of 0.00014 to 0.0039. Whereas, the values were much lower than 0.015 suggested by Simmons *et al*. (2003) as a critical value for Cd toxicity to human gut. However, considering the fact that grain Cd content was higher of super rice cultivars than that of hybrid ones and grains of extensive acidic paddy soils with high Cd bioavailability in South China, extension of these high-yielding super rice cultivars in the rice area where Zn is generally deficient (generally 50–80 mg/kg) may possess a serious problem of Zn-deficiency and Cd toxicity for human health, especially for subsistence rice diet farmers. An insufficient understanding of the agronomy of Cd and Zn, and the ability of rice to exclude Zn from grain, may have caused underestimation of health risk from soil and rice Cd in China, especially to subsistence rice consumers (Chaney *et al*., 2005; Wang *et al*., 2005). In fact, the "potential Zn hungry" still exists in most countryside (Wang *et al*., 2006). Assuming rice is consumed for an adult at 206 kg /year, dietary exposure of rice Cd from the super rice and common hybrid rice studied here would amount to 19 and 11 μg/d, respectively. Therefore, hardly health risk may arise from consumption of the hybrid rice grown in this soil as the tolerable daily dietary Cd intake could be as much as 60 μg/d using R_fD value (USEPA, 2000). Despite of a moderate Zn content (19.00 mg/kg), in the studied soil, none of the 110 cultivars had grain Zn concentration achieving the maximum permissible limit of 50 mg/kg. The calculated mean adult daily intake of Zn at 11 and 10 mg/d with hybrid rice and super rice cultivars, respectively, lower than the recommended Zn daily intake of 15 mg and especially of 20 mg for pregnant women, suggest a possible Zn deficiency with cultivation of these hybrid rice even in a neutral soil. Therefore, these studied hybrid rice, especially super rice, may impose a potential Zn deficiency in diet for the subsistence diet farmers adults, especially for the pregnant women.

3 Conclusions

Wide range of grain Cd was observed than of Zn among the 110 hybrid rice cultivars grown in a neutral, low Cd content paddy soil. Super rice cultivars exerted significantly higher grain Cd uptake with a higher ratio of Cd/Zn than the common hybrid rice cultivars. Thus, the super rice cultivars may possess a greater risk than those of hybrid ones from Cd exposure and Zn deficiency through dietary intake for human health, especially for subsistence diet farmers in South China where enhanced Cd bioavailability and Zn deficiency occurs due to low pH and poor soil nutrient status. However, a small portion of the common rice cultivars screened here had lower grain Cd and Cd/Zn ratio. This offers an opportunity to conduct a low-Cd cultivar breeding and cultivation for a safe high-yielding rice production while great care is needed for using super rice in rice production of South China with Cd contaminated and bioavailability enhanced soils.

Acknowledgments

This work was partially supported by the Ministry of Science and Technology of China (No. 2006BAD17B06) and the Ministry of Education of China (No. B200613). Special acknowledgment is given to Dr. R. L. Chaney from USDA for his comments and discussion of potential risk problem for subsistence rice diet farmers.

References

Cao R L, Jia X K, Zhang J S, 1999. The prevention and cure of paddy soil at Cd polluted sites. *Agricultural Technology of Tianjin*, 16(6): 12–17.

Chaney R L, Angle J S, McIntosh M S, Reeves R D, Li Y M, Brewer E P, Chen K Y, Roseberg R J, Perner H, Synkowski E C, Broadhurst C L, Wang S, Baker A J M, 2005. Using hyperaccumulator plants to phytoextract soil Ni and Cd. *Naturforschung Journal of Biosciences*, 60c: 190–198.

Chaney R L, Reeves P G, Ryan J A, Simmons R W, Welch R M, Angle J S, 2004. An improved understanding of soil Cd risk to humans and low cost methods to remediate soil Cd risks. *Biometals*, 17: 549–553.

Chang A C, Pan G X, Page A L, Asano T, 2002. Human Health-related Chemical Guidelines for Reclaimed Water and Sewage Sludge Applications in Agriculture. http://www.envisci.ucr.edu/downloads/chang/WHO_report.pdf.

CCFAC (Codex Committee on Food Additives and Contaminants), 2005. Codex Alimentarius Commission Report of the 37th Session of the Codex Committee on Food Additives and Contaminants. the Netherlands.

Gong W Q, Pan G X, 2006a. Issues of grain Cd uptake and the potential health risk of rice production sector of China. *Science and Technology Review*, 24(5): 43–48.

Gong W Q, Li L Q, Pan G X, 2006b. Cd uptake and accumulation in grains by hybrid rice in two paddy soils – Interactive effect of soil type and cultivars. *Environmental Science*, 24(8): 1647–1653.

Kukier U, Chaney R L, 2002. Growing rice grain with controlled cadmium concentrations. *Plant Nutrition*, 25: 1793–1820.

Li Y M, Chaney R L, Schneiter A A, Miller J F, 1995. Genotypic variation in kernel cadmium concentration in sunflower germplasm under varying soil conditions. *Crop Scicience*, 35: 147–141.

Li Z W, Li L Q, Pan G X, Chen J, 2005. Bioavailability of Cd in a soil-rice system in China: Soil type versus genotype effects. *Plant and Soil*, 271: 165–173.

Li Z W, Zhang Y L, Pan G X, Li J H, Huang X M, Wang J F, 2003. Grain contents of Cd, Cu and Se by 57 rice cultivars and the risk significance for human dietary uptake. *Environmental Science*, 24(2): 112–115.

Liu J G, Li K Q, Xu J K, Liang J S, Liu X L, Yang J C, Zhu Q S, 2003. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. *Field Crops Research*, 83: 271–283.

Liu J G, Zhu Q S, Zhang Z J, Xu J K, Yang J C, Wong M H, 2005. Variations in cadmium accumulations among rice cultivars and types and the selection of cultivars for reducing cadmium in the diets. *Journal of Scicence Food Agriculture*, 85: 147–153.

Lu R K, 2000. Methods of inorganic pollutants analysis. In: Soil and Agro-chenmical Analysis Methods. Beijing: Agricultural Science and Technology Press. 205–266.

Marr K M, Batten G D, Blakeney A B, 1995. Relations between minerals in Australian brown rice. *Journal of Scicence Food Agriculture*, 68: 285–291.

MOHC and SSC (Ministry of Health of China and State Standardization Committee), 2005. Guidelines for Limit Values of Pollutant Contents in Foods. GB2762-2005. 3rd release of State Standards.

Pongsakul P, Attajarusit S, 1999. Assessment of heavy metals of soils. *Soils Fertilizer*, 21: 71–82.

Reeves P G, Channey R L, 2004. Marginal nutritional status of zinc, iron, and calcium increases cadmium retention in the duodemum and other organs of rats fed rice-based diets. *Environmental Research*, 96: 311–322.

Simmons R W, Pongsakul P, Chaney R L, Saiyasitpanich D, Klinphoklap S, Nobuntou W, 2003. The relative exclusion of zinc and iron from rice grain in relation to rice grain cadmium as compared to soybean: Implications for human health. *Plant and Soil*, 257: 163–170.

USDA (United States Department of Agriculture), 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. United States Department of Agriculture, Natural Resources Conservation Service.

USEPA (U.S. Environment Protection Agency), 2000. Drinking Water Standards and Health Advisories. List of Substance, EPA 822-B-00-001, summer 2000.

Wang H Y, Stuanes A O, 2003. Heavy metal pollution in air-water-soil-plant system of Zhuzhou City, Hunan Province, China. *Water, Air, and Soil Pollution*, 47: 79–107.

Wang K R, Gong H Q, 1996. Comparative study on the difference of the uptake and redistribution of environmental Cd by two generic rices. *Agro-Environmental Protection*, 15(4): 145–149, 176.

Wang Q R, Cui Y C, Liu X M, Dong Y T, Christie P, 2003. Soil contamination and plant uptake of heavy metals at polluted sites in China. *Journal of Environmental Sciences and Health, Part B*, 38(5): 823–838.

Wang X L, Sato T, Xing B S, Tao S, 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Science of the Total Environment*, 350: 28–37.

Wang Z H, Zhai F Y, He Y N, Hu Y S, Wang H J, 2006. Analysis on the changes trend of dietary Zn ingestion in China. *Journal of Hygiene Research*, 35(4): 485–486.

Wu F B, Zhang G P, 2002. Genotypic differences in effect of Cd on growth and mineral concentrations in barley seedlings. *Bulletin of Environmental Contamination and Toxicology*, 69: 219–227.

Wu Q T, Chen L, Wang G S, 1999. Differences on Cd uptake and accumulation among rice cultivars and its mechanism. *Acta Ecologica Sinica*, 19(1): 104–107.

Yu H, Wang J L, Fang W, Yuan J G, Yang Z Y, 2006. Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. *Science of the Total Environment*, 370: 302–309.