

DISTRIBUTION CHARACTERISTICS, BIOACCUMULATION, AND SOURCES OF MERCURY IN RICE AT NANSI LAKE AREA, SHANDONG PROVINCE, CHINA

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ABSTRACT

Mercury (Hg) concentration in rice plant in Nansi Lake area, and rice growing environment was evaluated to elucidate the distribution characteristics, bioaccumulation, and sources of Hg. Results showed Hg distribution in rice followed the order: leaf > root > hull > bran > stem > brown rice > polished rice. Rice bran contained higher Hg concentration than in polished rice ($P < 0.05$). Bioaccumulation factors for Hg in rice ranged from 0.013 to 3.34, and 17.24% of root, 3.44% of the hull, 6.89% of the bran, 55.17% of the leaf exceeded 1. Principal components analysis indicated among these four growing factors (paddy soil, irrigation, atmospheric deposition and fertilizer), Hg concentrations in fertilizer played the most important role. Hg in atmospheric deposition could serve as the main controlling factor of rice leaf, Hg in paddy soils was the main factor of rice bran, root and stem, whereas, Hg in fertilizer was the main controlling factors of polished rice. Moreover, rice genotype had a certain effect on Hg accumulation of rice. The quality of polished rice in Nansi Lake area was safe, however, bran-made production consumption should be paid attention.

Keywords: Food safety, irrigation water, mercury, paddy soil, pollution distribution, rice.

INTRODUCTION

Mercury (Hg) is an extremely toxic element that can enter into the soil from various sources, such as sewage irrigation (Fang and Wang 2000), pesticides (Wang *et al.* 2003), and atmospheric deposition (Jackson 1997). Hg contamination in soil is hard to remove. Wiersma *et al.* (1986) showed that Hg in soil could be absorbed and enriched by crops. Moreover, Hg can migrate through long distances in the atmosphere, such that even people in areas with low Hg emissions may also suffer from Hg pollution. Unfortunately, low Hg exposure is often ignored, despite it being the cause of adverse effects on the cardiovascular, gastrointestinal and reproductive systems of humans (Zahir *et al.* 2006). Therefore, reducing the risks of low Hg exposure should gain worldwide attention.

The study area, Nansi Lake, is one of the most important rice production areas. It not only is the production and consumption area for coal, but also is one of the important industrial districts. Since the 1970s, natural and artificial factors have caused severe damage to wetland resources, leading to the function degeneration of wetland ecology. About 129 industrial pollution sources had been detected, and emission waste water amounted to 59.52 million tons, which included 0.23 tons of Hg per year (The Science and Technology Committee 1987). Therefore, the residents of this region are at risk of

Hg exposure. Feng and Han (1988) found that the hair Hg level of fishermen was five times higher than that of local farmers. This presumably caused by the consumption of Hg-contaminated aquatic products (Shi *et al.* 2006). Wang and Zhang (2009) confirmed that the surface sediment of Nansi Lake was polluted by heavy metals, especially by Hg. Hg in rivers and lakes can enter into the paddy field ecosystem through irrigation, and in paddy systems can transfer to plants, consequently becoming the Hg source of the food chain (Gnamuš *et al.* 2000).

In recent years, the departments at all levels have reinforced efforts to control the pollution of Nansi Lake area, and the environmental quality of lakes and rivers has been considerably improved. However, no empirical data is available as to whether the Hg pollution in rice in Nansi Lake has been mitigated or whether the health risks posed to residents have been reduced. Recent studies found that the contribution rate of intake of total Hg and methyl mercury (MeHg) through rice reached up to 42% and 96%, respectively (Feng *et al.* 2007; Zhang *et al.* 2010a). These results disprove the common notion that eating aquatic products is the primary source of human body exposure to MeHg. Rice consumption accounts for 22% to 30% of the dietary structure of Chinese residents, and approximately 50% of total Hg in dietary intake was from grains (primarily rice) (Li *et al.* 2006). Song *et al.* (2011) found that although rice consumption of Chinese residents in six provinces caused insignificant risks from Hg, the risk was high for

children. Therefore, we should investigate the health hazards associated with low-dose and long-term exposure to Hg through rice consumption, especially for pregnant women, children, and other sensitive groups.

In this study, we aimed to analyze Hg levels in rice and its growing environment at Nansi Lake area, to investigate Hg regional distribution characteristics which can illustrate Hg pollution sources and potential risks of the paddy field ecosystem, and to find the main factors which influence the Hg accumulation in rice. Moreover, the research result can be used to provide precise guidance for rice cultivation and the assessment of human health risk, and to supply powerful data support for the diet structure and food safety in this area.

MATERIALS AND METHODS

Study area: Nansi Lake is located in Shandong Province, China. The lake is one of the largest inland freshwater lakes in the east route of the National South-to-North Water Transfer Project (Ge *et al.* 2013). Moreover, it is an important rice-producing area.

Experimental Design: In this study, we selected the important rice-producing area-Nansi Lake as the study area to determine Hg levels in rice and its growing environment (paddy soil and irrigation) in the year of 2012. Six sampling areas (SZ, QH, XZ, WS, JX and TF) were chosen near Nansi Lake. The geographic coordinates of each location were determined. Four to six soil units were randomly selected far away from the roads in each sampling area (Fig. 1). The irrigation water of SZ is from Longgong River, that of XZ is from Xizhi River, that of JX is from Zhuzhaoxin River and Zhuzhao River, that of TF is from Guangfu River, and that of QH and WS is from Weishan Lake. All of the rivers are from Nansi Lake. The rice samples of the year 2008 were collected only rice grain at same site of SZ, QH, WS, and JX. According to the analysis, we studied the Hg level and the distribution characteristics in rice plant, and found main Hg pollution sources of rice plant of Nansi Lake area.

Sample Collection and Preparation: Rice samples were directly collected from paddy fields during October 2012. Soil samples were collected from rice roots (approximate depth of 0 cm to 20 cm). Irrigation water samples were collected from the corresponding river. All of the rice plant, soil and irrigation water samples were individually sealed into three successive polyethylene bags to avoid cross contamination. Soil samples were stored and transferred in ice packs, and irrigation water samples were immediately added to 1% nitric acid (v/v). At the laboratory, the subsamples of wet soil were homogenized after larger particles (e.g., stones and plant residues) removed. All of the soil samples in this study were air dried and then passed through a sieve. To determine Hg

concentration, portions of the samples were passed through a 0.125-mm sieve. Rice plant samples were divided into leaf, stem, hull, root, bran, and brown rice. All of the parts of rice were washed with ultrapure water for at least three times and dried at 40 °C to constant weight (Zhang *et al.* 2010b). Brown rice were ground into rice bran and polished rice. The polished rice was crushed and ground with a grinder. Atmospheric depositions were collected quarterly, and fertilizers were collected at the year of 2013 and 2014. All precautions were taken to avoid any cross-contamination during the process.

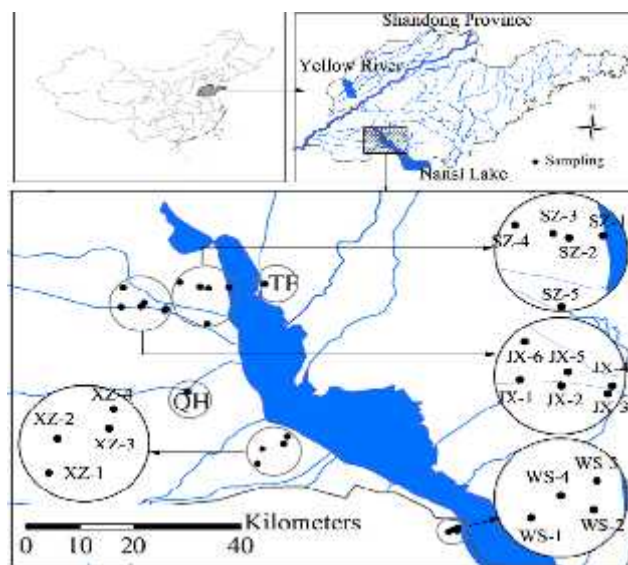


Fig. 1. The sample locations in the areas around Nansi Lake

Analytical Method: Hg concentration in soil and rice samples were determined by using Direct Mercury Analyzer (DMA-80, Milestone SRL, Italy) (Søvik *et al.* 2011). Quality assurance and control of the analyses were ensured by using sample replicates, reference material (National Research Center for Certified Reference Materials, China), control solutions, and blanks. The detection limit was 0.008 ng. The linear correlation factor (r) was higher than 0.9990. The relative standard deviation (RSD) was less than 2.0%, and the additive recoveries of samples were 95.1%~102.4%. Irrigation water samples were determined by atomic fluorescence spectrometry (AFS-933, Titan Instrument Co., Ltd, Beijing, China) coupled with flow injection analysis. The detection limit of AFS-933 is 0.001 $\mu\text{g L}^{-1}$. The RSD was less than 5.0%, and the recoveries in the range of 94.2%~100.2%. Quality control of data was achieved by using Hg (II) standard solution (National Research Center for Certified Reference Materials, China). Based on the analysis of the sample, the soil sample standard substance was GBW07450 and GBW07452 and rice standards (GBW10043) was quality control.

Data Analysis: All of the data was subjected to analysis of variance (ANOVA) by using SPSS 20.0 software (SPSS for Windows, Rel. 20.0. 2012. Chicago: SPSS Inc.) and principal components analysis (PCA) by CANOCO 5.0 software (2013).

RESULTS AND DISCUSSION

Hg levels in rice: Mean Hg concentration of Nansi Lake area in polished rice was $2.2 \mu\text{g kg}^{-1}$ which was lower than the mean value $3.4 \mu\text{g kg}^{-1}$ of Zhang *et al.* (2014) who studied 15 major rice grain-producing provinces of China (Fig. 2). In general, rice plant in Nansi Lake area, which was a low Hg exposure area, did not suffer Hg pollution. Hg concentrations in polished rice decreased compared with the data of 2008 (Table 1 and Fig. 2).

Table 1. Mercury concentrations in rice grain of the year of 2008 ($\mu\text{g kg}^{-1}$)

| Location | SZ | | | QH | | |
|----------|---------------|-----------|------------|---------------|-----------|------------|
| | polished rice | rice bran | brown rice | polished rice | rice bran | brown rice |
| Mean | 6.50 | 59.92 | 9.32 | 6.81 | 48.03 | 9.43 |
| SD | 1.52 | 13.13 | 1.67 | 1.22 | 7.47 | 1.31 |
| Mix | 4.88 | 36.41 | 7.30 | 5.04 | 37.20 | 7.53 |
| Max | 9.15 | 76.19 | 13.02 | 8.59 | 58.41 | 11.59 |
| Location | WS | | | JX | | |
| | polished rice | rice bran | brown rice | polished rice | rice bran | brown rice |
| Mean | 1.07 | 49.01 | 9.73 | 7.59 | 61.33 | 11.00 |
| SD | 1.15 | 5.88 | 1.03 | 1.83 | 25.54 | 2.90 |
| Mix | 4.69 | 41.45 | 7.99 | 4.99 | 25.73 | 7.16 |
| Max | 9.29 | 58.20 | 10.63 | 11.53 | 114.38 | 16.26 |

As shown in Fig.2, Hg concentrations in polished rice samples were lower than the limited amount for human ($20 \mu\text{g kg}^{-1}$, GB 2762-2005).

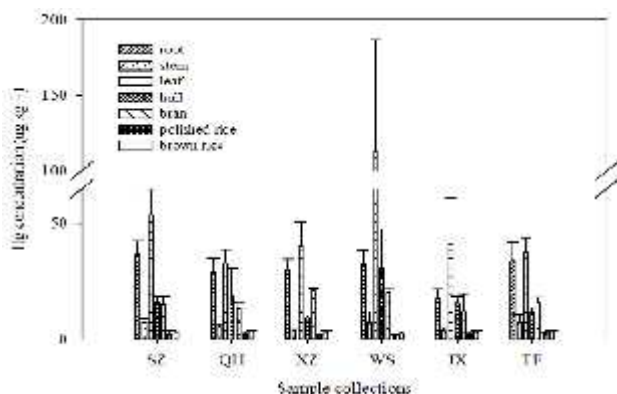


Fig. 2. Mercury concentrations in different parts of rice plants

Although the mass of rice bran occupied 6.3 % of the brown rice (Franz and Sampson, 2006), it contained 60% nutrition of the rice grain. Rice bran is rich in oleic acid, linoleic acid, and also contains vitamin E, dietary fiber, protein, phytic acid and other nutrients (Zullaikah *et al.* 2005), so it has become a valuable food material. Many literatures had shown that rice bran was usually, in addition to being used as animal feed, was also

used for lactic acid production and rice bran oil. However, only a few researches reported or covered the Hg concentration in rice bran. In this study, Hg concentrations in rice bran were 7.2 times higher than those in polished rice, and 27.6% of them exceeded the human consumption limited. Statistical analysis showed that Hg concentrations in rice bran samples were markedly lower than those in the year 2008 ($P < 0.05$). Fig. 3 showed that rice bran and polished rice for Hg had a negative linear correlation demonstrating Hg was easily enriched on the surface of brown rice.

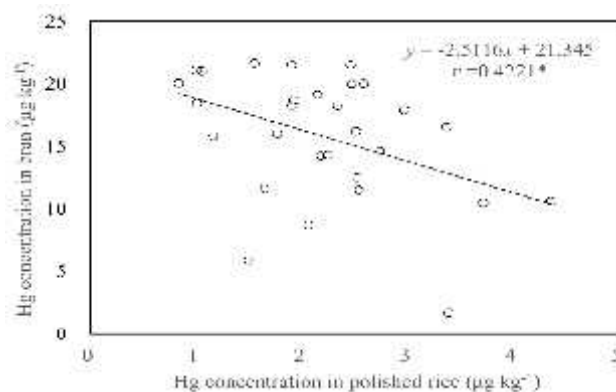


Fig. 3. The relationship between mercury concentrations in polished rice and bran (n=29) (* is in 0.05 level significance)

This result was consistent with Meng *et al.* (2014a). Studies shown that rice bran contained large amounts of phytic acid and protein which had strong chelation of Hg (Tuncel *et al.* 2014). Thus, if the bran were used as the raw material for rice bran oil and lactic acid, it would bring Hg enrichment in human beings. Therefore, considerable attention should be given to the consumption of bran-made food.

Hg concentrations in rice hull, root, and stem were all lower than the recommended amount of animal feed ($100 \mu\text{g kg}^{-1}$) (GB13078-2001). Statistical analysis indicated that Hg in rice root samples were significantly higher than those in polished rice ($P < 0.05$). Rice root and stem of Hg had a positive exponential correlation (Fig. 4), indicating that rice plants facilitated Hg uptake from root to rice stem.

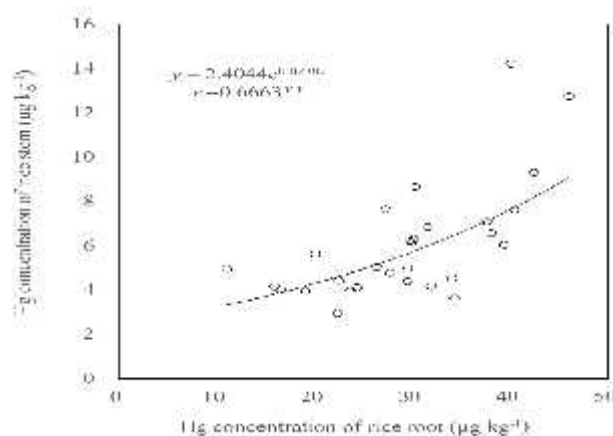


Fig. 4. The relationship between mercury concentrations in rice stem and rice root ($n=29$) (is in 0.01 level significance)**

This finding did not coincide with the results of Válega *et al.* (2009) who studied that root had an inhibition effect on Hg transferring to the aboveground part.

From Fig. 2 we found that leaf contained the highest Hg level, especially in WS, which because the coal power plant near the sample sites released Hg vapor into the atmosphere. Studies showed that rice stomata could absorb gases state Hg in the atmosphere, and then transferred it within the plant system (Rothenberg *et al.* 2011, Yin *et al.* 2013). Whole grains are rich in vitamins, plant fiber, low calorie, making them capable of preventing cardiovascular disease and enhancing cell activity. Consequently, an increasing number of people prefer to eating whole grains such as corn, wheat, and brown rice. Mean Hg in brown rice samples were $3.07 \mu\text{g kg}^{-1}$ in Nansi Lake area. These were lower than the average concentration of Chinese brown rice ($4.9 \mu\text{g kg}^{-1}$) (Zhang *et al.* 2014). Statistical analysis showed that Hg in brown rice samples were significantly lower than those

of the year 2008 ($P < 0.05$). The difference in Hg in brown rice between 2008 and 2012 was mainly caused by the decreased Hg in bran. The good quality of rice may be caused by the treatment of the south-to-north water diversion project (Wu *et al.* 2010).

Hg distribution in rice: Hg distribution in rice plant was in the following order: leaf > root > hull > bran > stem > brown rice > polished rice (Fig. 2). This finding was agreed with previous studies that Hg originated from ambient air through leaf surface absorption and from soil through root uptake in rice plant (Li *et al.* 2008; Li *et al.* 2009). In general, rice root could absorb more Hg (Meng *et al.* 2010). In our study, rice leaf contained the highest Hg level. Hg can exist in the atmosphere so that rice leaf may contain more Hg than root through gas exchange (Ericksen *et al.* 2003) and the deposition (Rea *et al.* 2001). Meng *et al.* (2014b) demonstrated in soil contaminated area, Hg concentration in root was higher than that in leaf. Hg in soil in Nansi Lake area did not exceed the recommend limit, but in recent years, air pollution was serious in this area where hazy weather frequently appeared. Aforementioned resulted that Hg in the atmosphere increased in this area. Therefore, rice leaf contained more Hg concentration than that in root. In the rice growth process rice absorbed Hg in the soil by root, but root had obvious retention effect of Hg upwards when it transport nutrition for grain (Cavllini *et al.* 1991). Therefore, Hg in rice stem, hull and grain were lower than those in root and leaf.

Hg concentrations in rice paddy soil and irrigation water: Hg concentrations in rice paddy soil and irrigation water are shown in Table 2. All of Hg in rice paddy soil and irrigation water samples were lower than the recommend Chinese national standard 0.5 mg kg^{-1} and 0.001 mg L^{-1} , respectively (GB15618-1995, GB 5084-92). Hg in rice paddy soil were significantly lower ($P < 0.01$) than those in the control sites in Guizhou Province in the study of Zhang *et al.* (2010b). Statistical results demonstrated that Hg in rice paddy soil had no regional difference. Whereas, Hg concentration in irrigation water did as followed: that in SZ was significantly lower than WS and JX, that in QH was significantly lower than XZ, WS and JX, that in XZ was markedly lower than WS and JX ($P < 0.05$). Although mean Hg level in rice paddy soil in WS were highest, whereas, the highest single sample spot was detected in TF ($95.67 \mu\text{g kg}^{-1}$). And TF also contained the highest Hg concentrations in irrigation water. TF area was a rice experimental plot irrigated by water from Guangfu River. Shen *et al.* (2008) showed that there were 56 industries discharged sewage into Guangfu River, and because the drainage basin was small, the whole river water quality was polluted seriously. In fact, Guangfu River has become the biggest sewage drains in Jining city.

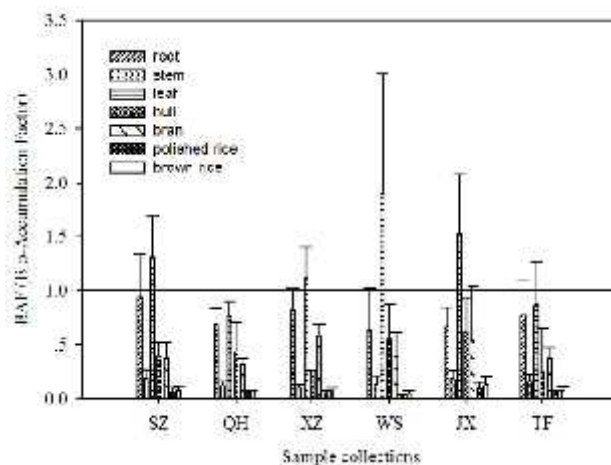
Table 2. Mercury concentrations in rice paddy soil and irrigation water

| Location | SZ | | QH | | XZ | |
|----------|-------|------------------|-------|------------------|-------|------------------|
| | soil | irrigation water | soil | irrigation water | soil | irrigation water |
| Mean | 43.89 | 0.036 | 42.87 | 0.038 | 36.62 | 0.042 |
| SD | 16.64 | 0.0032 | 3.03 | 0.024 | 5.35 | 0.001 |
| Min | 30.15 | 0.0331 | 39.48 | 0.036 | 29.47 | 0.0403 |
| Max | 72.02 | 0.0414 | 47.26 | 0.041 | 41.82 | 0.043 |
| Location | WS | | JX | | TF | |
| | soil | irrigation water | soil | irrigation water | soil | irrigation water |
| Mean | 62.96 | 0.054 | 28.07 | 0.0863 | 51.7 | 0.1027 |
| SD | 25.02 | 0.026 | 9.69 | 0.026 | 26.01 | 0.0082 |
| Min | 29.08 | 0.04 | 16.4 | 0.048 | 26.56 | 0.091 |
| Max | 83.53 | 0.093 | 39.14 | 0.13 | 95.67 | 0.11 |

The unit of Hg concentration of soil was $\mu\text{g kg}^{-1}$, and of irrigation water was $\mu\text{g L}^{-1}$

Relationships between Hg concentrations in rice and paddy soil/irrigation water: The site-specific bioaccumulation factors (BAFs, i.e., rice/soil concentration ratios) (Gnamuš *et al.* 2000) for polished rice (brown rice, bran, hull, leaf, stem, root) of all of the sample locations are shown in Fig. 5. BAF values exceeding 1 indicated intensive bio-accumulator. Approximately 17.24% of the root, 3.44% of the hull, 6.89% of the bran, and 55.17% of the leaf BAFs exceeded 1. BAFs of polished rice and brown rice were markedly lower than those in other parts of rice ($P < 0.05$) which showed that the migration ability of Hg from paddy soil to rice grain was weak. The variation of BAF in rice plant might be attributed to many reasons, such as, soil type, pH, organic matter, redox potential, and dissolved organic matter (Patra and Sharma 2000). Moreover, Zhu *et al.* (2008) indicated that genotypic variation was also an important rice property that affects the rice accumulation of Hg.

For principal components analysis (PCA), the percentage variances of Hg concentration explained by the first and second axes were 73.85% and 8.76%, respectively (the cumulative percentage variance explained by the two axes was 82.61%) (Fig. 6). Results indicated Hg in atmospheric deposition could serve as the main controlling factor of rice leaf through respiration and photosynthesis role. Hg in paddy soils was the main factor of rice bran, root and stem. Whereas, Hg in fertilizer was the main controlling factors of polished rice which would bring Hg pollution risk in edible rice parts. Therefore, Hg concentration in irrigation water was not the main source of rice plant. Many studies had found that soil Hg concentration had significant positive correlation with the residues of Hg in rice root and stem

**Fig. 5. The Bio-Accumulation Factor of rice plant**

(Zhang *et al.* 2010b; Qiu *et al.* 2013). Hg in fertilizer could enter paddy soils with rice fertilization and accumulated in rice plant which could rise Hg concentration in rice. Studies have shown that as the amount of phosphate fertilizer increased, rice plant absorption and accumulation of Hg increased significantly (Wang *et al.* 2013).

Moreover, QH (samples presented by star symbols in Fig.6) and TF (samples presented by diamond symbols in Fig. 6) were locations with specific breeds and gene. Those two area samples were not set in a cluster. The distribution of sampling points reflected the difference of property indicating that genotype affected the Hg level in rice.

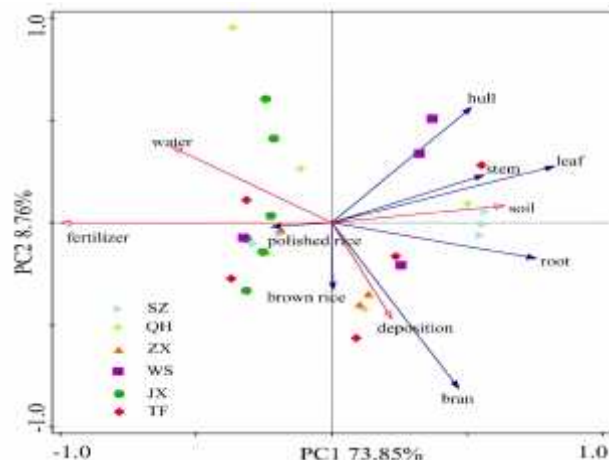


Fig. 6. Principal components analysis (PCA)

Studies reported that Hg concentrations had significant difference between rice genotypes and rice adsorption Hg had genotype stability (Qiu *et al.* 2008; Rothenberg *et al.* 2012). These findings demonstrated that screening low Hg accumulation genotype was a feasible and effective method to solve THg/MeHg accumulation in rice. Therefore, it was possible to screen rice genotypes to ensure rice quality in Hg-contained areas.

Conclusion: In general, the rice plant in Nansi Lake, which was a low Hg exposure area, did not suffer serious Hg pollution. Hg in all of the paddy soil and irrigation water samples were lower than the recommended Chinese national standard limited values. Hg concentrations in rice paddy soil had no significant regional difference ($P > 0.05$), whereas those in irrigation water did ($P < 0.05$). Polished rice contained lower Hg than our previous samples ($P < 0.05$). Hg in rice bran was significantly higher ($P < 0.05$) than those in polished rice. Therefore, we should pay more attention to coarse food grain and bran-made products. The distribution of Hg concentrations in rice plant was as follows: leaf > root > hull > bran > stem > brown rice > polished rice.

Leaf was an intensive accumulator for Hg. The migration ability of Hg from paddy soil to rice grain was weak. Genotype affected the properties of rice Hg concentration distribution. Hg in atmospheric deposition was the main source of rice leaf, Hg in paddy soils which was the main impact of rice bran, root and stem, whereas, Hg in fertilizer was the main controlling factors of polished rice.

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