Distribution Patterns of Inorganic Mercury and Methylmercury in Tissues of Rice (*Oryza sativa L.*) Plants and Possible Bioaccumulation Pathways

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Whole rice plants (*Oryza sativa L.*) were collected at different typical mercury (Hg) contaminated sites during regular harvest periods to investigate the distribution of inorganic mercury (IHg) and methylmercury (MeHg) in tissues. The whole rice plants were divided into rice seed (brown rice), hull, root, stalk and leaf. Elevated IHg and MeHg concentrations were observed in rice plants cultivated in Hg mining area compared to those obtained from the control site, which attributed to the Hg contamination of soil compartments by the historical large-scale Hg mining/smelting and ongoing artisanal Hg smelting activities. Our observations showed that Hg in ambient air was the potential source of IHg to the above ground parts, whereas IHg concentrations in root were restricted to Hg concentrations in paddy soil. The rice seed has the highest ability to accumulate MeHg compared to the other tissues. MeHg in paddy soil is a potential source to tissues of rice plant. Our study suggested that newly deposited Hg is comparatively more easily methylated than old mercury in soil.

KEYWORDS: Distribution; inorganic mercury; methylmercury; tissues; rice plant (*Oryza sativa L.*)

INTRODUCTION

Mercury (Hg) as a global and extremely toxic pollutant (*1*) has received considerable attention due to its ability to undergo methylation, accumulation, and biomagnification in aquatic food chains. Consuming fish, fish products and marine mammals is currently considered as the main pathway of human exposure to Hg and especially methylmercury (MeHg) posing a worldwide human health threat (*2*). Generally, Hg concentrations in most foodstuffs (except for fish) are below 0.020 mg kg^{-1}, and mainly present in inorganic forms (*3*).

Soil Hg can easily enter the food chain (*4*) and has attracted increasing attention. Rice paddy plantation is one of the most prevalent crop fields present in South and East Asia, where rice is usually the dominant foodstuff. Located in SW China, the Wanshan Hg mining area in Guizhou province is historically ranked as the largest Hg producing center in China. Geochemical investigations in Wanshan have revealed that the massive calcines and tailings introduced by the abandoned large-scale Hg mining, uncontrolled small-scale artisanal Hg mining and other mineral processing have resulted in heavily Hg-contaminated rivers and serious pollution of the irrigated rice paddies (*5−7*).

Regarding Hg pollution in Guizhou, Horvat et al. (*5*) first reported high MeHg concentrations in rice seeds in Wanshan Hg

mining sites and Qingzhen (a city impacted by a chemical plant), which ranged from 0.0080 to 0.14 mg kg^{-1} (mean: 0.026 mg kg^{-1}). Qiu et al. (*8*) found that the rice (*Oryza sativa L.*) grown at Hg mining areas in Wanshan contained MeHg levels up to 0.18 mg kg^{-1} in its edible portion. These authors explored that rice seeds can contain MeHg concentrations 2 to 3 orders of magnitude higher than MeHg in other local edible crop plants. In other Hg mining areas in Guizhou, such as Wuchuan in north of the province, rice with high levels of MeHg were also reported, ranging from 0.0031 to 0.018 mg kg^{-1} (*9*). Feng et al. (*10*) demonstrated that MeHg-contaminated rice was the dominant pathway of MeHg exposure to the residents in Wanshan Hg mining area. Significant brain damage in animals (rats) fed with Hg polluted rice from Wanshan Hg mining area was also observed (*11*).

From current available data in the open literature, it could be confirmed that rice seed has a high accumulation ability of MeHg. However, to date, the distribution patterns of inorganic mercury (IHg) and MeHg in stalk, leaf, hull and root of the rice plants have not been studied, and therefore, the understanding of the bioaccumulation pathways of different Hg species in rice plants is limited. Therefore, the primary objectives of this study were (1) to investigate the concentration and distribution of IHg and MeHg in tissues of rice plants and (2) to further understand the potential bioaccumulation pathways of both IHg and MeHg in rice plants.

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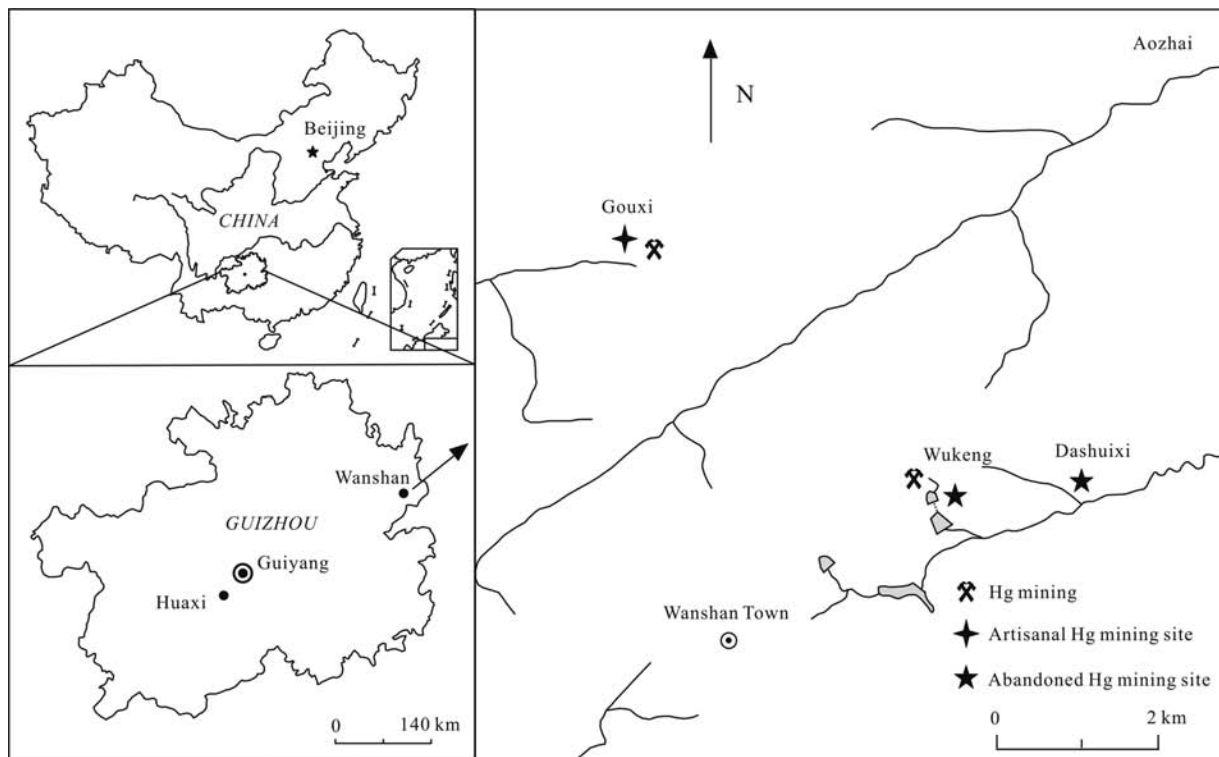
Figure 1. Map of the study area and sampling locations including abandoned Hg mining sites (Wukeng and Dashuixi), artisanal Hg mining site (Gouxi) and control site (Huaxi).

MATERIALS AND METHODS

Study Areas. Four typical areas, including an artisanal Hg mining site (Gouxi), two abandoned Hg mining sites (Wukeng and Dashuixi) and a control site (Huaxi), were selected for this study (**Figure 1**). The artisanal Hg mining site and the abandoned Hg mining sites are situated in Wanshan district, eastern Guizhou province. The control site of Huaxi is located in southwest of Guiyang city (about 30 km away from the city), the capital of Guizhou province (**Figure 1**).

The Wanshan district experiences a subtropical monsoon humid climate with an average annual rainfall of 1200−1400 mm and a perennial mean temperature of 17 °C. The historical Hg mining activities in the Wanshan area can be dated back to the Qin Dynasty (221 B.C.), and the large-scale mining activities were completely shut down in 2001. More than 2.0×10^4 tons of Hg and 1.0×10^8 tons of calcines and waste rocks were produced while the large-scale Hg mining activities were in operation between 1949 and the 1990s. Recently, artisanal Hg smelting activities were revived in the Gouxi area due to the increase of the Hg price in the world market and of domestic demands.

The Gouxi artisanal Hg mining area is located to the north of Wanshan town. Small-scale artisanal smelting was ongoing during the rice growing seasons in 2007 and 2008. The Wukeng and Dashuixi sites are located at the abandoned Hg mining site where large quantities of calcines were deposited along the river. The historical large-scale Hg smelting combined with current artisanal Hg smelting activities have resulted in serious Hg contamination to the ambient air, water, soil, sediment, and biota (*6, 7, 12*). Rice paddy fields at Wukeng, Dashuixi, and Gouxi were irrigated using polluted river water during the entire rice growing seasons and were heavily Hg-contaminated. Detailed information on Hg contamination in the environment of Wukeng, Dashuixi, and Gouxi can be obtained from other sources (*6−8, 12*).

Huaxi chosen as the control site experiences a similar climate in comparison to the Wanshan Hg mining area, but has no direct Hg point contamination source. The concentrations of gaseous element Hg in ambient air (2.12−12.8 ng m^{-3}) and total Hg (THg) in surface soil (0.25−0.30 mg kg^{-1}) representing the regional background levels were much lower than those found in Wanshan Hg mining area (*5−7, 12, 13*).

Sample Collection and Preparation. Eleven, twenty-two and thirteen whole rice plant samples as well as corresponding soil samples (10−20 cm in depth) were collected from the control site, the abandoned Hg mining sites, and the artisanal Hg mining site (**Figure 1**) during the harvest seasons in 2007 and 2008, respectively. In detail, each rice sample comprised a composite of at least five subsamples of the rice plants within an area of about 5−10 m^2 in the same paddy field, and the amount of each rice sample was recorded. Soil samples were also collected by hand with a disposable polyethylene glove at the same site as the rice plant samples. Rice grains were separated from the rice plant using a scalpel. The other parts of the plant were divided into three fractions, including root, stalk, and leaf. All samples were cleaned using drinking water in situ followed by deionized water rinses after being brought back to the laboratory, and finally air-dried and stored in polyethylene bags to avoid cross-contamination. The seeds (brown rice) were separated from their hulls using a pestle and mortar in the laboratory. The mass of all rice plant tissues was recorded after the plants were completely dry. All the tissues of rice samples were ground to 150 meshes per inch (IKA-A11 basic, IKA, Germany) for THg and MeHg analyses. Previous studies observed that ethyl mercury (EtHg) could not be detected in different kinds of foodstuffs, including rice flour, wheat flour and dogfish liver (*14, 15*), even when the researchers used different detection methods. In detail, Shi et al. (*14*) demonstrated that MeHg was present in twenty-five rice samples cultivated in fifteen provinces of China, but EtHg was not found in any sample. The sum of the concentration of IHg and MeHg was in agreement with the THg concentration of Hg determined in rice flour, wheat flour and dogfish liver (*15*). So in this study the concentration of IHg in tissues of rice plant was calculated by the difference between the concentration of THg and MeHg in the sample. The soil samples were sealed and double-bagged in situ and within 24 h transported in an ice-cooled container to a refrigerator kept at −17 °C prior to freeze-drying. Subsequently the freeze-dried soil samples were homogenized to a size of 150 meshes per inch with a mortar before chemical analysis.

Precautions were taken in order to avoid any cross-contamination during the processing. The grinder was thoroughly cleaned after each sample processing. The powdered samples were subsequently packed into plastic dishes, sealed in polyethylene bags and stored in a refrigerator within desiccators for further laboratory analysis.

Analytical Methods. All reagents used in this study were at least of guaranteed reagent and were purchased from Shanghai Chemicals Co. (Shanghai, China).

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Table 1. List of Certified Reference Materials Used in the Present Study and the Results Obtained

producer	CRM	n	Hg speciation	obtained value	certified value
NRC CRM[a]	GBW08508	10	THg (mg kg^{-1})	0.038 ± 0.001	0.038 ± 0.003
NIST[b]	SRM-2710	5	THg (mg kg^{-1})	32.4 ± 0.8	32.6 ± 1.8
NRCC[c]	TORT-2	10	THg (mg kg^{-1})	0.27 ± 0.02	0.27 ± 0.06
NRCC[c]	TORT-2	10	MeHg (mg kg^{-1})	0.15 ± 0.004	0.152 ± 0.013
IAEA[d]	IAEA-405	5	MeHg (mg kg^{-1})	0.0053 ± 0.0005	0.00549 ± 0.00053

[a] NRC CRM: National Research Centre for Certified Reference Materials. [b] NIST: National Institute of Standards and Technology. [c] NRCC: National Research Council Canada. [d] IAEA: International Atomic Energy Agency.

For THg analysis, 0.2−0.3 g of rice plant tissue sample was prepared and digested with a freshly prepared mixture of HNO$_3$/H$_2$SO$_4$ (v/v 4:1) present in a water bath (95 °C) (16, 17). Concerning soil, 0.1−0.2 g samples were digested in a similar setup using a fresh mixture of HCl and HNO$_3$ (1:3, v/v). A suitable volume of aliquot from the digested sample was taken for THg analysis by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran 2500, Tekran Inc., Canada) preceded by BrCl oxidation, SnCl$_2$ reduction, purge and thermoreduction of Hg (16).

For MeHg analysis, 0.3−0.5 g of a rice plant sample was digested using a KOH−methanol/solvent extraction technique (18). In this process, samples were first digested with 25% KOH/CH$_3$OH and heated at 75 °C in a water bath for 3 h. After completion, the digests were acidified with concentrated HCl. For soil samples, 0.3−0.4 g of soil sample was prepared using CuSO$_4$−methanol/solvent extraction (18). Then MeHg in samples was extracted with methylene chloride as well as back-extracted from the solvent phase into water and aqueous phase ethylation. The ethyl analogue of MeHg, methylethyl mercury (CH$_3$CH$_3$CH$_2$Hg), was separated from solution by purging with N$_2$ onto a Tenax trap. The trapped CH$_3$CH$_3$CH$_2$Hg was then thermally desorbed, separated from other Hg species by an isothermal gas chromatography (GC) column, decomposed to Hg^0 in a pyrolytic decomposition column (800 °C) and analyzed by CVAFS (Brooks Rand model III, Brooks Rand Laboratories, Seattle, WA) following method 1630 (18, 19).

THg in water samples were quantified using dual amalgamation cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran 2500, Tekran Inc., Canada) within 28 days after sampling following approved methodologies (16).

Three sampling campaigns were conducted during 2008 in order to establish the concentrations of total gaseous mercury (TGM) present in ambient air at the control site, the abandoned Hg mining sites, and the artisanal Hg mining site during the rice growing seasons. TGM is normally made up Hg^0 vapor (>95%) together with minor fractions of gaseous molecular compounds. In this study an automated Hg vapor analyzer (LUMEX, RA-915+, Ohio Lumex Co., Twinsburg, OH) was employed measuring Hg^0 vapor concentrations in air (20). This instrument is based on the Zeeman cold vapor atomic absorption spectrometry technique with a detection limit of 2 ng m^{-3}. Average Hg^0 concentrations during a 20 s interval were retrieved and stored in a portable computer, and measurements at each sampling site were carried out continuously for at least two hour periods. Precipitation samples were collected at all sampling sites during the rice growing seasons in 2007 and 2008 and analyzed for THg following approved methodologies (21).

Quality Control and Statistical Analysis. Quality control for THg and MeHg determination in samples was conducted using duplicates, method blanks, matrix spikes, and certified reference materials. The method detection limits (3σ) were 10 ng kg^{-1} for THg and 2 ng kg^{-1} for MeHg in tissues of rice samples as well as in soil samples. The relative standard deviation for analysis of duplicate samples was ≤7.8% for THg and MeHg. Recoveries for matrix spikes ranged from 96 to 110% for THg analysis, and from 86 to 108% for MeHg, respectively. The certified international reference materials, including National Research Centre for Certified Reference Materials, rice (GBW08508), National Research Council Canada, TORT-2 (Lobster Hepatopancreas), National Institute of Standards and Technology, Montana soil (SRM-2710) and International Atomic Energy Agency, sediment (IAEA-405), were used for quality control of rice plant and soil sample analysis. **Table 1** lists the reference materials utilized and the corresponding analytical results.

Statistical analysis was performed using SPSS 13.0 software. Relationships between covariant sets of data were subjected to regression analysis. Correlation coefficients (r) and significance probabilities (p) were computed for the linear regression fits. The principal component analysis (PCA) was performed for our data set.

RESULTS AND DISCUSSION

IHg in Tissues of Rice Plants. Concentrations and distribution of IHg in tissues of rice plants collected from the artisanal Hg mining site (Gouxi), the abandoned Hg mining sites (Wukeng and Dashuixi), and the control site (Huaxi) are shown in **Table 2** and **Figure 2**. The highest mean concentrations of IHg in seed (0.27 ± 0.16 mg kg^{-1}) were observed at the artisanal Hg mining site, which were higher than those at abandoned Hg mining sites (0.18 ± 0.16 mg kg^{-1}) as well as the control site (0.0033 ± 0.0013 mg kg^{-1}). The average concentrations of THg in seed collected from both the artisanal Hg mining site (0.31 ± 0.17 mg kg^{-1}) and the abandoned Hg mining sites (0.18 ± 0.16 mg kg^{-1}) significantly exceeded the maximum permissible limit of 0.02 mg kg^{-1} issued by Chinese National Standard Agency (22). On the contrary, the average THg concentration in seed collected from the control site (0.0062 ± 0.0020 mg kg^{-1}) was much lower than the permissible limit.
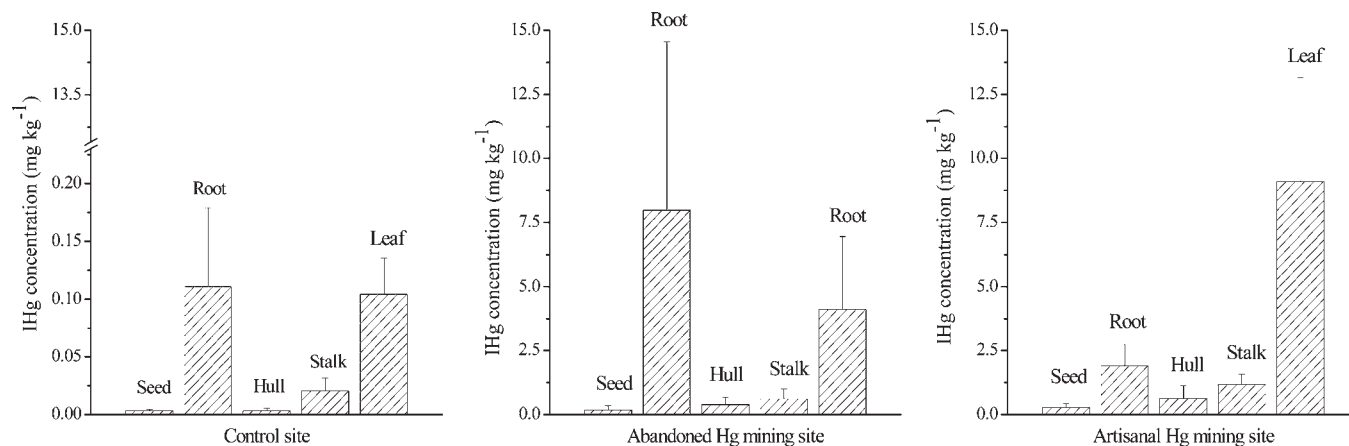
The IHg concentrations in hull at the artisanal Hg mining site (0.63 ± 0.48 mg kg^{-1}) and the abandoned Hg mining sites (0.39 ± 0.28 mg kg^{-1}) were significantly higher than those at the control site (0.0032 ± 0.0024 mg kg^{-1}). The highest concentration of IHg in hull reaching to 1.6 mg kg^{-1} was present at the artisanal Hg mining site, where TGM concentrations in the ambient air reached up to 1652 ng m^{-3} (**Table 3**). It implies that the IHg in hull might originate from air.

The average IHg concentrations in root at the abandoned Hg mining sites and the artisanal Hg mining site were 8.0 ± 6.6 mg kg^{-1} ranging from 1.1 to 24 mg kg^{-1} and 1.9 ± 0.82 mg kg^{-1} ranging from 1.0 to 3.2 mg kg^{-1}, respectively, which were significantly higher than those at the control site (mean concentration of 0.11 ± 0.068 mg kg^{-1}, ranging from 0.031 to 0.29 mg kg^{-1}). Previous studies showed that the root Hg level was directly associated with soil Hg concentrations (23). Significantly higher THg levels were observed in rice paddy soil at the abandoned Hg mining sites (131 ± 145 mg kg^{-1}) compared to those at the artisanal Hg mining site (22 ± 8.4 mg kg^{-1}) and the control site (0.31 ± 0.04 mg kg^{-1}) (**Table 3**). The significantly positive correlation between IHg levels in root and THg levels in soil (**Table 4**) suggested that Hg in paddy soil is the major source of IHg in root. Hence, compared to the control site the elevated IHg in rice root in Wanshan Hg mining area, especially at the abandoned Hg mining sites, definitely results from the serious Hg-contamination in rice paddy fields (5−7).

The highest IHg concentration in stalk samples was present at the artisanal Hg mining site (1.2 ± 0.41 mg kg^{-1}), followed by the abandoned Hg mining sites (0.61 ± 0.40 mg kg^{-1}), then the control site (0.021 ± 0.011 mg kg^{-1}). Although THg concentrations in rice paddy soil in abandoned Hg mining sites were significantly higher than those at the artisanal Hg mining site (**Table 3**), the IHg concentrations in stalk at abandoned Hg mining sites were significantly lower than those at the artisanal

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Table 2. Concentrations of IHg and MeHg in Tissues of Rice Plant from Different Sampling Sites

sampling sites	tissues	Hg concn, mean $\pm$ SD, mg kg^{-1} (range)		
		IHg	MeHg	MeHg/THg, %
control site (Huaxi) (n = 11)	seed	0.0033 $\pm$ 0.0013 (0.0020−0.0058)	0.0029 $\pm$ 0.0010 (0.0018−0.0045)	47 $\pm$ 8 (35−64)
	hull	0.0032 $\pm$ 0.0024 (0.0013−0.0077)	0.00062 $\pm$ 0.00018 (0.00039−0.0011)	16 $\pm$ 6 (5−28)
	root	0.11 $\pm$ 0.07 (0.031−0.29)	0.0021 $\pm$ 0.0007 (0.0011−0.0030)	2.5 $\pm$ 1.5 (0.38−5.9)
	stalk	0.021 $\pm$ 0.011 (0.010−0.042)	0.00060 $\pm$ 0.00027 (0.00030−0.0011)	3.0 $\pm$ 0.7 (2.2−4.2)
	leaf	0.10 $\pm$ 0.03 (0.060−0.14)	0.00055 $\pm$ 0.00032 (0.00020−0.0010)	0.49 $\pm$ 0.19 (0.19−0.87)
abandoned Hg mining sites (Wukeng and Dashuxi) (n = 22)	seed	0.18 $\pm$ 0.16 (0.021−0.53)	0.0070 $\pm$ 0.0032 (0.0038−0.018)	8.1 $\pm$ 6.8 (1.6−23)
	hull	0.39 $\pm$ 0.28 (0.027−0.87)	0.0024 $\pm$ 0.0010 (0.00090−0.0041)	1.8 $\pm$ 2.1 (0.17−6.8)
	root	8.0 $\pm$ 6.6 (1.1−24)	0.0057 $\pm$ 0.0017 (0.0032−0.0085)	0.15 $\pm$ 0.12 (0.025−0.41)
	stalk	0.61 $\pm$ 0.40 (0.13−1.3)	0.0011 $\pm$ 0.0005 (0.00050−0.0023)	0.27 $\pm$ 0.20 (0.052−0.77)
	leaf	4.1 $\pm$ 2.8 (0.88−9.4)	0.00058 $\pm$ 0.00011 (0.00040−0.00088)	0.028 $\pm$ 0.026 (0.0049−0.074)
artisanal Hg mining site (Gouxi) (n = 13)	seed	0.27 $\pm$ 0.16 (0.11−0.56)	0.032 $\pm$ 0.014 (0.018−0.062)	11 $\pm$ 2.1 (7.4−15)
	hull	0.63 $\pm$ 0.48 (0.23−1.6)	0.0064 $\pm$ 0.0020 (0.0034−0.0010)	1.6 $\pm$ 1.1 (0.47−3.7)
	root	1.9 $\pm$ 0.8 (1.0−3.2)	0.012 $\pm$ 0.002 (0.0085−0.015)	0.72 $\pm$ 0.33 (0.36−1.3)
	stalk	1.2 $\pm$ 0.4 (0.63−1.7)	0.0037 $\pm$ 0.0013 (0.0020−0.0064)	0.32 $\pm$ 0.08 (0.20−0.52)
	leaf	9.1 $\pm$ 4.1 (5.0−15)	0.0017 $\pm$ 0.0006 (0.00078−0.0027)	0.025 $\pm$ 0.017 (0.0057−0.055)

Figure 2. IHg concentration in different parts of rice plant collected from artisanal Hg mining site, abandoned Hg mining sites and control site (with standard deviations; dry weight).

Hg mining site (**Table 2**). With respect to analytical data in roots mentioned above, the accumulation and retention of IHg in stalk probably was influenced by a number of factors, and TGM concentration in the ambient air may be one of the most important variables.

The highest concentration of IHg in leaf was found at the artisanal Hg mining site, with an average concentration of 9.1 $\pm$ 4.1 mg kg^{-1} and a range of 5.0−15 mg kg^{-1}, which were significantly higher than those at the abandoned Hg mining sites (mean: 4.1 $\pm$ 2.8 mg kg^{-1} with a range of 0.88−9.4 mg kg^{-1}) and the control site (mean: 0.10 $\pm$ 0.032 mg kg^{-1} with a range of 0.060−0.14 mg kg^{-1}). During our sampling campaigns, TGM concentrations in ambient air at the artisanal Hg mining site,

reaching up to 1652 ng m^{-3} with a mean of 198 $\pm$ 183 ng m^{-3}, were much higher than those at the abandoned Hg mining sites (32 $\pm$ 36 ng m^{-3}) and the control site (6.2 $\pm$ 3.0 ng m^{-3}) (**Table 3**). It indicated that high concentrations of TGM in ambient air at the artisanal Hg mining site are the major source for the elevated IHg levels in leaf. Our data are in good agreement with the results reported by Fay and Gustin (23) that the leaves of plants can accumulate Hg from air.

IHg concentrations in rice tissues exhibited the following distribution patterns: root > leaf > stalk > hull > seed at the abandoned Hg mining sites; root > leaf > stalk > seed > hull at the control site; and leaf > root > stalk > hull > seed at the artisanal Hg mining site, respectively (**Figure 2**).

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Table 3. Hg in Precipitation, Ambient Air and Rice Paddy Soil at Control Site, Abandoned Hg Mining Sites and Artisanal Hg Mining Site

sampling sites	precipitation, mean ± SD, THg, ng L^{-1} (range)	ambient air, mean ± SD, Hg^0, ng m^{-3} (range)	soil, mean ± SD, mg kg^{-1} (range)	
			THg	MeHg
control site	24 ± 14 (3.4−54)	6.2 ± 3.0[a] (2.0−12)[a]	0.31 ± 0.04 (0.24−0.38)	0.00040 ± 0.00010 (0.00024−0.00058)
abandoned Hg mining sites	577 ± 371 (68−1035)	32 ± 36 (6.0−290)	131 ± 145 (31−527)	0.0017 ± 0.0007 (0.0008−0.0032)
artisanal Hg mining site	2157 ± 1446 (365−4760)	198 ± 183 (12−1652)	22 ± 8 (13−36)	0.0032 ± 0.0005 (0.0026−0.0042)

[a] Data from Zheng (*13*).

Table 4. Pearson's Correlation Matrix, Giving the Linear Correlation Coefficients (*r*) among the Hg Levels in Tissues of Rice Plant and Soil (*n* = 46)[a]

	seed[b]	hull[b]	stalk[b]	leaf[b]	root[b]	soil[b]	seed[c]	hull[c]	stalk[c]	leaf[c]	root[c]	soil[c]
seed[b]	1											
hull[b]	0.89***	1										
stalk[b]	0.86***	0.87***	1									
leaf[b]	0.90***	0.88***	0.95***	1								
root[b]	0.41**	0.39**	0.39**	0.28	1							
soil[b]	0.27	0.23	0.27	0.18	0.92***	1						
seed[c]							1					
hull[c]							0.81***	1				
stalk[c]							0.94***	0.82***	1			
leaf[c]							0.47***	0.66***	0.55***	1		
root[c]							0.78***	0.83***	0.84***	0.70***	1	
soil[c]							0.76***	0.78***	0.72***	0.60***	0.80***	1

[a] ***Correlation is significant at the 0.001 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). [b] IHg in fractions of rice plant and soil. [c] MeHg in fractions of rice plant and soil.

Table 5. PCA Factor Loadings and Percent Variance of the Data Explained by Each Factor from Concentrations of Hg in Tissues of Rice Plant and Soil

IHg	F1-PCR	F2-PCR	MeHg	F1-PCR	F2-PCR
seed	0.93	−0.21	seed	0.910	−0.34
hull	0.92	−0.25	hull	0.94	−0.048
stalk	0.94	−0.20	stalk	0.93	−0.23
leaf	0.93	−0.31	leaf	0.75	0.64
root	0.62	0.77	root	0.93	0.10
soil	0.50	0.85	soil	0.87	−0.003
% of variance explained	68%	26%	% of variance explained	80%	9.8%
cumulative of total variance	94%		cumulative of total variance	89.8%	

Elevated IHg concentrations in tissues of rice plant in the Wanshan Hg mining area compared to the control site evidently reflected the combined uptake of Hg from the soil and water by roots, and from atmosphere by leaves (*6, 7, 12*). Fay and Gustin (*23*) reported that (1) air Hg level was the key indicator of leaf Hg concentration especially for the deciduous plants, (2) stalk Hg level was impacted by both air and soil Hg concentrations, and (3) root Hg level was directly associated with soil Hg concentrations. As mentioned above, the different patterns of IHg levels in tissues of rice plant among the artisanal Hg mining site, the abandoned Hg mining sites, and the control site resulted from the different levels of Hg in paddy soil and in ambient air.

As shown in **Table 4**, significantly positive correlations between IHg in leaf, seed, hull, and stalk were observed. However, the correlations between IHg concentration in root and other fractions of rice plant (seed, $r = 0.41$, $p < 0.01$, $n = 46$; hull, $r = 0.39$, $p < 0.01$, $n = 46$; stalk, $r = 0.39$, $p < 0.01$, $n = 46$; leaf, $r = 0.28$, $p > 0.05$, $n = 46$) were consistently weaker. Principal component analysis (PCA) was performed for our data set, and it is shown in **Table 5** that cumulatively 94% percent of the total variance was explained by two principal components. IHg in above ground parts of rice plants had all high loadings in the first factor (F1) extracted explaining about 68% of the total variance. Preliminary

F1 was attributed to an indicator of the ambient air Hg impact. The second component still with an eigenvalue >1 explaining 26% of the total variance had higher loadings of IHg in root as well as in soil. The PCA analysis thus implies that Hg in ambient air is a potential source to the above ground parts (seed, leaf, stalk and hull), and Hg in soil is a potential source to root.

In this study the roots were cleaned using drinking water in the field followed by deionized water rinses after being brought back to the laboratory. In this case the iron plaque of roots was probably not removed from the root during the process of sample preparation. Hence the IHg levels in root represented the IHg both in root and in iron plaque. It implied that the root surface acted as a potential Hg barrier (iron plaque), which blocks Hg ion mass transport through the root system to the above ground parts such as leaf, stalk, seed and hull (*24*).

MeHg in Tissues of Rice Plants. The concentrations and distribution of MeHg in tissues of rice plant collected from the artisanal Hg mining site (Gouxi), the abandoned Hg mining sites (Wukeng, Dashuixi), and the control site (Huaxi) are showed in **Table 2** and **Figure 3**. The highest concentrations of MeHg in tissues of rice plant were all observed at the artisanal Hg mining site, with mean values of 0.032 ± 0.014 mg kg^{-1} in seed, 0.012 ± 0.0018 mg kg^{-1} in root, 0.0064 ± 0.0020 mg kg^{-1} in hull, 0.0037 ± 0.0013 mg kg^{-1} in stalk,

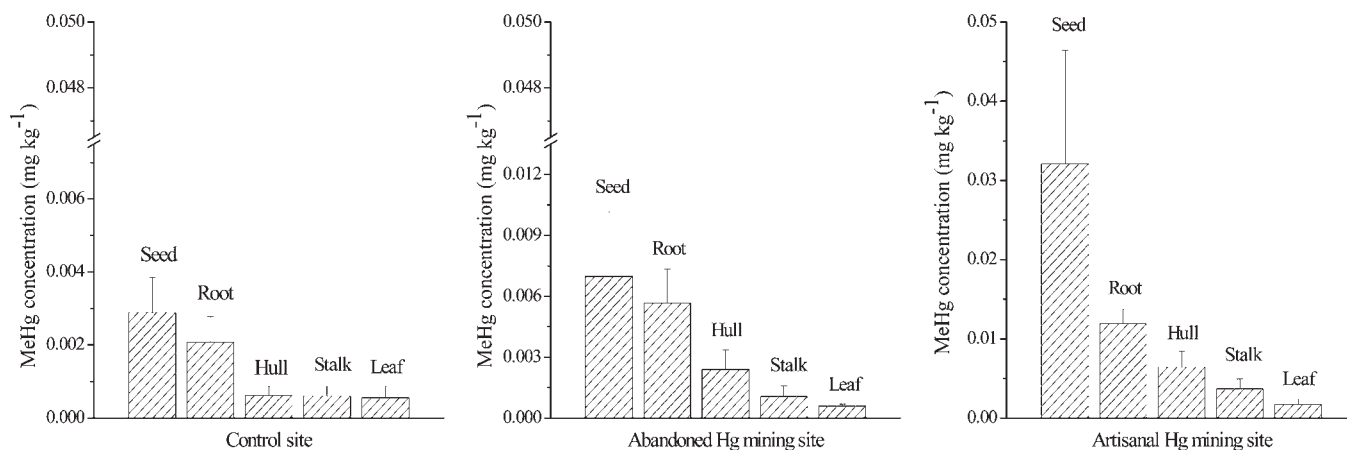**4956** *J. Agric. Food Chem.,* Vol. 58, No. 8, 2010

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Figure 3. MeHg concentration in different parts of rice plant collected from artisanal Hg mining site, abandoned Hg mining sites and control site (with standard deviations; dry weight).

and 0.0017 ± 0.00061 mg kg^{-1} in leaf, respectively. MeHg concentrations in tissues of rice plants at the abandoned Hg mining sites were slightly lower, with mean values of 0.007 ± 0.0032 mg kg^{-1} in seed, 0.0057 ± 0.0017 mg kg^{-1} in root, 0.0024 ± 0.0010 mg kg^{-1} in hull, 0.0011 ± 0.00052 mg kg^{-1} in stalk, and 0.00058 ± 0.00011 mg kg^{-1} in leaf, respectively. The lowest values of MeHg in tissues of rice plants were observed at the control site, with mean values of 0.0029 ± 0.00097 mg kg^{-1} in seeds, 0.0021 ± 0.00070 mg kg^{-1} in root, 0.00062 ± 0.00018 mg kg^{-1} in hull, 0.00060 ± 0.00027 mg kg^{-1} in stalk, and 0.00055 ± 0.00032 mg kg^{-1} in leaf, respectively.

Generally, THg concentrations in most crops are usually below 0.020 mg kg^{-1}, which is the maximum permissible limit recommended by Chinese National Standard Agency (22), and mainly in inorganic forms (3). However, in this study elevated ratios of MeHg/THg in seed, with a mean of $47 \pm 8.0\%$ at the control site, $11 \pm 2.1\%$ at the artisanal Hg mining site and $8.1 \pm 6.8\%$ at the abandoned Hg mining sites, were observed compared to the remaining tissues of rice plant. Furthermore, the mean concentration of MeHg in seed (0.032 ± 0.014 mg kg^{-1}) at the artisanal Hg mining site was significantly higher than the food standard for THg (0.020 mg kg^{-1}), posing a potential threat to the health of the local residents (10).

Although the IHg concentrations in root at the abandoned Hg mining sites were significantly higher than those at the artisanal Hg mining site and the control site due to the elevated THg concentrations in soil, the highest MeHg concentrations in both root and soil were present at the artisanal Hg mining site as shown in **Tables 2** and **3**. A significantly positive correlation was observed between MeHg concentrations in rice plants and the corresponding soil (**Table 4**). These observations implied that MeHg in soil was the main source of MeHg in tissues of rice plant.

Wetlands are widely known as sites for MeHg production due to the fact that the environmental conditions are favorable for net Hg methylation (25), and therefore are net sink for THg but as net source of MeHg (26). Previous study showed that the ephemeral wetland can be more conductive to MeHg production and bioaccumulation (27). The rice paddy field is shallowly flooded (about 30 cm) during the rice growing season, and then flooded again postharvest in fall and winter to speed the decomposition of stalk. Furthermore, the irrigation water, precipitation and ongoing smelting activities provided sufficient Hg to the rice paddy field in the Wanshan Hg mining area (6, 12). Hence rice paddy soil, as a typical ephemeral wetland, has a high potential of Hg methylation, which results in the accumulation of MeHg to rice plant.

The average MeHg concentrations showed statistically significant differences between the fractions of rice plant (seed, hull, root, stalk and leaf) (K−W test, $p < 0.001$). Regardless of the sampling sites, the seeds contained the highest concentrations of MeHg. Seed to leaf concentration quotients for MeHg reached up to 15 ± 13, followed by those of seed to stalks (7.1 ± 2.7), seed to hull (4.3 ± 2.4), and seed to root (1.7 ± 0.97), respectively. Furthermore, as shown in **Table 2**, the maximal ratios of Hg as MeHg were present in seed compared to hull, root, leaf, and stalk for all sampling sites. Quotients for the various tissues of rice plant to soil MeHg concentration were calculated to clarify the bioaccumulation potential of MeHg (preferable uptake of mercury from soil). The bioaccumulation factors (BAF, i.e. seed/soil concentration ratios) (4) of MeHg in seed can reach up to 7.0 ± 3.9, which are much higher than those in root (4.2 ± 2.1), hull (1.8 ± 0.82), stalk (1.1 ± 0.75) and leaf (0.72 ± 0.73). These results concluded that rice seed has the highest ability to accumulate MeHg.

Significantly positive correlations were observed between MeHg levels in seed, hull, stalk, leaf, and root for all of the sampling sites (**Table 4**). Further statistic analysis showed that MeHg concentration in root significantly positively correlated with seed ($r = 0.78, p < 0.001, n = 46$), hull ($r = 0.83, p < 0.001, n = 46$), leaf ($r = 0.70, p < 0.001, n = 46$) and stalk ($r = 0.84, p < 0.001, n = 46$) (**Table 4**). Hence, it is implied that the source of MeHg in tissues of rice plant should be unique.

Schwesig et al. (28) have jointly reported that organic Hg in plant can be transported much more easily than inorganic Hg. Moreover, a recent study has revealed that the phytochelatins, small peptides that detoxify plants from heavy metals, can sequester Hg^{2+}, but not MeHg (29). PCA showed that one PC with eigenvalue > 1 was extracted as shown in **Table 5**. This had high loadings in all of the parameters, of which MeHg (leaf) was slightly lower (~ 0.75). The result can be interpreted as MeHg in tissues of rice plant being firmly associated with MeHg level in soil. The results of the statistical analysis as shown above, therefore, suggest that MeHg in paddy soil could be the only source to root, and other tissues of the rice plant.

Our analytical data showed that MeHg levels in tissues of rice plants followed the trend seed > root > hull > stalk > leaf for all sampling sites (**Table 2** and **Figure 3**), which is different from the distribution pattern of IHg. This observation implied that the sources/mechanisms of IHg and MeHg uptakes into the tissues of rice plant are completely different. As concluded above, the distribution patterns of IHg in tissues of rice plant are restricted by both the Hg in ambient air, which is the potential
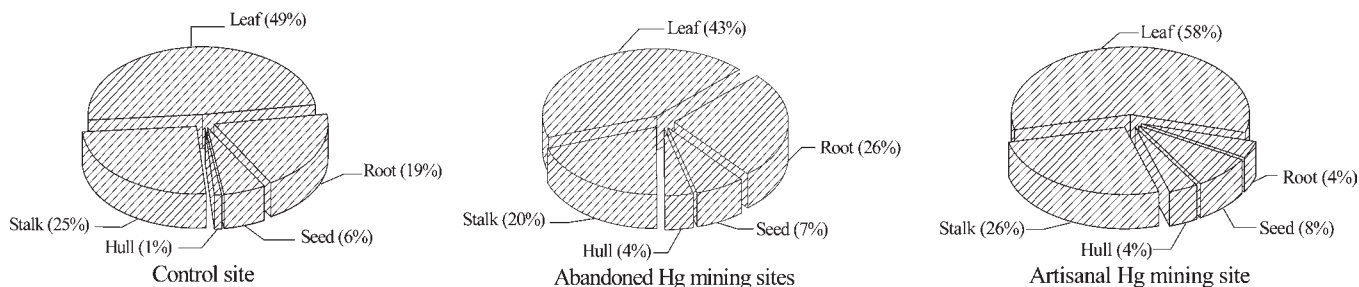
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Figure 4. The relative distribution of IHg in tissues from rice plants at the various experimental sites.
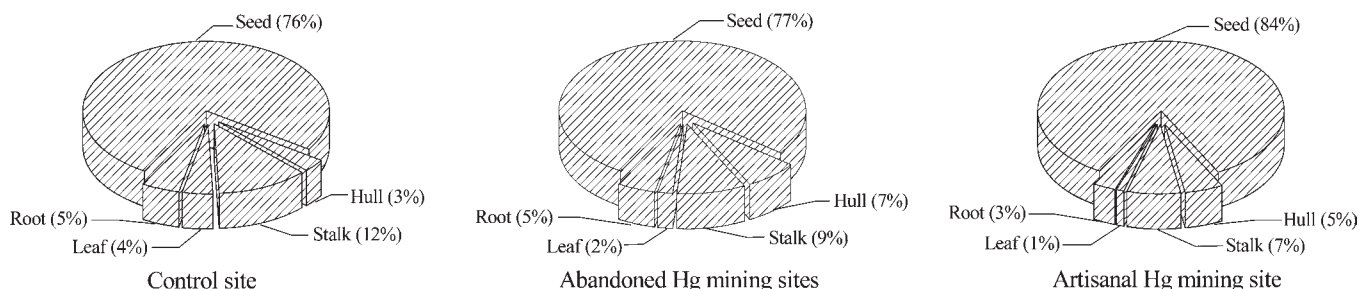

Figure 5. The relative distribution of MeHg in tissues from rice plants at the various experimental sites.

source to the above ground parts, and the Hg in soil, which is the is the potential source to root. However, the MeHg in soil impacts the distribution pattern of MeHg in tissues of rice plant for all the sampling sites, and is the only source to tissues of rice plant.

Although THg concentrations in soil at the abandoned Hg mining sites were significantly higher than those at the artisanal Hg mining site and the control site as shown in **Table 3**, the highest MeHg concentrations in soil occurred at the artisanal Hg mining site (0.0032 ± 0.00045 mg kg^{-1}). The artisanal Hg smelting activities which emitted a large amount of Hg to the ambient air (*7*) were in operation during the entire rice growing season at the artisanal Hg mining site. The concentrations of Hg^0 at the artisanal Hg mining site were significantly higher than those at the abandoned Hg mining sites and the control site (**Table 3**). THg concentrations in precipitation at the artisanal Hg mining site reached 2157 ± 1446 ng L^{-1}, which was much higher than those at the abandoned Hg mining sites (577 ± 371 ng L^{-1}) and the control site (24 ± 14 ng L^{-1}) (**Table 3**). Harris et al. (*30*) demonstrated that an increase in Hg loading at rates resembling atmospheric deposition resulted in an increase in MeHg production and concentrations in aquatic fresh water biota. Hence, we may consider that the higher MeHg concentrations in soil at the artisanal Hg mining site can be attributed to the newly deposited Hg, which is more readily transformed to MeHg (*31*). However, more research is needed to verify this hypothesis.

Distribution of IHg and MeHg in Rice Plant Tissues. The relative distributions of IHg and MeHg in different tissues of rice plant are shown in **Figure 4** and **Figure 5**. Our data showed that the above ground parts of rice plant accumulated the majority of both IHg and MeHg in the whole rice plants. Among the tissues of rice plant, IHg content was highest in leaf (**Figure 4**). The highest IHg content in above ground parts of rice plant (leaf, hull, leaf and stalk) was present at the artisanal Hg mining site, presumably due to the serious Hg contamination in the ambient air. On the contrary, the highest IHg content in root occurred at the abandoned Hg mining sites, suggesting that root IHg was directly associated with soil Hg concentrations. On the other hand, regardless of sampling sites, most of the MeHg is accumulated in the seeds as shown in **Figure 5**, which is about 10, 19, 21, and 62 times (mean data) higher than that of stalk, hull, root and

leaf, respectively. The results further proved that rice seed has the highest ability to bioaccumulate MeHg compared to other parts of rice plant.

Our study confirmed the occurrence of elevated IHg and MeHg in tissues of rice plant cultivated in Wanshan Hg mining area compared to a control site, which can be linked to the historical large-scale Hg mining/smelting and ongoing artisanal Hg smelting activities. The elevated levels of IHg in leaf measured at the artisanal Hg mining site were specifically mediated by the enhanced loading of atmospheric mercury. The elevated IHg concentrations in rice root tissues in the Wanshan Hg mining area, especially at the abandoned Hg mining sites compared to the control site, can be attributed to the serious Hg-contaminated rice paddies. The highest values of IHg in stalks were found at the artisanal Hg mining site, implying the effect of high levels of Hg^0 in air. MeHg concentrations in tissues of rice plants followed the trend seed > root > hull > stalk > leaf, which confirmed that the rice seed has the highest ability to bioaccumulate MeHg. MeHg concentrations in tissues of rice plant at the artisanal Hg mining site were significantly higher than those at both the abandoned Hg mining sites and the control site due to high Hg deposition fluxes resulting from the ongoing artisanal Hg mining activities. This may indicate that the newly deposited Hg can be more readily methylated in soil.

ABBREVIATIONS USED

MeHg, methylmercury; THg, total mercury; IHg, inorganic mercury; EtHg, ethyl mercury; $CH_3CH_3CH_2Hg$, methylethyl mercury; TGM, total gaseous mercury; CVAFS, cold vapor atomic fluorescence spectrometry; GC, gas chromatography; BAF, bioaccumulation factors; PCA, principal component analysis.

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