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Localization and Speciation of Mercury in Brown Rice with Implications for Pan-Asian Public Health

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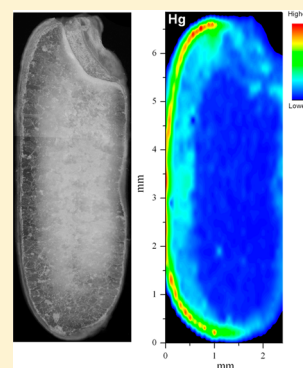
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S Supporting Information

ABSTRACT: Cultivation of paddy rice for human consumption is a dominant agricultural activity throughout Asia. High levels of mercury (Hg) in rice grain pose a potential threat to human health, although the extent of risk is dependent on the chemical speciation of Hg inside the grain. We have investigated the speciation and localization of Hg in three fractions of rice grain (hull, bran, and white rice) collected from a Hg-contaminated region in China. On a mass basis, the majority of inorganic mercury (IHg) in a rice grain is found in hull and bran. However, the majority of the more toxic species methyl mercury (MeHg) is found in edible white rice. Our data show that during grain processing, most of the IHg (~78%) is eliminated, but the majority of the MeHg remains in the food product (~80%). Synchrotron radiation microscopic X-ray fluorescence (SR- μ XRF) mapping shows strong localization of Hg at the surface of brown rice grains, corresponding to the pericarp and aleurone layer. We infer that this Hg is predominantly IHg absorbed from the atmosphere. Based on X-ray absorption near-edge spectroscopy (XANES) data we propose that IHg in bran is primarily bound to cysteine, and is associated with phytochelatin. Consequently, IHg is largely immobile and restricted to the outer layers of rice grain. MeHg in bran is primarily bound to cysteine and is associated with proteins. However, this MeHg–cysteine association behaves like a mobile nutrient and is actively transported to the endosperm during seed ripening. Concentration of MeHg–cysteine in white rice has implications for public health. There is growing evidence for Hg contamination of rice throughout Asia due to point and diffuse sources of Hg pollution. The magnitude of the associated risk must be quantified through better understanding of the localization and speciation of mercury in rice. Our work makes an effort to contribute to this understanding.



1. INTRODUCTION

Rice (*Oryza sativa* L.) has been highlighted in recent literature as a bioaccumulator plant species of methylmercury (MeHg). Of particular concern is the growing understanding that MeHg behaves as a mobile nutrient in rice, and will concentrate in the ripening seed of rice plants.^{1–5} The reason for this unique bioaccumulation may be related to the fact that rice, unlike other cereal crops, is grown in an ephemeral wetland. The associated anaerobic biogeochemical conditions provide an environment that is suitable for the bacterial methylation of Hg in the soil where a source for the contaminant exists.¹ Widespread contamination of paddy soils with Hg has occurred in many parts of the world due to large- and small-scale Hg mining activities, the irrigation of paddy fields with Hg-contaminated water, and due to diffuse vectors associated with industrial activity and the combustion of coal for energy. MeHg concentrations in rice can be enhanced even in cases where no point source of Hg contamination can be defined.^{4,6}

The consumption of fish, fish products, and marine mammals is generally considered to be the main global pathway for human exposure to MeHg. However, recent studies have shown that rice consumption can be the major pathway of MeHg exposure in inland Hg mining areas and in certain Hg-

contaminated areas of Southwest China.^{4,7} Rice is one of the major stable food crops in the world, with daily intake up to 0.5 kg (dry weight) in many Asian countries.⁸ Consequently, paddy rice cultivation is one of the most prevalent agricultural land uses in South and East Asia. The considerable attention worldwide to the uptake, metabolism, transport, and accumulation of Hg in rice is justifiable.

Current understanding is that soil is the primary source of MeHg to rice plant tissues.^{1,2,5} Li et al.⁹ established that MeHg in rice seeds exists exclusively as methylmercury-L-cysteinate (MeHg–cysteine). This is the same complex that is thought to store MeHg in fish and is thought to be responsible for the transfer of MeHg across the blood-brain and placental barriers.

Hg²⁺ and MeHg⁺ ions preferentially bind to organic matter (including living organisms) via reduced sulfur groups (thiol groups).¹⁰ The chemical speciation of Hg defines the bioavailability and toxicity of this element in biological samples.¹¹ For example, lighter dialkylmercury derivatives of

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Hg (such as Me_2Hg) are recognized as extremely toxic even at low levels, whereas mercuric selenide (HgSe) has relatively low toxicity and the formation of HgSe will act as a detoxification agent in the liver of marine mammals.¹⁰ MeHg^+ associated with glutathione is believed to play an important role in metal detoxification within plants; while, when present as an L-cysteine complex, MeHg^+ may readily transfer across cell membrane barriers.¹² The complexation of Hg with biomolecules (including complexation to hydrosulfide or thiol ligands), therefore, plays a key role in the mechanism of distribution, transport, and accumulation of Hg in biota.

Despite the abundance of literature on the effect of contaminated air, soil, and water on Hg and MeHg uptake by plants, the compartmentalization of accumulated Hg in rice grain is poorly understood. A kernel of rice consists of a hull and a nutritious bran coat layer enclosing the endosperm and inner embryo. Before the rice can be packaged or cooked, the inedible hull is removed yielding brown rice. Brown rice may be eaten in this state, or further processed into rice bran and regular milled rice. The latter is often referred to as “white” or “polished” rice, and is the most common type of rice consumed. Consequently, the Hg content in the raw rice grain does not readily correspond to the amount of Hg retained in cooked rice. Rothenberg et al.¹³ determined the concentration of THg and MeHg in brown and white rice, although the bran and hull layers were not separated and analyzed for Hg concentration in this work. Recently, Qiu et al.¹⁴ reported elevated MeHg in rice bran compared to the other rice fractions. While these results are interesting, they do not afford comprehensive understanding of the speciation and localization of Hg throughout rice grain. Such understanding is critical in defining a better model for human risk that may be associated with the ingestion of MeHg-contaminated rice.

To expand our knowledge of the distribution of IHg and MeHg in rice, we quantified the presence of Hg in three fractions of rice grain. We used X-ray absorption near-edge spectroscopy (XANES) to examine the Hg speciation in rice grains as this technique has proved ideally suited to the identification of chemical forms of heavy metals within biological specimens. Furthermore, synchrotron microscopic X-ray fluorescence (SR- μ XRF) was used to map the Hg localization patterns in the rice grain. The primary objectives of our study were to (1) investigate the speciation and localization of Hg in rice grain; and (2) to gain better understanding of the mechanism of Hg transport and accumulation in rice plants.

2. MATERIALS AND METHODS

2.1. Study Area. Rice analyzed in this study was collected from paddy fields at three locations in Guizhou Province, China (Supporting Information (SI) Figure S1). Two of these locations have a recorded history of Hg contamination and are situated within the Wanshan Hg mining district: an artisanal Hg mining site (Gouxi), and two abandoned Hg mining sites (Wukeng and Dashuixi). The control site for the study with no point source of Hg contamination is located in Huaxi, 300 km away from the mining region. Detailed information concerning each sampling location is shown in the SI (Table S1).

2.2. Sample Collection and Preparation. Eighty nine composite ripe rice grain samples were collected by hand from the control site ($n = 10$), the abandoned Hg mining sites ($n = 39$), and the artisanal Hg mining site ($n = 40$) during the harvest seasons of 2006–2009 (SI Table S2). Rice grain samples were divided into three fractions: hull, bran, and white

rice. All samples were ground to less than 150 mesh (IKA-A11 basic, IKA, Germany) for THg and MeHg analysis. In addition, approximately 150 μm thick longitudinal and latitudinal sections of brown rice were prepared and placed on Kapton tape for SR- μ XRF analysis. XANES spectra of selected rice bran samples and reference compounds were obtained using beamline 1W1B of the Beijing Synchrotron Radiation Facility (BSRF). A detailed description of sample collection and preparation is given in the SI.

2.3. Analytical Methods. Detailed information concerning analytical methods are described in the SI, including THg/MeHg analysis of fractions of rice grain, SR- μ XRF analysis of thin sections (brown rice), XANES analysis of selected rice bran samples, and statistical analysis of the resulting analytical data.

2.4. Quality Control. Quality control measures consisted of method blanks, triplicates, matrix spikes, and certified reference materials (Table S3) as described in the SI.

3. RESULTS AND DISCUSSION

3.1. Distribution of Inorganic Mercury and Methylmercury in Rice Grain. *Inorganic Mercury and Methylmercury Concentrations in Rice Grain.* There was a significant difference between the concentration of both IHg and MeHg in each rice fraction for all the sampling sites ($p < 0.001$) (Table 1). The highest mean concentration of IHg was recorded in

Table 1. Concentrations of IHg and MeHg in Fractions of Rice Grain from Different Sampling Sites (Mean \pm S.D, $\mu\text{g kg}^{-1}$)

sampling sites	fractions	Hg concentration		MeHg/ THg(%)
		IHg	MeHg	
Control Site ($n = 10$)	hull	6.2 ± 0.51	0.26 ± 0.05	4.1 ± 0.73
	bran	24 ± 1.4	3.0 ± 0.48	11 ± 1.8
	white rice	2.7 ± 0.25	2.4 ± 0.16	48 ± 3.3
abandoned Hg mining Sites ($n = 39$)	hull	66 ± 50	1.1 ± 0.68	2.1 ± 1.7
	bran	139 ± 84	11 ± 4.5	8.3 ± 4.4
	white rice	7.9 ± 3.6	5.7 ± 2.2	44 ± 13
artisanal Hg mining site ($n = 40$)	hull	643 ± 820	3.8 ± 2.7	1.1 ± 0.97
	bran	1909 ± 1590	42 ± 24	3.7 ± 2.9
	white rice	89 ± 73	22 ± 14	26 ± 16

bran at each site, and followed the order bran > hull > white rice. The highest mean concentration of MeHg was again recorded in bran for each site, but followed the order bran > white rice > hull (SI Figure S2).

We calculated the Hg concentration ratio for adjacent fractions of rice grain to further evaluate the spatial distribution of IHg and MeHg within the grains (SI Table S4). Specifically, we calculated the concentration ratios for bran to hull, and bran to white rice. The relative ordering of these two ratios gives an indication of where in the rice grain these two Hg species are predominantly stored. For IHg, the bran to hull concentration ratio was lower than the bran to white rice ratio, for all three sites. However, for MeHg, the bran to hull ratio was higher than the bran to white rice ratio for all three sites. The ratios indicate that IHg is predominantly restricted to the outer bran

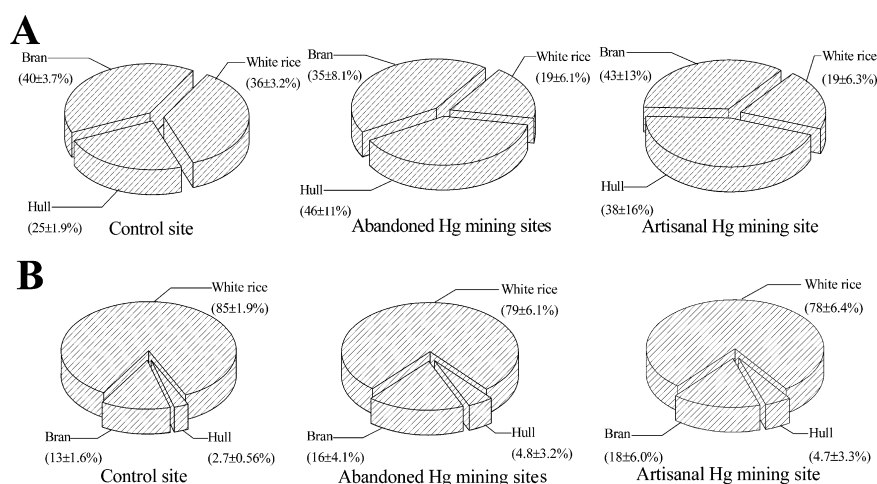


Figure 1. Relative distribution of IHg (A) and MeHg (B) in fractions of rice grain collected across three experimental areas.

coating, whereas MeHg can be mobilized throughout the rice grain, and can become concentrated in the white rice. We interpret these concentration ratios to infer a difference in the mechanism for transport and storage of the two Hg species in rice grain. Previous work has defined atmospheric Hg as the principal source of IHg to the aboveground parts of rice plant.^{1,15} However, MeHg in paddy soil is believed to be the dominant source of MeHg in rice.^{1,2} We therefore suggest that the source of mercury could be a key factor that determines the Hg concentration ratio apparent for adjacent fractions of rice grain.

The distribution of IHg and MeHg in rice grains (Table 1 and SI Figure S2) is in agreement with the results of a previous study that investigated the relative presence of Hg in fractions of rice grain.^{13,14} The data show that the inedible bran coating accumulates the highest concentration of both IHg and MeHg species. Literature shows that Hg^{2+} and MeHg^+ ions are preferentially present in biotic tissues as complexes of sulfur-containing biomolecular ligands.¹⁰ Furthermore, reported chemical analyses show that the concentrations of protein in rice bran is much higher than that in white rice and rice hull, suggesting the enrichment of protein in bran.¹⁶ Therefore, an explanation for the enrichment of Hg in bran may be the high affinity of heavy metal ions to side chain ligands of protein¹⁷ that are dominant in the bran as a consequence of the comparatively higher protein content.¹⁶

The THg concentration in white rice collected from the artisanal Hg mining site ($111 \pm 75 \mu\text{g kg}^{-1}$) exceeded the maximum permissible limit of $20 \mu\text{g kg}^{-1}$ recommended by the Chinese National Standard Agency.¹⁸ This food standard was not, however, exceeded for rice collected from the abandoned Hg mining sites ($14 \pm 4.4 \mu\text{g kg}^{-1}$) and control site ($5.1 \pm 0.23 \mu\text{g kg}^{-1}$). Mercury is generally thought to exist in food crops as less toxic inorganic forms.¹⁹ Food safety guidelines are therefore set considering THg: there is no safe level of MeHg in food. However, our data for the artisanal mining site show that the MeHg concentration itself exceeded the food safety level. Across each of the three sites, the ratio of MeHg to IHg (expressed as MeHg% of THg) is greater for white rice than for the hull or bran, ranging from 3.6 to 72% of THg. In contrast, MeHg is 0.43–24% of THg in the bran and 0.058 to 7.6% of THg in the hull across the three sites. This relative concentration of MeHg in white rice compared to the other

rice fractions again infers differential transport, distribution, and sources of IHg and MeHg throughout the ripening rice grain.

Mercury Mass in the Various Fractions of Rice Grain. Although rice bran has the highest ability to accumulate both IHg and MeHg species, the bran only constitutes $\sim 8.0\%$ of the rice weight; white rice constitutes the major part of rice grain dry mass ($71 \pm 3.1\%$). Therefore, with respect to food safety, the total amount of Hg in the various fractions of rice grains is more important than the relative concentration of the Hg species.

There was an unequal distribution in the total amount of IHg and MeHg between the three fractions of rice grain (Figure 1). For the control site, there was a relatively even distribution of IHg content between the three rice fractions. For the two contaminated sites, this distribution was shifted toward the hull and the bran. In contrast, the dominant fraction for MeHg storage was white rice, and there was no evidence for differential storage across the three sites. Therefore, the consequence of grain processing (the removal of hull and bran) is the elimination of most of the IHg mass from rice ($\sim 78\%$). However, there is little reduction in the mass of MeHg ($\sim 20\%$).

The unequal mass distribution of IHg and MeHg between the three analyzed rice fractions again indicates that the extent of Hg mobility within plants is a function of Hg speciation. Previous studies have described the behavior of MeHg in plants as similar to that of a mobile plant nutrient that can be translocated to ripening seed.^{2,20,21} Assuming that the atmosphere is the major source of IHg in rice grain^{1,15} (see SI), we propose that the coating layers (hull and bran) have considerable capacity to retain IHg and limit translocation into the rice kernel. In contrast, MeHg in paddy soils is taken up through roots, translocated to above-ground parts of the rice plant, and eventually unloaded into the white rice during the ripening phase. Different sources of IHg and MeHg to fractions of rice grain could partly explain the divergent mass distribution of IHg and MeHg between the three analyzed rice fractions. However, further work is needed to verify this hypothesis.

3.2. Spatial Distribution of Mercury and Selected Elements in the Grain. SR- μ XRF elemental maps of longitudinal and latitudinal sections of the rice grain (brown rice) illustrate the spatial distribution of Hg as well as other selected elements Ca, Cd, Cu, Fe, K, Mn, Zn, and Se (Figure 2). The SR- μ XRF maps do not differentiate between IHg and

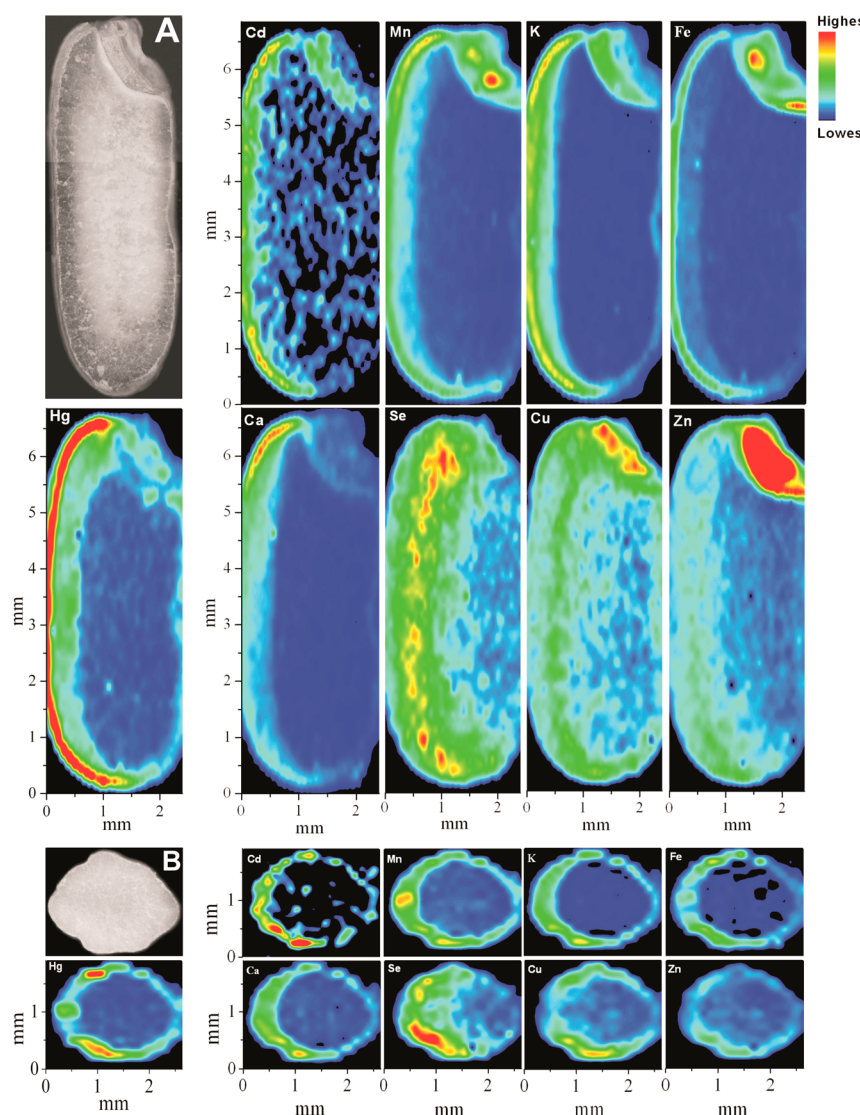


Figure 2. Elemental maps of longitudinal (A) and latitudinal (B) sections of rice grain (ventral side on right). The SR- μ XRF signals for map (A) and (B) were collected at 50 and 100 μ m steps, respectively. The areas mapped for (A) and (B) were 6800 μ m \times 2400 and 2600 μ m \times 1900 μ m, respectively.

MeHg. The maps present THg distribution throughout rice grains.

SR- μ XRF revealed a spatial disparity in the distribution of Hg throughout the grain (Figure 2). The longitudinal (Figure 2A) and latitudinal sections (Figure 2B) expose a prominent localization of Hg at the surface of the brown rice grain corresponding to the pericarp and aleurone layer, and a defined concentration gradient from the epidermal layer toward the center of the grain. This concentration gradient is in agreement with the Hg concentration analyzed in the various fractions of the rice grain (Table 1). Furthermore, longitudinal section imaging quantified a slightly elevated level of Hg in the embryo of rice grain. However, this enrichment is minor compared to the Hg concentration level in the outer layer of the grain.

Figure 2 indicates that the spatial distribution of a number of other elements also varies throughout brown rice grain. The elements Ca, Fe, K, Mn, and Cd, similarly to Hg, are highly localized at the surface of the rice grain. Numerous studies on elemental location in rice grain have highlighted the concentration of nutrient elements such as Fe, Ca, P, and K to the epidermal layer.^{22,23}

Analysis of the longitudinal and latitudinal sections of brown rice shows that Hg, Ca, Cd, Fe, K, and Mn are not uniformly concentrated around the grain, but are particularly enriched in the dorsal side. Del Rosario et al.²⁴ reported that the dorsal side of rice kernel had a thicker aleurone layer than the ventral area, with a relative cell thickness ratio of 3:1. The longitudinal (Figure 2A) and latitudinal (Figure 2B) sections we present in this paper confirm the idea of uneven thickness in the bran layer, with the thicker portion (dorsal area) being concentrated in Hg.

Copper and Se exhibit a different spatial distribution. Longitudinal imaging shows that these elements are not specifically enriched at the epidermal layers of rice grain, but exhibit peak concentrations in the interior of white rice (Figure 2). Latitudinal images show a general distribution of Se throughout the dorsal side of the grain which is opposite to the very restricted distribution of Hg in the grain. The images suggest that there is some overlap of Se and Hg in parts of the rice grain. However, when considered together, we propose that these images support the hypothesis of a different mechanism for storage of these two elements in plants.

Zhang et al.,²⁵ reported that Se could play an important role in limiting the bioaccessibility, absorption, and translocation/bioaccumulation of Hg in rice plants. These authors suggested an antagonistic effect of Se on Hg uptake by rice. We speculate that the strong localization of Se in the interior of the endosperm could restrict the accumulation of Hg in white rice but not in rice bran. The formation of unavailable MeHg selenocysteine and mercuryselenide (HgSe) complexes in organisms has been attributed to the high binding affinity between Se and Hg^{25,26} where there is colocalization of Hg and Se in rice seed. Clearly, more research is needed to better understand the spatial distribution and implications of Hg and Se storage in rice grain.

During the milling process, the pericarp and most of the aleurone layer is removed, while simultaneous polishing also removes the germ leaving milled white rice.²² Removal of the pericarp and aleurone layer results in a considerable loss of elements contained within these parts of a rice grain, with a consequential effect on the nutrient content of the food. Removal of these layers also eliminates a large portion of IHg but not MeHg or Se. We believe that this observation may be important in the context of public health.

3.3. Speciation Analysis of Mercury in Rice Grain by XANES. Due to the relatively low concentration of Hg (IHg and MeHg) in white rice, Hg speciation analysis by XANES was restricted to two rice bran samples, which contained a concentration of Hg that was appropriate for analysis using this technique. The analytical techniques CVAFS and GC-CVAFS were used to determine the THg and MeHg concentrations in the two rice bran samples following the methods described in SI. The results indicated that the THg concentration in sample bran-1 and bran-2 was 3412 $\mu\text{g kg}^{-1}$ and 7365 $\mu\text{g kg}^{-1}$, respectively. The corresponding MeHg concentration was 100 $\mu\text{g kg}^{-1}$ and 33 $\mu\text{g kg}^{-1}$, respectively.

Normalized Hg-L_{III}-edge XANES spectra of standard compounds and the two rice bran samples are shown in SI Figure S3. The absorption spectra for the Hg standards red HgO, HgSO₄, (CH₃COO)₂Hg, yellow HgO, and HgCl₂, all contain a distinct peak near 12.28 keV, which was missing from the spectra of MeHg-cysteine, (α -, β -)HgS, Hg-cysteine, Hg-dicysteine (or Hg-cystine hereafter), and HgSe, as well as the spectra of the two rice bran samples. Therefore, the normalized Hg-L_{III}-edge XANES spectra of the rice bran samples show a superficial resemblance to MeHg-cysteine, (α -, β -)HgS, Hg-cysteine, Hg-dicysteine, and HgSe owing to the absence of the intense peak. The Hg-L_{III}-edge XANES spectra of the two bran rice samples (bran-1 and bran-2) are displayed in SI Figure S4. These two spectra are very similar, with differences between them essentially consisting only of noise. This could suggest that Hg speciation within rice bran is homogeneous.

A superimposition of the XANES spectra of sample bran-1 with the standards MeHg-cysteine, (α -, β -)HgS, Hg-cysteine, Hg-dicysteine, and HgSe is presented in Figure 3. This superimposition shows that the spectra of Hg-cysteine, MeHg-cysteine and, to a lesser extent, Hg-dicysteine, closely match the spectra of the bran sample. This is reasonable, as rice bran, unlike white rice, is protein rich and Hg has high affinity for protein -SH functional group (e.g., -cysteine and -dicysteine).

To derive quantitative information on the Hg speciation in rice bran, linear combination fitting (LCF) was performed on the normalized XANES spectra using the possible combinations of Hg-cysteine, Hg-dicysteine, and MeHg-cysteine spectra.

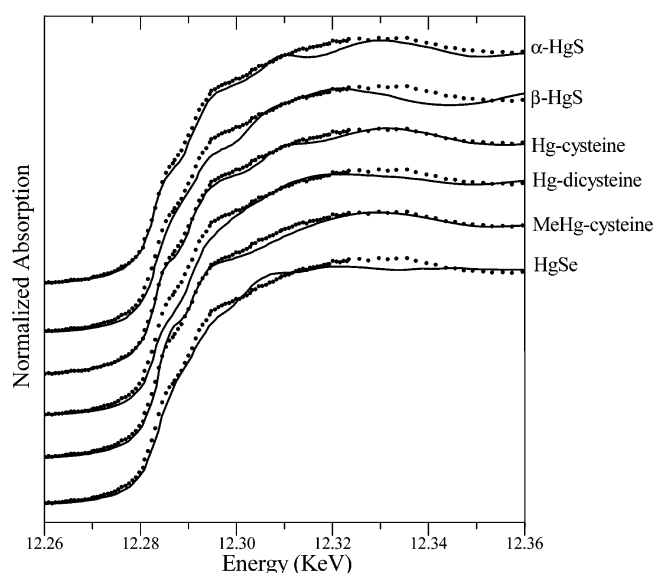


Figure 3. Comparison of the mercury L_{III} XANES spectra of rice bran with the spectra of selected standard compounds. The points show the superimposed spectrum of the rice bran sample on the spectra for the standard compounds (solid lines).

The LCF procedure determined that the spectra of MeHg-cysteine and Hg-cysteine can accurately reproduce the spectra of the bran samples (Figure 4). We infer that this fitting defines the existence of both IHg and MeHg as cysteine derivatives in the rice bran. XAFS scans of the two rice bran samples revealed that approximately 76–77% of the total Hg was present as IHg (Hg-cysteine), with the remainder present in the organic form (MeHg-cysteine) (Table 2). These results are in general agreement with the analysis by GC-CVAFS, although the proportion of MeHg determined by XANES is larger than that assessed using CVAFS. We consider that the discrepancy between the two data sets is due to the relatively low level of Hg in the samples, close to the limit of detection for XANES. Our results agree with the consideration that while synchrotron techniques provide an indication of the separate species present in the sample, they are unable to provide an accurate assessment of their proportion in the whole sample.²⁷

3.4. Possible Mechanism for Hg Accumulation in Rice Grain. Inorganic Mercury. Sulfhydryl amino acids, thiol-containing proteins, and enzymes are abundant in biological systems and play an important role in intracellular activities by controlling or detoxifying metal ions. In the context of Hg metallomics, the peptides containing cysteine and glutamate (glutamic acid) are mechanistically important for heavy metal detoxification in plants and other organisms.²⁸ In this process, phytochelatin (PCs), being enzymatically synthesized cysteine-rich peptides, act as detoxification agents. PC synthase is primarily regulated by activation of the enzyme in the presence of heavy metals.²⁸ PC biosynthesis is induced until the activating metal ions are chelated by the surplus of PCs formed. The formation of metal-PCs complexes occurs in vitro as well as in vivo.²⁹ Evidence for a variety of mercuric complexes of PCs in root and shoot has been established in experiments where rice plants were exposed to MeHg⁺ or Hg²⁺. These PCs can effectively chelate Hg²⁺, but not MeHg⁺.³⁰ Therefore, PCs appear to play an important role in the plant tolerance of IHg while their influence on MeHg detoxification in rice plant is unclear and possibly minor.

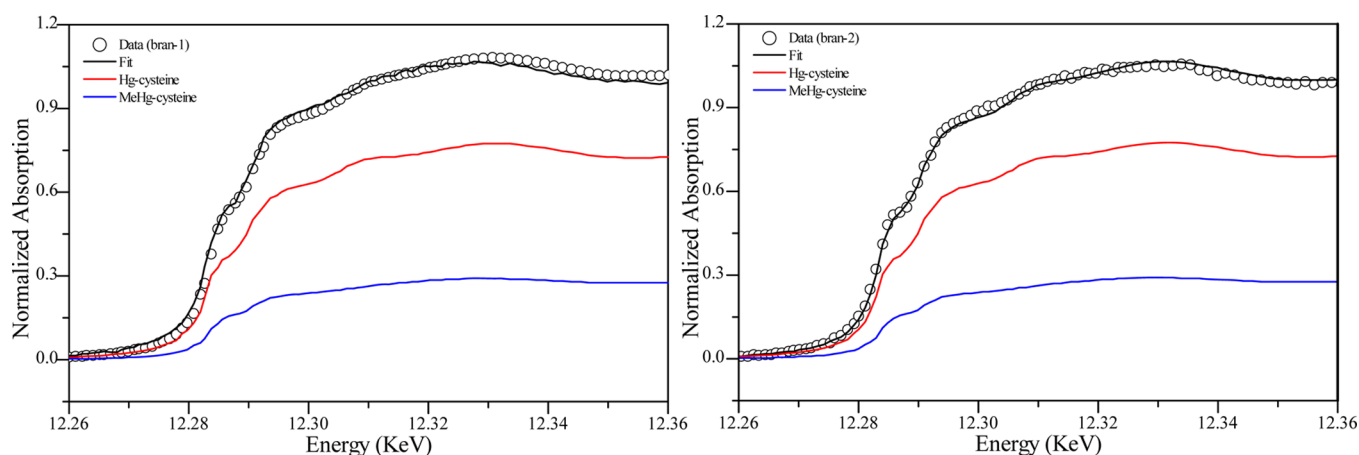


Figure 4. Linear combination analysis of mercury L_{III} XANES spectra of two rice bran samples.

Table 2. Relative Proportion of Mercury Species in Rice Bran Samples As Assessed by Hg L_{III} XANES Least Fitting

samples	Hg-cysteine (%)	MeHg-cysteine (%)	R-Factor	chi-square	reduced chi-square
Bran-1	77 ± 0.5	24 ± 0.5	0.000 19	0.011	0.000 13
Bran-2	76 ± 0.5	25 ± 0.5	0.000 27	0.016	0.000 16

Elemental Hg is mildly lipophilic,³¹ exhibiting foliage-air exchange via stomata as well as uptake by nonstomatal pathways.³² Oxidized Hg^{II} compounds that are either wet or dry deposited to leaves from the atmosphere or produced on leaf surface by Hg^0 oxidation,³³ can traverse the underlying cuticle by epicuticular dissolution, and eventually diffuse into the epidermal cells.³⁴ In previous work, we have highlighted the role of atmospheric Hg^0 as a principal source of IHg accumulated in the fractions of rice grain.^{1,2,15} Based on our XAFS data we assume that a significant fraction of IHg assimilated from the atmosphere is stored in the rice grain as cysteine complexes (Hg-cysteine), and plausibly as Hg-PCs in cytosol.²⁹ We believe the Hg-PC complex will be subsequently actively transported from cytosol to the vacuole,³⁵ since the vacuole is most likely the ultimate storage compartment for Hg ions that enter the cytosol of a given plant cell.²⁹ Consequently, IHg cannot be transported to the endosperm through the ovular vascular trace, which is recognized as a bottleneck for nutrient unloading in rice.³⁶ This theory is consistent with the observation that IHg in brown rice is largely compartmentalized to the outer layers, which can be seen as a self-protection or detoxification mechanism of the rice plant against serious Hg vapor exposure. However, we believe that the mechanism of Hg detoxification in rice plants is more complex than the simple chelation of Hg ions by PCs. We propose that Hg ions can activate PCs synthase, be chelated by the PCs synthesized, and then aggregate to form a more complex metallic structure in vacuole.²⁹ Further research should be conducted to validate this hypothesis.

Methylmercury. To our knowledge, this study is the first XANES evidence of the presence and dominance of a specific MeHg-S compound, namely MeHg-cysteine, in rice bran. Owing to MeHg's well-known affinity for thiol,^{10,37} we believe the assumption that MeHg is bound to thiol in rice bran is a reasonable one. Using HPLC-ICP-MS, Li, et al.⁹ revealed that MeHg in rice seeds (brown rice) was present almost exclusively

as MeHg-cysteine, a complex that is thought to be responsible for the transfer of MeHg across the blood-brain and placental barriers. As shown in section 3.1, the majority of the MeHg in rice grain ($80 \pm 6.2\%$) is accumulated in the white rice. Hence, our XANES observations when interpreted with Li, et al.'s findings, suggest that the chemical form of MeHg in rice bran is similar to that in white rice. Our results are also consistent with previous research on MeHg speciation in fish muscle^{10,38} and in other plant tissues.³⁷

The presence of MeHg in rice as MeHg-cysteine does not necessarily infer the presence of the free MeHg-cysteine complex. Cysteine moieties are likely to be part of a larger peptide and may be present in many proteins. We therefore speculate that free MeHg-cysteine might be incorporated into proteins in rice grain. An analogy between the process of protein and MeHg accumulation in rice grain during the rice growing seasons has been proposed.^{2,20,21} According to this analogy, MeHg and protein bioaccumulate in the above-ground part of the rice plant before rice seeds start to form, and then synchronously translocate and accumulate in seeds as the rice ripens. We speculate that MeHg bound to cysteine is mobile in the same way as free cysteine (or as any other amino acid), and that MeHg is incorporated into proteins together with cysteine.

The outer part of brown rice is constituted by bran, which is made up of living cells rich in protein and the concentration of protein in rice bran is much higher than in white rice and hull.¹⁶ Using literature data, the ratio of protein for bran to hull and bran to white rice can be calculated as approximately 6 and 2, respectively, and these ratios are comparable to the concentration ratios of MeHg in bran to hull and bran to white rice discussed in section 3.1. We believe that these ratios provide further evidence that MeHg in rice grain is likely associated with protein. Our data suggest that despite binding to proteins, MeHg can be transported to the endosperm through a single ovular vascular trace.³⁶ This phenomenon has been widely detected in rice grain for organic selenium³⁹ and organic dimethylarsinic acid²⁷ and would explain the apparent mobile behavior of MeHg, but not IHg, in rice.

3.5. Pan-Asian Context and Risk to Human Health.

The latest report on global Hg issued by UNEP⁴⁰ describes a continual increase of Hg emission to air from Hg-emitting industrial sectors, especially within Asian countries. Paddy rice is one of the most prevalent agricultural crops in southern and eastern Asia, where rice provides the dominant staple food. Mercury in ambient air is the key factor that controls the

concentration of both IHg and MeHg in rice grain, and therefore, elevated levels of airborne Hg could consequently be a diffuse pollution risk to large areas of cultivated land. This diffuse pollution risk could indirectly lead to a large-scale increase in the concentration of MeHg in rice grain across Asia. Evidence for MeHg contamination of rice is not restricted to the Hg mining areas of Guizhou province. At Xunyang, another active mercury mine in Northwest China, similarly high levels of MeHg in rice (ranging from 8.2 to 80 $\mu\text{g kg}^{-1}$) have been reported.^{14,41} Recently, Krisnayanti et al.⁴² reported MeHg concentrations as high 100 $\mu\text{g kg}^{-1}$ in rice grain collected from an artisanal gold mining area in Indonesia. Furthermore, evidence for high levels of MeHg in rice grain has been reported in cases where no point source of Hg contamination can be defined (presumably derived from coal combustion).^{4,6} These studies indicate that the accumulation of MeHg in rice may be a more widespread phenomenon than is currently understood.

Unlike fish, rice does not contain long-chain polyunsaturated fatty acids, which are essential for the development of neural pathways and the brain.⁴³ Therefore, the negative health effects that may be associated with MeHg exposure through rice consumption cannot be offset by the essential nutrient content of the food. We believe that the issue of MeHg in rice should receive at least as great attention as MeHg in fish due to the potential seriousness that exposure may have on pan-Asian public health.⁴⁴ As MeHg–cysteine can be readily transported across the blood-brain barrier,¹² the presence of this compound in rice raises critical questions on the risk that ingestion of contaminated rice may have on the human population.

A potential prophylaxis to MeHg poisoning is Se, an essential trace element for mammals that has a known antagonistic effect on Hg toxicity.⁴⁵ For example, selenium compounds can effectively prevent mortality caused by a high dose of IHg in rats, and can decrease the toxicity of MeHg in fish.⁴⁶ It is plausible that where the distribution of Se in rice mirrors that of MeHg (Figure 1-B and Figure 2), then Se may have a moderating effect on MeHg toxicity during digestion of rice meals. Fortunately rice grain samples collected throughout the Wanshan mining area also contain elevated levels of selenium due to elevated concentrations of this metalloid in soil.^{25,47} These findings may be important to local residents exposed to high levels of MeHg as a result of the cultivation of rice in an artisanal mining area. Research is currently assessing the possible use of Se as a remediation agent in paddy fields to restrict the accumulation of Hg in rice grain and to reduce Hg any toxicity in humans. Intervention may be necessary in areas subject to point- and diffuse-source mercury contamination to protect public health.

■ ASSOCIATED CONTENT

■ Supporting Information

Description of sampling location; detailed information concerning sample collection and preparation; protocol for THg/MeHg analysis in fractions of rice grain, X-ray Absorption Near-Edge Structure Analysis in rice bran, synchrotron radiation microscopic X-ray fluorescence analysis in brown rice; statistical analysis of analytical data; the QC/QA protocol; factors controlling mercury levels in rice grain; 4 Tables; 4 Figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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