

INORGANIC MERCURY ACCUMULATION IN RICE (*ORYZA SATIVA* L.)BO MENG,[†] XINBIN FENG,^{*†} GUANGLE QIU,[†] DINGYONG WANG,[‡] PENG LIANG,[‡] PING LI,[†] and LIHAI SHANG[†][†]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, People's Republic of China[‡]College of Resources Environment, Southwest University, Chongqing, People's Republic of China

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Abstract—To investigate the source and process of inorganic mercury (IHg) accumulation in rice, we monitored the concentrations of IHg in tissues of rice plants (*Oryza sativa* L.) from four experimental plantation plots. Biweekly during the rice-growing season, tissues of rice plants, corresponding soil, precipitation, and irrigation water samples were collected. The sampling data support the following: (1) the atmosphere is the principal source of IHg to the aboveground parts of the rice plant; (2) both the atmosphere and soil contribute to IHg content in stalks, but the former source tends to be more important; and (3) soil is the major source of root IHg content. These observations and the fact that the gradually increasing concentration and mass of IHg in stalks and leaves during the rice-growing season suggested that atmospheric Hg could be absorbed by and incorporated into the aboveground parts of the rice plant and that limited or no Hg emission to the air or translocation to the soil occurred after deposition of atmospheric Hg. The root surface acted as a potential Hg barrier and consequently reduced the translocation of Hg ion mass through the root system to the aboveground parts. Accumulated IHg in aboveground parts of rice plants cannot be transported to seeds, which is completely different from the case of methylmercury. Environ. Toxicol. Chem. 2012;31:2093–2098. © 2012 SETAC

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INTRODUCTION

Mercury (Hg) is a toxic global element that could pose risk to terrestrial ecosystems and human health. Rice grown on Hg-contaminated paddy soil can accumulate high levels of Hg in grains, which can eventually be transferred to consumers, thus posing a health risk [1–3]. The rice paddy is one of the most prevalent agricultural crop fields in southern and eastern Asia, where rice is the dominant staple food. China emerges as the largest rice producer in the world, with an output of approximately 197 million metric tons per year, accounting for as much as 29% of the global total (<http://faostat.fao.org/site/339/default.aspx>).

The Wanshan Hg mining area, located in Guizhou Province, southwest China, was ranked as the largest domestic Hg-producing center. Geochemical investigations in Wanshan have revealed that massive calcines and waste tailings introduced by large-scale Hg mining, uncontrolled small-scale artisanal Hg mining, and other mineral processing have resulted in serious Hg pollution of the surrounding arable landscape, where rice paddies dominate [4–7].

It is generally considered that human exposure to Hg is mainly through fish products [8], but information on Hg accumulation in food crops is scarce. It was recently reported that in some contaminated areas, terrestrial food chain transfer could be significant, such as Hg accumulation in rice grains [3,4,9,10]. The seminal work by Horvat et al. [4] revealed that rice seeds collected from the Wanshan Hg mining site contained high levels of total Hg (THg) and methylmercury (MeHg) (THg 569 $\mu\text{g kg}^{-1}$, MeHg 145 $\mu\text{g kg}^{-1}$). Qiu et al. [3] observed that rice grown in Wanshan has a span of THg and MeHg concentrations up to 1,120 and

174 $\mu\text{g kg}^{-1}$, respectively. At Wuchuan, another Hg mining center in northeast Guizhou Province, rice with high levels of Hg (THg 113 $\mu\text{g kg}^{-1}$, MeHg 13 $\mu\text{g kg}^{-1}$) was also reported [6]. Furthermore, elevated Hg concentrations (THg 25 $\mu\text{g kg}^{-1}$, MeHg 4.7 $\mu\text{g kg}^{-1}$) were found in rice grain at the control site, which was adjacent to the Wanshan Hg mining area [10]. In these regions of Guizhou, rice rather than fish is the primary pathway of MeHg exposure to residents [1–3].

Recently, the study of Hg accumulation in rice has received considerable attention worldwide [1–3,4,6,9–13]. Zhang et al. [10] specified that, on average, the bioaccumulation factors for MeHg in rice were more than 800 times (with a maximum of 40,000 times) higher than those for inorganic Hg (IHg). Krupp et al. [11] observed that phytochelatin, a group of small peptides in rice plants, can sequester Hg^{2+} but not MeHg. A recent study indicated that MeHg in rice seeds exists almost exclusively as $\text{CH}_3\text{Hg-L-cysteinate}$ (CH_3HgCys), a complex that is thought to be responsible for the transfer of MeHg across the blood–brain and placental barriers [12]. Schwesig and Krebs [14] reported that MeHg in plants can be transported much more easily than IHg. Available data indicate that the sources/mechanisms of IHg and MeHg uptake into the tissues of rice plants should be different [9–12].

Previous research has mostly focused on THg and MeHg in ripened rice seed. Our previous study [13] observed that soil is the potential source of MeHg to tissues of rice plants. Roots firstly absorbed MeHg from soil, and then MeHg was translocated to the aboveground parts of the plant. Finally, most of the MeHg accumulated in stalks and leaves were transferred to seeds during the ripening period. At present, the dynamic process of IHg translocation within rice plants is unknown. To date, the distribution of IHg in rice plants during the rice-growing season has not been investigated, and the understanding of the bioaccumulation pathways of IHg in rice is still limited. To better understand the mechanisms and processes controlling IHg accumulation in rice plants, we measured IHg

All Supplemental Data may be found in the online version of this article.

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concentrations in tissues of rice plants collected from four designated experimental plots during a complete rice-growing season.

MATERIALS AND METHODS

Site description

A detailed description of the sampling sites, Gouxi (a typical artisanal Hg mining site) and Huaxi (a regional background control site), can be found in the Supplemental Data. Briefly, the Hg mining center of Gouxi, located in the Tongren District, eastern Guizhou Province, is one of the largest artisanal Hg mining centers in China (Supplemental Data, Fig. S1). The control site of Huaxi, located southwest of Guiyang city (about 30 km from the city) (Supplemental Data, Fig. S1), has no direct point sources of Hg contamination.

Experimental design and chemical sampling protocol

To investigate the dynamic process of IHg accumulation in rice plants, four experimental plots of rice were carefully designed. Briefly, the first plot was a control site paddy (CS-P) consisting of a rice paddy field located at Huaxi (the control site) receiving a comparatively low Hg input by atmospheric deposition. The second plot was a control site-box (CS-B, dimension $1.5 \times 1.5 \times 0.4$ m) located at Huaxi but filled with topsoil (0–10 cm) collected from an Hg-contaminated paddy field in Gouxi. The third plot was an artisanal site paddy (AS-P) located at Gouxi with a high Hg soil concentration. The fourth plot was an artisanal site-box (AS-B) with the same dimension as the CS-B and filled with topsoil (0–10 cm) collected from the Huaxi paddy field with a low level of Hg. A detailed description of the four experimental plots can be found in the Supplemental Data, Table S1.

The type of rice cultivar (hybrid rice, Jinyou) that is widely grown throughout Guizhou Province was selected for the present study. Germinated seeds were cultivated in a paddy field at the control site for one month. Subsequently, plants were transplanted into the soil of the four plots (with a density of 25×25 cm). Experimental plots were cultivated during the period June through September 2007.

Sample collection and preparation

During the rice-growing season (June–September 2007), five sampling campaigns were conducted. Due to the fact that rice plants before 30 d of growth were unavailable, the first campaign took place one month after rice seedlings were planted out, while the remaining ones were accomplished at days 45, 60, 75, and 90. For each experimental plot, rice plants, corresponding soil from the root zones (10–20 cm depth, corresponding to the root zone of the rice), irrigation water, and precipitation were collected. Rice plants were divided into three parts: root, stalk, and leaf. Concerning the later sampling campaigns (at days 75 and 90) coinciding with rice ripening, rice grains were in addition manually separated using a scalpel to prevent rice grain loss.

Rice tissue samples were cleaned in situ using drinking water, followed by ultrasonically assisted rinses with deionized water after being brought back to the laboratory, and finally air-drying and storage 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. Tissue samples were ground with a mixer (IKA-A11 basic, IKA) to a size of 150 per inch mesh. A

detailed description of sample collection and preparation is given in the Supplemental Data. Subsequently, concentrations of THg and MeHg in the treated tissue samples (dry wt) were determined. Total Hg was determined as well for irrigation water, precipitation, and rice paddy (dry wt) samples.

Analytical methods

The protocol for THg/MeHg analysis of rice plant tissues and THg analysis of soil and water samples is described in the Supplemental Data. Investigations with differing techniques have established that ethyl mercury species are not present in detectable amounts in provisions including rice flour, wheat flour, and dogfish liver [15,16], in one of the studies in such a way that the sum of IHg and MeHg concentrations compared favorably with that of THg [15]. Therefore, the concentration of IHg in rice plant tissues was calculated here as the difference between the concentrations of THg and MeHg in the sample [9].

Quality control

Quality-control measures consisted of method blanks, field blanks, triplicates, matrix spikes, and parallel analysis of several certified reference materials, as described in the Supplemental Data, Table S2.

RESULTS AND DISCUSSION

THg in irrigation water, wet precipitation, and rice paddies

In a companion paper [13], concentrations of THg in irrigation water, precipitation, and rice paddies during the rice-growing season were documented. Briefly, the average THg concentration in the irrigation water of plot AS-P ($4,200 \pm 4,900$ ng L⁻¹) was more than three orders of magnitude higher than that of plots AS-B (14 ± 2.7 ng L⁻¹) and CS (-P and -B, 5.4 ± 1.9 ng L⁻¹) due to the supply of seriously Hg-contaminated water from the massive calcines and tailings in the Hg mining area. During the rice-growing season, the THg concentration in precipitation at Gouxi was highly elevated ($2,900 \pm 1,400$ ng L⁻¹) in comparison with Huaxi (27 ± 17 ng L⁻¹), indicating that both wet and dry depositions are responsible for Hg input to surface areas in Gouxi, whereas Huaxi as a control site has no direct point sources of Hg contamination. In all, (1) owing to the highly elevated Hg concentration in precipitation (wet and dry depositions) compared to that in the local drinking water at Gouxi, the relative importance of airborne Hg input to plot AS-B should be considered large; (2) with respect to AS-P, Hg input from both precipitation and irrigation water would be considerable; (3) for CS-P and CS-B, the contributions of Hg from both precipitation and irrigation water (local drinking water) were trivial because of very low Hg concentrations [9].

The average THg concentration in soil for AS-P and CS-B before rice planting was 11 ± 3.4 and 30 ± 1.1 mg kg⁻¹, respectively, which was significantly higher than that of CS-P (0.29 ± 0.023 mg kg⁻¹) and AS-B (0.44 ± 0.028 mg kg⁻¹). During the rice-growing season, THg in soil at plots CS-B and AS-P was consistently above the domestic environmental quality standard for agricultural soil (1.5 mg kg⁻¹) [17], which is a result of the historically large-scale Hg smelting activities combined with current artisanal Hg retorting. However, the THg concentrations in soil at CS-P and AS-B were significantly lower than the limit of 1.5 mg kg⁻¹. The concentration of THg in the soil of CS-P, CS-B, and AS-P showed a narrow scale variation with time. On the contrary, the corresponding AS-B concentration–time series of THg increased by a factor of approximately 2.5 from

an initial value of $0.44 \pm 0.028 \text{ mg kg}^{-1}$, indicating the profound effect of atmospheric deposition.

Inorganic Hg in tissues of rice plants during the rice-growing season

The variation of IHg concentration in rice plant tissues is displayed in Figure 1 for the various experimental plots as a function of time. A statistical summary of these data in tabulated form can be retrieved from Table 1 and the Supplemental Data, Table S3.

As stated, rice seedlings were cultivated in a batch before being transplanted. Hence, the rice seedlings set out in the field experiment contained the same level of IHg. However, the average concentrations of IHg in roots at CS-B, AS-P, and AS-B were significantly higher than those at CS-P throughout the rice-growing season (Table 1). As shown in our companion paper [13], significantly higher THg levels were observed in paddy soil at CS-B ($29 \pm 3.6 \text{ mg kg}^{-1}$), AS-P ($13 \pm 1.9 \text{ mg kg}^{-1}$), and AS-B ($0.76 \pm 0.36 \text{ mg kg}^{-1}$) compared to those at plot CS-P ($0.32 \pm 0.060 \text{ mg kg}^{-1}$) throughout the rice-growing season.

Previous studies reported that IHg levels in roots were significantly correlated with the corresponding soil Hg concentration [9,18]. Root and soil Hg concentrations exhibited a positive correlation for the four experimental plots ($r = 0.80$,

Table 1. Mean concentrations (\pm SD, mg kg^{-1}) of inorganic mercury in tissues of rice plants (dry wt with standard deviations) during the rice-growing season

Tissue	CS-P	CS-B	AS-P	AS-B
Root	0.14 ± 0.031	3.2 ± 0.78	2.9 ± 0.48	1.9 ± 0.46
Stalk	0.029 ± 0.018	0.13 ± 0.067	1.1 ± 0.47	2.3 ± 1.2
Leaf	0.079 ± 0.037	0.20 ± 0.069	9.4 ± 4.5	11 ± 5.5
Hull	0.0073 ± 0.0021	0.023 ± 0.020	1.1 ± 0.32	1.5 ± 0.14
Seed	0.0042 ± 0.0011	0.035 ± 0.020	0.43 ± 0.074	0.51 ± 0.13

CS-P = control site paddy; CS-B = control site-box; AS-P = artisanal site paddy; AS-B = artisanal site-box.

$p < 0.001$, $n = 58$; Table 2). This is consistent with the observation that Hg in paddy soil is the major source of IHg in roots, as we suggested in a previous paper [9]. During the rice-growing season, IHg levels in the roots of CS-P, CS-B, and AS-P showed a narrow variation with time. The feature of the root IHg concentration–time series of plot AS-B (Fig. 1) is compatible with the effect of a steady increase of Hg level in the corresponding soil [13].

The highest IHg concentration in stalk samples was present at plot AS-B, followed by AS-P, then CS-B and CS-P during the rice-growing season (Table 1). Concerning the leaf, there was a more remarkable difference (K-S test, $p < 0.001$) between IHg levels at the Gouxu site compared to that at the control site. Statistical analysis revealed a positive correlation ($r = 0.29$, $p < 0.05$, $n = 58$) between IHg in the stalk and soil. However, the correlation was consistently weaker than that between IHg in the root and soil (Table 2).

Gouxu is a typical artisanal Hg mining site, where total gaseous mercury (TGM) in ambient air ($1,652 \text{ ng m}^{-3}$) was elevated by one to three orders of magnitude compared to the control site of Huaxi ($6.2 \pm 3.0 \text{ ng m}^{-3}$) [9,19]. Although concentrations of IHg in soil collected from CS-B were significantly higher than those from AS-P and AS-B [13], the IHg concentrations in stalks at CS-B were significantly lower than those at AS-P and AS-B (Fig. 1), indicating that atmospheric Hg rather than soil Hg is the major source of IHg in the stalk.

A previous study demonstrated that TGM decreased sharply with distance downwind from mining smelting workshops (by one to three orders of magnitude) [6]. Plots AS-B and AS-P were situated at a distance of approximately 100 and 400 m from the location where the small-scale artisanal smelting was still ongoing in Gouxu during our sampling period. Hence, it is reasonable to believe that plot AS-B is more impacted by elevated TGM concentrations compared to AS-P [6], which may be the key reason for the twofold higher IHg level in stalks from AS-B compared to AS-P. Both plots CS-P and CS-B were cultivated at the control site, which was

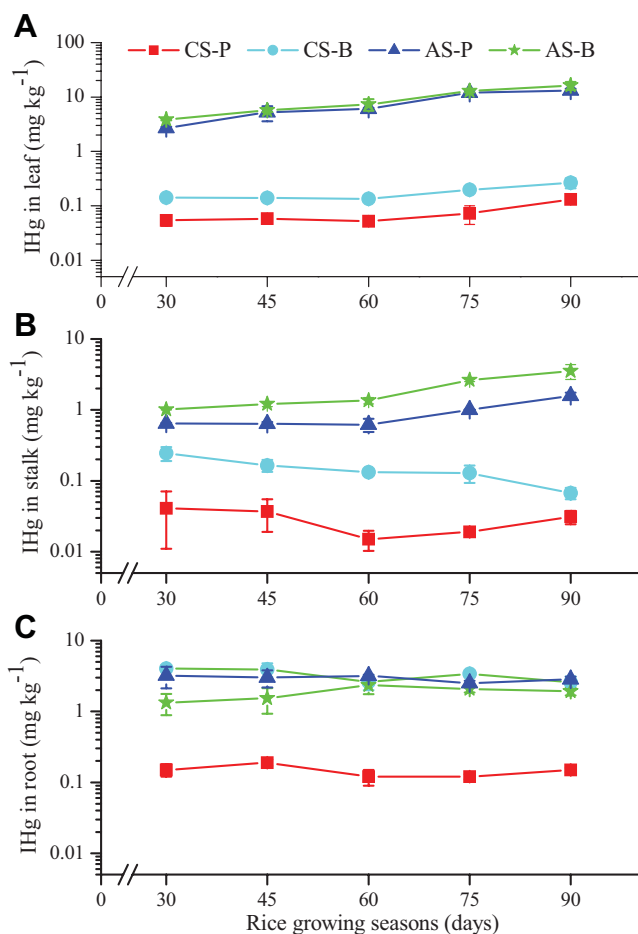


Fig. 1. Concentration of inorganic mercury (IHg; dry wt with standard deviations) in (A) leaf, (B) stalk, and (C) root of rice plants collected from four experimental plots as a function of cumulative time of experiment (rice plants before day 30 were unavailable). CS-P = control site paddy; CS-B = control site-box; AS-P = artisanal site paddy; AS-B = artisanal site-box.

Table 2. Pearson's correlation matrix, giving the linear correlation coefficients (r) among inorganic mercury levels in leaf, stalk, root, and soil during the rice-growing season ($n = 58$)

	Root	Stalk	Leaf	Soil
Root	1			
Stalk	0.13	1		
Leaf	0.20	0.91 ^a	1	
Soil	0.80 ^a	0.29 ^b	0.24	1

^a Correlation is significant at the 0.001 level (two-tailed).

^b Correlation is significant at the 0.05 level (two-tailed).

exposed to a relatively low level of TGM ($6.2 \pm 3.0 \text{ ng m}^{-3}$) [19]. However, a statistically significant difference in IHg levels in stalks was found between plots CS-P and CS-B (K-S test, $p < 0.001$), which was attributed to the high Hg content in the soil of CS-B ($29 \pm 3.6 \text{ mg kg}^{-1}$) compared to that of CS-P ($0.32 \pm 0.060 \text{ mg kg}^{-1}$) [13], indicating that a fraction of the overall IHg in the stalk originated from the soil via uptake by the roots.

Furthermore, based on the significant difference between the concentrations of IHg in stalks exposed to low levels of ambient air Hg⁰ vapor (CS-P and CS-B) and to Hg-enriched air (AS-P and AS-B), the accumulation and retention of IHg in stalks are influenced by atmospheric as well as soil sources [18]; however, the former may dominate. Moreover, a previous study [20] indicated that stalks in general accumulated Hg as a function of air Hg concentration rather than following a soil Hg content parameterization.

Leaves sampled from rice plants grown in low-Hg soil of AS-B had IHg concentrations comparable to those of leaves of rice plants grown in Hg-contaminated soil of AS-P. If soil-leaf translocation of Hg was a significant pathway, then leaf IHg levels would logically reflect differences in soil Hg concentrations. However, the fact that leaf IHg concentrations for plots AS-B and AS-P (as well as for CS-P and CS-B) represented similar concentrations regardless of soil Hg concentration (Fig. 1) is strong evidence that uptake of gaseous Hg⁰ from the air was the predominant pathway of IHg accumulation in the leaves of rice plants.

Furthermore, no correlation between IHg levels in leaves and soil was found ($r = 0.24$, $p > 0.05$, $n = 58$) during the rice-growing season, implying that the IHg in leaves is not controlled by soil Hg content. Fay and Gustin [18] reported that plant foliage can accumulate Hg from the air and that the air Hg level was a good indicator of leaf Hg concentration, especially for deciduous plants. Similarly, the mean (range) IHg leaf concentration from plot AS-B was significantly higher than that from CS-B (K-S test, $p < 0.001$) and obviously an effect of the very high Hg⁰ levels in ambient air of the artisanal Hg mining site [9]. Hence, our findings are consistent with previous studies that demonstrated that Hg in leaves of other plants was mainly accumulated from the air and not from the soil [21,22]. Analogous to the distribution patterns of IHg in stalks, the slightly higher IHg concentrations in leaves at plot AS-B compared to AS-P may result from the strong concentration gradient of Hg⁰ in the air near Hg retorting activities.

It is well known that geologically Hg-enriched areas are important atmospheric Hg sources [23]. Moreover, Wang et al. [24,25] observed that Hg emission flux from Hg-enriched soil can reach up to 27,827 and 5,493 $\text{ng m}^{-2} \text{ h}^{-1}$ in Wanshan and Wuchuan Hg mining districts, respectively, and that the Hg emission flux was significantly positively correlated with the soil Hg level. During the rice-growing season, the average THg concentration in soil for CS-B was $29 \pm 3.6 \text{ mg kg}^{-1}$, which was significantly higher than that of CS-P ($0.32 \pm 0.060 \text{ mg kg}^{-1}$) [13]. Hence, the slightly higher IHg levels in leaves collected from CS-B compared to CS-P could be speculated to derive from a ground-level vertical gradient of Hg⁰ concentration in the air as the soil of plot CS-B acts as a localized source.

Throughout the rice-growing season, the concentrations of IHg in stalks and leaves at plots CS-P and CS-B showed a narrow variation with time (Fig. 1). On the contrary, there was a steady increase in the concentration of IHg in stalk and leaf tissues for plots AS-P and AS-B, which were exposed to elevated atmospheric Hg vapor throughout the rice-growing

season (Fig. 1). Before the seeds started to form (around day 60), the growth of rice was mostly focused on the increase in biomass of the stalk and leaf. Hence, IHg concentrations in stalks and leaves increased slowly (Fig. 1), which could be partly attributed to the dilution of IHg with rice growth. After the seeds started to form (at day 60), the biomass gain of the rice plant was mostly confined to the grain. Still, there was a continuous assimilation of IHg into the stalk and leaf in the presence of an elevated Hg⁰ level in the air. Consequently, a sharp increase in stalk and leaf IHg concentrations for plots AS-P and AS-B was observed from days 60 to 90 (Fig. 1 and Supplemental Data, Table S3). Furthermore, the stalk and leaf IHg concentrations reached maximum when the rice was harvested (Fig. 1). Generally, the stalk and leaf were used to feed the livestock in the countryside in Guizhou Province.

As the dominant form of Hg in the atmosphere (>95%) [26], Hg⁰ is a mildly lipophilic compound [27] that could diffuse through the cuticle similar to volatile lipophilic compounds [28]. Moreover, compounds containing Hg(II)—either deposited to leaves from the atmosphere or produced at the leaf surface via Hg⁰ oxidation [29]—could traverse the epicuticular dissolution into the underlying cuticle and diffuse to epidermal cells [30]. Therefore, the gradual increase of leaf IHg concentration over time implied that leaves absorbed Hg from the air directly and that atmospheric Hg could be retained and incorporated into leaf and stalk tissues.

The mean concentrations of IHg in hulls at plots AS-P and AS-B were significantly higher than those from CS-P and CS-B (K-S test, $p < 0.001$) (Table 1). Generally, THg in most food crops is below a concentration of 0.020 mg kg^{-1} , which is the maximum limit recommended by the Chinese National Standard Agency [31]. However, the mean concentration of IHg in ripe rice seed obtained from AS-B and AS-P reached 0.51 ± 0.13 and $0.43 \pm 0.074 \text{ mg kg}^{-1}$, respectively, when the rice was harvested, posing a potential threat to the health of local residents [1–3]. On the contrary, the average IHg concentration in seed collected from plots CS-P ($0.0042 \pm 0.0011 \text{ mg kg}^{-1}$) and CS-B ($0.035 \pm 0.020 \text{ mg kg}^{-1}$) was lower or slightly higher than the permissible limit.

As shown in Table 2 and our previous study [9], significantly positive correlations were observed among IHg concentrations in the leaf, seed, hull, and stalk during the rice-growing season. However, the correlations between IHg concentration in the root as well as in the soil and other parts of rice plants (leaf, seed, hull, and stalk) were weaker, with the relationship between IHg concentration in the stalk and soil being an exception (Table 2) [9]. Linear correlation analysis further suggested that Hg in ambient air is the predominant source for the seed, leaf, and hull. Furthermore, IHg in the stalk was influenced by both the air and soil. Finally, the Hg in the soil was the major source of IHg in the root. Previous studies established that the majority of Hg in the aboveground parts of terrestrial plants is obtained from the atmosphere and that translocation of Hg from the roots to the stalk is low (<5%) or nonexistent [22,32], which supports our observations.

IHg mass in tissues of rice plants during the rice-growing season

The mass of IHg ($\mu\text{g plant}^{-1}$) in the tissues of rice plants collected from each plot throughout the rice-growing season is summarized in Figure 2 and the Supplemental Data, Table S4.

Despite the distribution patterns of IHg, concentration and mass in the tissues of rice plants were not unique for the four experimental plots, although some general trends were observed. Both concentrations of IHg and the mass of IHg in

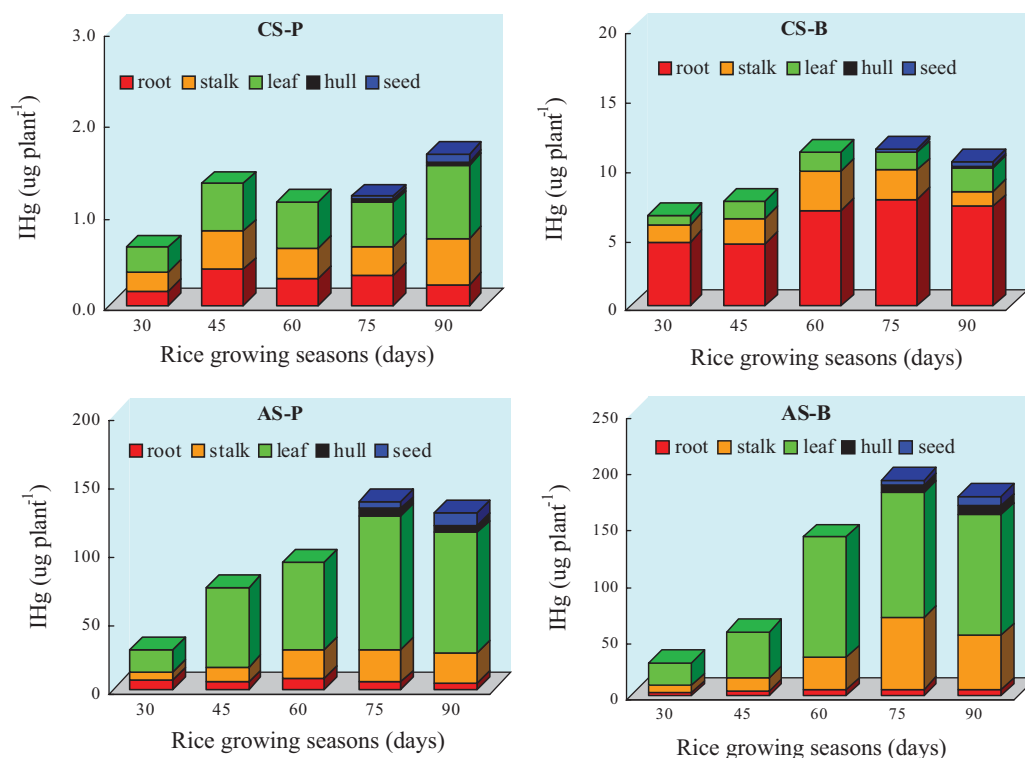


Fig. 2. Inorganic mercury (IHg) mass in tissues of rice plants collected from four experimental plots as a function of cumulative time of experiment. CS-P = control site paddy; CS-B = control site-box; AS-P = artisanal site paddy; AS-B = artisanal site-box.

the stalk and leaf at plots AS-P and AS-B steadily increased during the rice-growing season, reaching a maximal level during the period of ripening of the rice grain (between days 75 and 90) (Figs. 1 and 2; Supplemental Data, Tables S3 and S4). Atmospheric Hg^0 vapor uptake into seedling foliage depends on the ambient Hg concentration, with multiple studies suggesting the existence of an atmospheric Hg compensation point concentration, at which no net Hg exchange occurs between the atmosphere and leaf [33]. Moreover, previous studies have also indicated that experimentally identified compensation point concentrations are often above the background level of TGM in ambient air, ranging from 1 to 2 ng m^{-3} [34]. For plots CS-P and CS-B, both the concentration and mass of the IHg in the stalk and leaf represented low levels and showed a narrow variation with time, probably due to the atmospheric Hg^0 concentration at the control site of Huaxi similar to the compensation point for the aboveground portions of rice plant tissues. However, further study is required to confirm this hypothesis. Furthermore, the mass of IHg in the whole rice plant from plot CS-B was significantly higher than that from CS-P (Fig. 2), which can be attributed to the elevated IHg mass in roots at plot CS-B in comparison with plot CS-P throughout the rice-growing season. The present observations indicate that the mechanisms of IHg and MeHg uptake into rice plant tissues are divergent [13].

It is known that iron plaque is commonly formed on the rice root surface and acts as a physical barrier to heavy metal uptake [35,36]. In the present 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 reported in the present study for roots include a contribution from the iron plaque. Moreover, a recent study also found that

the formation of Hg -phytochelatin complexes would trap the Hg in the roots rather than translocate it from the roots into the aboveground parts of the rice plant [11], explaining the low concentration of IHg in the stalk and leaf at plot CS-B, even though the rice was exposed to Hg -enriched soil. These observations indicate that the root surface acted as a potential Hg barrier, which sequesters IHg and consequently reduces the translocation of Hg ion mass through the root system to aboveground parts such as the leaf, stalk, seed, and hull. This process might be a self-protection or detoxification mechanism of the rice plant against IHg exposure [11].

The gradual increase in concentration and mass of IHg in the stalk and leaf at plots AS-P and AS-B may indicate that accumulated IHg was not available for emission from the leaf and stalk to the atmosphere or translocation from the stalk and leaf to the other tissues of the rice plant, such as the seed, hull, and root. Atmospheric Hg may be absorbed by and incorporated into the leaf and stalk by a mechanism of Hg vapor entering the leaf and stalk tissues via stomatal or nonstomatal pathways and gas and particles being sorbed at the surface and in some cases traversing the cuticle, possibly reaching the epidermis [21]; then, limited or no Hg reemission to the air occurred after deposition of atmospheric Hg . However, the exact mechanism of IHg accumulation in rice is still unknown.

SUPPLEMENTAL DATA

Fig. S1. Site description.

Table S1. Summary of the experimental design.

Table S2. List of certified reference materials used in the present study and corresponding analytical result.

Table S3. Concentrations of IHg in tissues of rice plant (dry wt with standard deviations) during the rice growing season.

Table S4. IHg mass (ng plant⁻¹) in tissues of rice plant (dry wt with standard deviations) during the rice growing season. (254 KB DOC)

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