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Isotopic Fractionation and Source Appointment of Methylmercury and Inorganic Mercury in a Paddy Ecosystem

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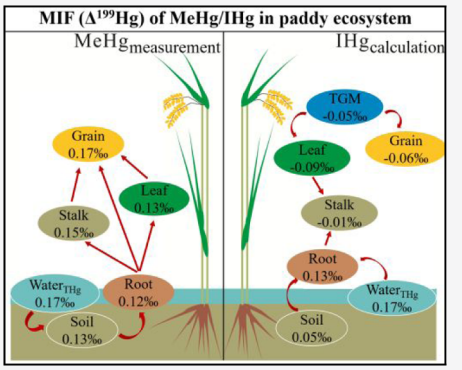
ABSTRACT: Bioaccumulation of methylmercury (MeHg) in rice grains has been an emerging issue of human health, but the mechanism of bioaccumulation is still poorly understood. Mercury (Hg) isotope measurements are powerful tools for tracing the sources and biogeochemical cycles of Hg in the environment. In this study, MeHg compound-specific stable isotope analysis (CSIA) was developed in paddy soil and rice plants to trace the biogeochemical cycle of Hg in a paddy ecosystem during the whole rice-growing season. Isotopic fractionation was analyzed separately for MeHg and inorganic Hg (IHg). Results showed distinct isotopic signals between MeHg and IHg in rice plants, indicating different sources. δ^{202}Hg values of MeHg showed no significant differences between roots, stalks, leaves, and grains at each growth stage. The similar Δ^{199}Hg values of MeHg between rice tissues (0.14 ± 0.08‰, 2SD, n = 12), soil (0.13 ± 0.03‰, 2SD, n = 4), and irrigation water (0.17 ± 0.09‰, 2SD, n = 5) suggested that the soil−water system was the original source of MeHg in rice plants. Δ^{199}Hg values of IHg in the paddy ecosystem indicated that water, soil, and atmosphere contributed to IHg in grains, leaves, stalks, and roots with varying degree. This study demonstrates that successful application of MeHg CSIA can improve our understanding of the sources and bioaccumulation mechanisms of MeHg and IHg in the paddy ecosystems.



KEYWORDS: *methylmercury, mercury isotope, paddy ecosystem, accumulation*

1. INTRODUCTION

Mercury (Hg) is a well-known global pollutant existing as various chemical species across environmental media. Its organic form, methylmercury (MeHg), is a neurotoxin and public concern because of its high bioaccumulation potential in the aquatic food chain.[1] Fish consumption is recognized as the main pathway of human MeHg exposure.[2] However, Hg-methylation also occurs in flooded rice paddies and rice grains can bioaccumulate a substantial amount of MeHg, causing severe human exposure concerns.[3,4] The unique flooded anaerobic environment in paddy fields is conducive to Hg-methylation, but the process may be affected by many factors, such as redox potential, pH, and dissolved organic carbon, sulfur, iron, and dissolved Hg contents.[5,6] Rice consumption accounts for 94−96% of the probable daily intake of MeHg for local residents in Hg-contaminated areas in China.[4] The knowledge of the sources and bioaccumulation processes of MeHg in rice is urgently needed in assessing Hg pollution-caused environmental and human health issues because understanding the sources of Hg is the primary prerequisite of pollution prevention and control. Enriched Hg isotopes were artificially added into paddy soil or ambient air to trace the contributions of Hg from the soil or atmosphere.[7,8] Previous studies measuring Hg concentrations in rice tissues (e.g., roots, stalks, leaves, and grains) indicated paddy soil as the main source of MeHg in rice tissue, whereas rice plants received inorganic mercury (IHg) both from the soil and the atmosphere.[9,10] However, no direct evidence was provided to reveal the mechanisms of bioaccumulation of MeHg and IHg in rice plants.

Mercury-stable isotopes are useful tools to trace Hg sources and environmental processes. Mercury has seven natural stable isotopes, including ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg, which undergo both mass-dependent fractionation (MDF, reported as δ^{202}Hg) and mass-independent fractionation (MIF, mainly reported as Δ^{199}Hg or Δ^{201}Hg) via various physical, chemical, and biological processes.[11−13] MDF occurred in almost all environmental processes, such as microbial methylation and demethylation,[11,14] abiotic methylation,[15] and Hg uptake by plants.[16,17] MIF is caused by the magnetic isotope effect[13] and the nuclear volume effect,[18]

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while biotic and dark abiotic reactions do not produce significant amounts of MIF.[12] Previous studies have demonstrated the occurrence of high MDF during Hg metabolism in plant and uptake of Hg by foliage from atmosphere, with the resulted MDF shift of −2.89‰.[16,17] However, significant MIF was unlikely to occur during Hg metabolism processes in plants.[14,15] The absence of MIF during Hg metabolism processes suggests that Hg isotopes could be geochemical tools to trace the sources of Hg (soil vs atmosphere) in plant tissues. The two sources of Hg in plants, that is, soil and atmosphere, are characterized by distinct "δ^{202}Hg−Δ^{199}Hg" signals.[16] Based on the well-defined MIF signals of soil and atmospheric Hg sources, Yin et al.[16] used an isotope-based binary mixing model to quantify the contributions of soil and atmospheric Hg to Hg in rice tissues. Meng et al.[19] found that MeHg and IHg showed different distribution patterns in rice tissues and MeHg was translocated to rice grains from roots, stalks, and leaves during the ripening period. Significant differences of the translocation pathways and sources for MeHg and IHg have been found in rice plants,[7,9,19−21] and thus, studying the isotopic composition of MeHg and IHg in rice tissues would help us better understand the sources and translocation processes separately. The selective extraction method (SEM) and ethylation combined gas chromatography (GC) separation method were recently used to separate MeHg from IHg in biota samples and soil samples, respectively, which demonstrated significantly different isotopic signatures between MeHg and IHg.[22−25]

In this study, we adopted both the SEM and ethylation/GC separation method to separate MeHg from the biological and environmental samples for Hg isotopic analysis, which also enabled the calculation of the isotopic composition of IHg by THg isotope analysis. Besides paddy soil and total gaseous mercury (TGM) samples, irrigation (river and paddy) water samples were also collected as the end member in the Wanshan Hg mining area. The objectives of this study are to (1) establish extraction methods of MeHg in soil and rice tissues to achieve direct measurements of isotopic values in MeHg; (2) understand the isotopic fractionation of MeHg and IHg in rice plants during the whole rice-growing season; and (3) quantify the sources of MeHg and IHg in rice plants based on MIF data.

2. MATERIALS AND METHODS

2.1. Experimental Design and Sample Collection. A rice paddy field (14 m × 19 m) in Gouxi area (27°23′11″ N, 109°11′28″ E) was selected as the study site, which is located in the Wanshan Hg mining area, Guizhou Province, Southwestern China (Figure S1). Although Hg mining activities in Wanshan have been ceased since 2003, Hg contamination in this area is still a serious environmental issue because of previously released and accumulated Hg in various environmental media.[23,26,27]

Sample collections were conducted in 2017 during different rice-growing stages, including tillering (15 days), elongation (45 days), heading (75 days), and ripening (105 days) stages (Figure S2). Rice plants were collected at each stage following the schedule listed in Table S1. At the tillering stage, 41 rice plants were collected and mixed into one sample because of the small mass of these plants. At each of the other stages, one sample was also obtained by mixing three rice plants. The rice plants were separated into root, stalk, leaf, and grain portions. The rice tissues were washed with bottled pure water three times in situ and then with deionized water three times in laboratory. Rhizosphere soil samples around the root (depth 0−20 cm) were also collected at each rice-growing stage. The soil samples were packed in clean plastic bags and stored in a refrigerator (−20 °C) in laboratory. Rice plants and soil samples were freeze-dried, ground to 200 mesh, homogenized, sealed in plastic bags, and stored at room temperature.

The nearby river was the main source of irrigation water for this paddy field. Water samples from this river were collected at three growing stages until the rice plant mature. Water samples were also collected from the surface of the paddy field during the elongation and heading stages. At the ripening stage, paddy fields were dry before the harvest. All the water samples from the river and paddy field were collected using precleaned borosilicate glass bottles. Each water sample was filtered through a 0.45 μm filter in situ, and 4% (v/v) HNO_3 and 0.5% (v/v) BrCl were added to the filtrate. TGM samples were collected at 1 m above the paddy water using chlorine-impregnated activated carbon traps,[28] with a gas flow rate of 2 L/min. Each TGM sample lasted for 12 h and a total of six samples were collected during a continuous 72 h period at the ripening stage.

2.2. THg Measurement and Digestion. Approximately 0.1 g of the soil sample (dry weight) was digested with aqua regia (HCl/HNO_3, 3:1 v/v, 5 mL) and approximately 0.5 g of the rice plant sample (dry weight) was digested with 10 mL of HNO_3 in a water bath at 95 °C for 3 h.[16] The digested solutions were analyzed for THg concentration by BrCl oxidation, $SnCl_2$ reduction, and cold vapor atomic absorption spectrometry (F732-S, Huaguang, China).[29] Quality control consisted of method blanks, certified reference materials (CRM) (CC580, estuarine sediment; BCR482, lichen), and duplicate samples. The average THg concentration in the method blanks was 0.04 ng/mL. The recoveries of THg for CC580 (n = 2) and BCR482 (n = 4) averaged at 99 and 93%, respectively. The relative percentage differences of THg in duplicated samples were <6%. The above-mentioned digested solutions were also stored in a refrigerator (4 °C) for THg isotope analysis, as described in the next section.

THg concentrations in water samples were determined following $SnCl_2$ reduction and cold vapor atomic fluorescence spectrophotometer (CVAFS, Brooks Rand) detection.[30] Because of the relatively low THg concentrations in water samples, preconcentration was performed for THg isotope analysis. Briefly, THg in water samples was reduced to Hg(0) by $SnCl_2$, and Hg(0) was purged and captured on gold traps using Ar gas (400 mL/min). The gold traps were heated at 400 °C to release Hg(0) and finally Hg(0) was oxidized by a trapping solution with reverse aqua regia (HNO_3/HCl, 3:1 v/v, 20%) with an Ar carrier gas flow of 25 mL/min. Preparation of the working standard solution (10 ng/mL Hg with 1% HNO_3, Brooks Rand, USA) followed the whole procedures of the preconcentration processes to ensure the recovery. The recoveries of THg in the working standard solutions averaged at 96 ± 4% (SD, n = 5). The trapping solutions of water samples were oxidized with 0.5% (v/v) BrCl and stored in the refrigerator at 4 °C for THg isotope analysis.

The carbon traps that captured the TGM samples were processed using a double-stage offline combustion-trapping technique[31] and the released Hg(0) was trapped by a reverse aqua regia trapping solution (HNO_3/HCl, 3:1 v/v, 20%). Suitable amounts of the trapping solutions were used for THg concentration measurement by CVAFS, following the

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Table 1. Hg Isotopic Composition of THg and MeHg Fractions in Standard Solution and CRMs (Mean ± 2SD)

name	fraction	matrix	n	δ^{202}Hg(‰)	Δ^{199}Hg(‰)	Δ^{200}Hg(‰)	Δ^{201}Hg(‰)
UM-Almadén	THg	standard solution	10	−0.57 ± 0.08	−0.02 ± 0.05	0.00 ± 0.06	−0.04 ± 0.10
CC580	THg	sediment	2	−0.51 ± 0.08	0.00 ± 0.05	0.02 ± 0.06	−0.02 ± 0.10
BCR482	THg	lichen	4	−1.68 ± 0.09	−0.69 ± 0.03	0.03 ± 0.06	−0.65 ± 0.10
STD[a]	MeHg	standard solution	2	−0.76 ± 0.08	0.05 ± 0.05	0.02 ± 0.06	0.04 ± 0.10
TORT−2	MeHg	lobster	3	0.54 ± 0.10	1.12 ± 0.07	0.05 ± 0.06	0.89 ± 0.14

[a]MeHg standard solution.

previously reported method.[30] The TGM concentrations were calculated by the mass of Hg in trapping solutions divided by the pumped volume of the air sample. The remaining solutions were oxidized with 0.5% (v/v) BrCl and stored in the refrigerator at 4 °C for THg isotope analysis. We only measured the THg isotope, rather than MeHg isotope, in dissolved Hg for water samples and in TGM for atmospheric samples.

2.3. MeHg Measurement and Extraction. For rice plants, approximately 0.2−0.3 g of the sample (dry weight) was digested with the methanolic KOH/solvent extraction technique.[32] The digests were then diluted to 50 mL using 18.2 MΩ·cm water (Milli-Q Integral System), ethylated, purged, and analyzed by GC−CVAFS.[33] The HNO_3 leaching/solvent extraction method was used to measure MeHg concentrations in soil samples.[32,34] CRMs (TORT-2, lobster hepatopancreas; CC580, sediment) were prepared using the same procedures, which yielded MeHg recoveries of 97 ± 4% (n = 3) and 93 ± 3% (n = 3), respectively. The relative percentage differences in duplicated samples averaged at 9 and 7%, respectively.

A modified SEM was used for MeHg isotope analysis in rice tissues.[22] Briefly, approximately 0.2−1.0 g (dry weight) of the rice tissue sample was mixed with 5 mL of NaBr (30% w/w in 4 mol/L H_2SO_4), 10 mL of $CuSO_4$ (2.5% w/w in water), and 10 mL of toluene (>99% purity) in a 50 mL centrifuge tube. The tube was shaken at 1500 rpm with a high-speed oscillator (CM-1000, Japan) for 6 h to ensure that all MeHg was converted to MeHgBr. The toluene phase was then removed to a 15 mL centrifuge tube and mixed fully with an aqueous $Na_2S_2O_3$ solution (0.01 mol/L, 4 mL) to convert MeHgBr to a stable MeHg−thiosulfate complex. After centrifugation at 8000 rpm (Avanti J-26 XP, Japan) for 1 h, the bottom layer $Na_2S_2O_3$ solution was pipetted into a precleaned 5 mL centrifuge tube. Suitable amount of the solutions was diluted with Millipore water or 10% (v/v) HNO_3 + 0.5% (v/v) BrCl for MeHg or THg analysis, respectively, whereas the remaining solutions were oxidized with 10% (v/v) HNO_3 + 0.5% (v/v) BrCl and stored at 4 °C for isotope analysis.

CRM TORT-2 (lobster hepatopancreas) was prepared using the same procedures to investigate the accuracy and precision of the SEM. We used the recovery and purity of MeHg to determine the efficiency of extraction. The recovery was the ratio of MeHg in the extraction solution to the initial MeHg concentration in the rice tissue. The purity was the percentage of MeHg to the THg concentration in the extraction solution. The recoveries and purities of MeHg in TORT-2 averaged at 92 ± 5 and 102 ± 5% (SD, n = 5), respectively. The averages of recovery and purity in rice tissues were 102 ± 9 and 94 ± 11% (SD, n = 15), respectively.

The detailed method for separating MeHg from THg in soil samples was described in our recent study.[25] Typically, approximately 10 g of sample (dry weight) was evenly divided into 12 centrifuge tubes, which were treated by HNO_3 leaching/solvent extraction, ethylation, and purging. All organic Hg was captured by 12 Tenax traps and then desorbed and transferred onto a GC column. Finally, MeHg was separated by a Teflon solenoid valve and absorbed by a reverse aqua regia trapping solution (HNO_3/HCl, 3:1 v/v, 20%). Because there was no available CRM for analyzing Hg isotopes of soil MeHg, MeHg spikes were prepared using the same procedures, from digestion to separation, mentioned above, to verify the inexistence of isotope fractionation. The isotopic composition of the Hg spikes in the trapping solution (δ^{202}Hg = −0.76 ± 0.08‰, Δ^{199}Hg = 0.05 ± 0.05‰, n = 2) was similar to the previously reported values (MeHg standard solution, diluted from a MeHg stock solution; Brooks Rand, USA, δ^{202}Hg = −0.74 ± 0.14‰, Δ^{199}Hg = 0.02 ± 0.07‰),[25] indicating that no significant MDF and MIF occurred during the sample preparation processes. The trapping solutions were oxidized with 0.5% (v/v) BrCl and stored at 4 °C for MeHg isotope analysis.

2.4. Mercury Isotope Analysis. The prepared solutions were diluted to 1 ng/mL Hg for isotope analysis using a Nu Plasma II multicollector-inductively coupled plasma mass spectrometer at the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Science.[35] NIST SRM 3133 solution, with Hg concentration and acid matrices matched to the sample solution, was measured before and after sample measurement. δ^{202}Hg, Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg values were calculated relative to NIST SRM 3133 following the protocol by Blum and Bergquist.[36] MDF results were expressed in delta notation (δ) and calculated using eq 1

$$\delta^{xxx}\mathrm{Hg}_{\mathrm{sample}}(‰) = [(^{xxx/198}\mathrm{Hg}_{\mathrm{sample}}/^{xxx/198}\mathrm{Hg}_{\mathrm{NIST3133}}) - 1] \times 1000 \quad (1)$$

where xxx is 199, 200, 201, or 202.

The MIF was defined using capital delta (Δ) notation and calculated using eqs 2−4

$$\Delta^{199}\mathrm{Hg} = \delta^{199}\mathrm{Hg} - \delta^{202}\mathrm{Hg} \times 0.252 \quad (2)$$

$$\Delta^{200}\mathrm{Hg} = \delta^{200}\mathrm{Hg} - \delta^{202}\mathrm{Hg} \times 0.502 \quad (3)$$

$$\Delta^{201}\mathrm{Hg} = \delta^{201}\mathrm{Hg} - \delta^{202}\mathrm{Hg} \times 0.752 \quad (4)$$

UM-Almadén secondary standard solutions (1.0 ng/mL Hg) were measured every 10−20 samples, yielding average values of −0.57 ± 0.08, −0.02 ± 0.05, and −0.04 ± 0.1‰ for δ^{202}Hg, Δ^{199}Hg, and Δ^{201}Hg, respectively (2SD, n = 10). The results were well consistent with the accepted values.[36,37] The isotopic composition of THg and MeHg in CRMs (Table 1) was consistent with previous studies.[13,22,24,25,38]

The isotopic composition of IHg in the rice tissue samples was calculated using eqs 5 and 6

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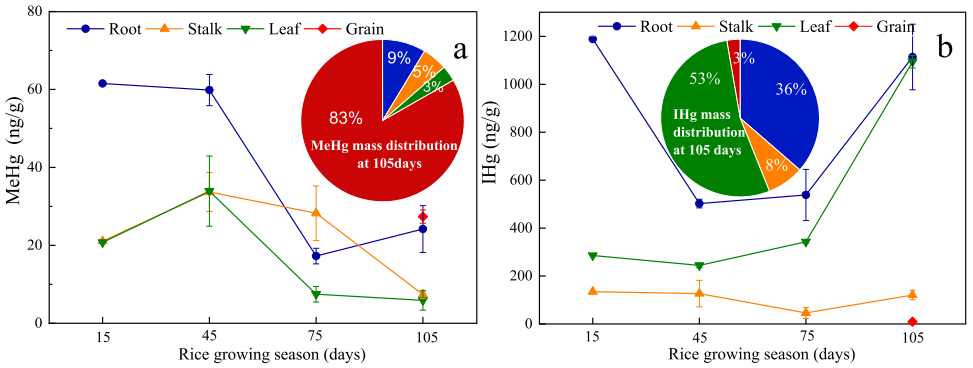

Figure 1. MeHg (a) and IHg (b) concentrations in rice tissues throughout the rice-growing season.

$$\delta^{202}Hg_{THg} = \delta^{202}Hg_{MeHg} \times R + \delta^{202}Hg_{IHg} \times (1 - R) \quad (5)$$

$$\Delta^{199}Hg_{THg} = \Delta^{199}Hg_{MeHg} \times R + \Delta^{199}Hg_{IHg} \times (1 - R) \quad (6)$$

R represents the ratio of MeHg to THg in the sample. $\delta^{202}Hg_{THg}$, $\delta^{202}Hg_{MeHg}$, and $\delta^{202}Hg_{IHg}$ represent the $\delta^{202}Hg$ of THg, MeHg, and IHg in the sample, respectively. $\Delta^{199}Hg_{THg}$, $^{199}Hg_{MeHg}$, and $\Delta^{199}Hg_{IHg}$ represent the $\Delta^{199}Hg$ of THg, MeHg, and IHg in the sample, respectively. Data uncertainties (±2SD) reflect the larger of either the external precision of the replication of UM-Almadén secondary standard or sample replicates. Because all the $\Delta^{200}Hg$ values of THg and MeHg were near 0, we did not discuss $\Delta^{200}Hg$ data in this study.

The contributions of IHg were calculated in leaves, grain, and stalks according to eqs 7 and 8

$$\Delta^{199}Hg_{IHg-tissue} = \Delta^{199}Hg_{atm/water} \times f_{atm/water} + \Delta^{199}Hg_{IHg-root/soil} \times f_{IHg-root/soil} \quad (7)$$

$$f_{atm/water} + f_{root/soil} = 1 \quad (8)$$

where $\Delta^{199}Hg_{IHg-tissue}$ represents $\Delta^{199}Hg$ of IHg in rice tissue; $\Delta^{199}Hg_{atm}$ represents $\Delta^{199}Hg$ of TGM; $\Delta^{199}Hg_{water}$ represents $\Delta^{199}Hg$ of water; $\Delta^{199}Hg_{IHg-root}$ represents $\Delta^{199}Hg$ of IHg in roots; $\Delta^{199}Hg_{IHg-soil}$ represents $\Delta^{199}Hg$ of IHg in rhizosphere soil; and f_{atm}, f_{water}, f_{root}, and f_{soil} are fractions of IHg from ambient air, water, roots, and soil, respectively.

3. RESULTS AND DISCUSSION

3.1. THg, MeHg, and IHg Concentrations. TGM concentrations at the study site ranged from 3.5 to 18.3 ng/m^3, which were much lower than those observed at the same site in 2011 (211 ng/m^3 on average).[16] The main sources of TGM in Wanshan area are atmospheric Hg emission from the mine wastes and soils.[29] The significant decline in TGM concentrations could be attributed to environmental control and remediation actions implemented by local government in recent years, such as ceasing the artisanal Hg mining activities, restoring mine waste heaps ecologically, and remedying soil Hg extensively.[39,40]

The average THg concentration in the filtered irrigation (paddy and river) water was 21.1 ± 5.2 ng/L (average ±SD, n = 5), which was also much lower than previously reported values in 2016 (paddy water: 105 ± 58 ng/L; river water: 39 ± 9.4 ng/L).[5] THg and MeHg concentrations in rhizosphere soil were 13433 ± 1285 ng/g (n = 4) and 2.5 ± 0.8 ng/g (n = 4), respectively, and the proportions of MeHg in THg (MeHg %) were <0.02%. THg concentrations in rice roots, stalks, leaves, and grains were 876 ± 370, 130 ± 39.5, 473 ± 426, and 40.0 ng/g, respectively. MeHg concentrations in rice roots, stalks, leaves, and grains were 40.7 ± 23.2, 22.6 ± 11.4, 17.0 ± 13.1, and 27.4 ng/g, respectively. MeHg % in rice tissues decreased in the following order: grains (74%) > stalks (20 ± 14%, n = 4) > leaves (5 ± 5%, n = 4) > roots (5 ± 4%, n = 4).

MeHg concentrations in rice tissues generally decreased with the growing season, with the exception of the case of grain. After the heading stage (75 days), MeHg contents in roots, stalks, and leaves gradually decreased (Figure S3). Such a decreasing trend has been reported in our previous study, which showed the transfer of MeHg from stalks and leaves to grains after the heading stage.[41] This explains the much higher MeHg bioaccumulation capacity by grains than other plant tissues, and MeHg mass in grains accounted for 83% of the total MeHg of all the plant tissues (Figure 1a). In contrast, the IHg concentration in rice tissues increased throughout the whole growing season (Figure 1b). The different temporal patterns between MeHg and IHg concentrations in rice plants indicated their different migration and transformation processes inside the plants.

3.2. Hg Isotopic Composition in Paddy Soil, Water, and Air. The isotopic composition of Hg in rice tissue, paddy soil, irrigation water, and the atmosphere was listed in Tables S2 and S3. $\delta^{202}Hg$ and $\Delta^{199}Hg$ of soil THg averaged at −1.30 ± 0.27‰ (n = 4, 2SD; −1.47 to −1.14‰) and 0.05 ± 0.01‰ (n = 4, 2SD; 0.04 to 0.06‰), respectively. Because of the extremely high proportion of IHg in THg (~99.98%) in soil, the calculated results of $\delta^{202}Hg$ and $\Delta^{199}Hg$ of IHg were equivalent to the THg measurements (Tables S2 and S3). The $\delta^{202}Hg$ and $\Delta^{199}Hg$ of MeHg in soil averaged at −1.55 ± 0.47‰ (n = 4, 2SD; −1.78 to −1.22‰) and 0.13 ± 0.03‰ (n = 4, 2SD; 0.10 to 0.14‰), respectively. The $\delta^{202}Hg$ values of soil MeHg were lower than those of soil IHg (or THg), which were consistent with previously reported results. This is because methylation enriches light Hg isotopes in the produced MeHg and methylation rather than demethylation is the dominant process in paddy soil.[24,25] Our finding was different from the result obtained by Janssen et al., which showed the heavier $\delta^{202}Hg$ values of MeHg rather than the THg pool in estuarine sediments.[24] In this study, no significant temporal change of $\delta^{202}Hg$ was observed for soil IHg (or THg). However, $\delta^{202}Hg$ of soil MeHg showed a decline of 0.56‰ with the growing season. In addition, MeHg in paddy

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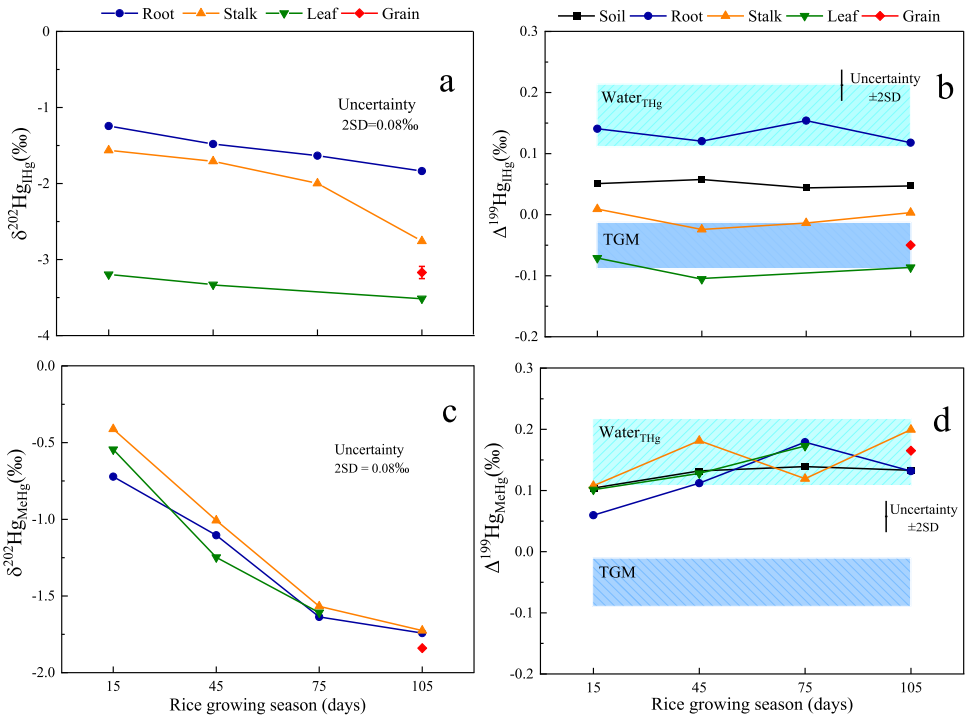

Figure 2. δ^{202}Hg (a) and Δ^{199}Hg (b) values of IHg, δ^{202}Hg (c) and Δ^{199}Hg (d) values of MeHg in different rice tissues and paddy ecosystem throughout the rice-growing season.

soil showed small but significantly positive Δ^{199}Hg values (0.13 ± 0.03‰, n = 4, 2SD), which were significantly higher than those of IHg (0.05 ± 0.01‰, n = 4, 2SD). Generally, microbial methylation by iron and sulfate-reducing bacteria will not result in significant MIF.[14] The isotopic signal of IHg in the soil might be unable to represent the part of the IHg that was methylated, because the IHg in the soil was hardly available to be methylated. The IHg ("old Hg") had become tightly bound to soil complexes over time, such as Hg−OM, Hg−S−OM, and Hg−N−OM complexes, which prevented IHg from methylation in soil.[5,42] The spiked Hg (referring to "new Hg") was easily absorbed by vegetation than the ambient Hg (referring to "old Hg") in the wetland.[43] In addition, the atmospheric Hg deposition seemed to be the primary factor regulating net MeHg production in the paddy field.[42,44] In the paddy ecosystem, Hg in irrigation water and from the atmospheric Hg deposition can be considered as "new Hg" relative to Hg in the soil. Therefore, δ^{202}Hg and Δ^{199}Hg values of soil MeHg were mostly influenced by the Hg isotopic characteristics in the paddy water.

δ^{202}Hg, Δ^{199}Hg, and Δ^{201}Hg values of THg in river water and paddy water averaged at −0.82 ± 0.27, 0.17 ± 0.09, and 0.10 ± 0.15‰ (2SD, n = 5), respectively. Odd isotopes were preferentially retained in the reactors during the photoreduction progresses of Hg, including MeHg and IHg photoreduction.[13] Even though IHg was the main speciation of Hg in paddy water, MeHg in the pore water may be transported to soil surface and progressed photoreduction during the water cycle.[44,45] We speculated that Hg in paddy water inherited the signals from the photoreduction of IHg and MeHg. Our results showed that Δ^{199}Hg values of THg in paddy water were consistent with those of MeHg, rather than IHg, in paddy soil, which indicated that paddy water may be an important media for Hg-methylation in the paddy ecosystem. Therefore, both dissolved Hg in irrigation water and Hg from atmospheric deposition might be important factors regulating MeHg in the paddy ecosystem.

In this study, δ^{202}Hg and Δ^{199}Hg of TGM averaged at −0.80 ± 0.63‰ (2SD, n = 6; −0.95 to −0.37‰) and −0.05 ± 0.07 (2SD, n = 6; −0.09 to −0.02‰), respectively, which were significantly higher than those previously reported for the same area (Gouxi) in 2011 (δ^{202}Hg: −2.32 to −2.14‰; Δ^{199}Hg: −0.34 to −0.3‰).[16] The increase in isotope values of TGM at Gouxi was in accordance with the sharp decrease in TGM concentrations (211 ng/m^3 in 2011 to 3.5−18.3 ng/m^3 in 2017).[16,46] The isotope values of TGM in this study were close to those of Hg(0) emissions from mine wastes in Almadén mining district, Spain (δ^{202}Hg: −1.15 ± 0.38‰, Δ^{199}Hg: 0.03 ± 0.14‰),[47] which indicated that Hg emissions from mine wastes might be the most important source of atmospheric Hg in Gouxi area.

3.3. MDF and MIF of IHg in Rice Tissues and IHg Sources. δ^{202}Hg values of IHg in roots (−1.53 ± 0.49‰, 2SD, n = 4), stalks (−2.01 ± 1.07‰, 2SD, n = 4), leaves (−3.26 ± 0.08‰, 2SD, n = 2), and grains (−3.16‰, n = 1) were significantly lower than those in paddy soil (−1.30 ± 0.27‰, 2SD, n = 4), paddy water (−0.82 ± 0.27‰, 2SD, n = 5), and TGM (−0.80 ± 0.63‰, 2SD, n = 6). δ^{202}Hg of IHg in all rice tissues decreased in varying degrees with the growing season (Figure 2a), suggesting bioaccumulation of lighter Hg isotopes in rice tissues. However, at different sampling stages, δ^{202}Hg of IHg in stalks and grains were in the range of those of roots and leaves, while roots received the majority of IHg from the soil.[16,19] A negative shift of δ^{202}Hg in IHg (~0.21‰) was observed between rhizosphere soil and roots, suggesting that significant MDF occurred during the uptake of IHg by roots. Similar shifts in δ^{202}Hg have been reported in the soil−root system in paddy fields at the Gouxi area.[16] Light isotopes were preferentially absorbed by plants because transport through ion channels and/or electrogenic pumps in root cell membrane

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preferred the uptake of the lighter isotope because of its greater diffusion coefficient.[48,49] Unlike roots, leaves mostly uptake IHg from Hg(0) in ambient air.[50,51] A shift of −2.4‰ in δ^{202}Hg between leaf IHg and TGM was observed, which was consistent with the previous results that negative shifts of −3.0 to −1.0‰ in δ^{202}Hg occurred between air and leaves.[12,52,53]

In order to identify relative contributions of IHg in rice tissues from air, soil, and water, a mixing model was applied based on Δ^{199}Hg data (eqs 7 and 8). IHg in roots (0.13 ± 0.03‰, n = 4), stalks (−0.01 ± 0.03‰, n = 4), leaves (−0.09 ± 0.05‰, n = 2), and grains (−0.05‰, n = 1) showed different Δ^{199}Hg values. However, Δ^{199}Hg values in rice tissues remained consistent during the whole rice-growing season (Figure 2b). The absence of MIF during Hg bioaccumulation in rice plants has led to the use of a Δ^{199}Hg-based binary mixing model to calculate the relative contributions of Hg from potential sources, such as the atmosphere and soil, in a previous study.[16] However, this previous study did not consider Hg from paddy water, which may be an important source of Hg in rice plants. Our data showed that the average Δ^{199}Hg of IHg in roots (0.13 ± 0.05‰) fell in between those of water (0.17 ± 0.09‰) and soil (0.05 ± 0.01‰). Assuming that roots received IHg exclusively from paddy water and soil, the above-mentioned binary mixing model would predict 58−83 and 17−42% contributions from water and soil, respectively, to the IHg in rice roots.

The average Δ^{199}Hg of IHg in leaves (−0.07 ± 0.02‰, SD, n = 4), grains (−0.05‰, n = 1), and stalks (0.02 ± 0.02‰, SD, n = 4) were much lower than those in roots (0.13 ± 0.03‰, n = 4). Therefore, these tissues also received IHg from the atmosphere because only TGM showed a negative Δ^{199}Hg signal among the three potential sources.

Results shown in Figure 3 indicated that nearly ∼100% of IHg in leaves and grains were from the atmosphere. Using the enriched isotope tracing approach, Strickman and Mitchell (2016) indicated that rice leaf and grain uptake IHg entirely from the atmosphere.[7] The Hg isotopic results of sawgrass verified that the majority (>90%) of THg in leaves was assimilated from the atmosphere, rather than from soil.[54] According to the contributions from water and soil to IHg in the roots, it was calculated that 12−24, 5−10, and 66−83% of IHg in stalks was from water, soil, and atmosphere, respectively. The individual isotopic characteristics of IHg in each tissue of the rice plants (Figure 2a,b) indicated that the transport of IHg between tissues was restricted. The tissues under the water (roots and small parts of stalks) were controlled by the IHg of soil and water, and the tissues above the water (leaves, grains, and most parts of stalks) were controlled by the IHg in the atmosphere. Results presented above supported that Hg isotope measurements are powerful methods for tracking the sources and procedures of Hg and certainly increased our understanding of the biogeochemical cycle of IHg in the paddy ecosystem.

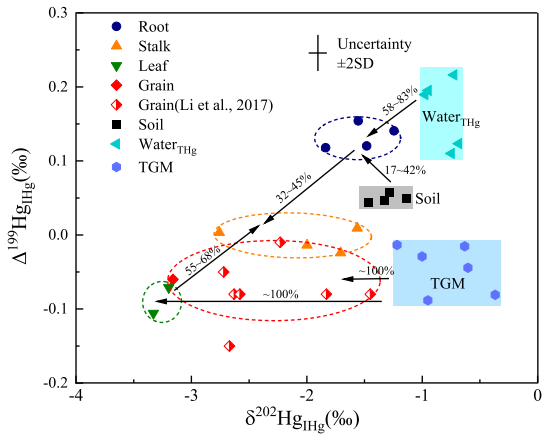

Figure 3. δ^{202}Hg and Δ^{199}Hg values in IHg of the paddy ecosystem and source appointment results. The squares represented the isotopic characteristics of each end member. The circles represented the isotopic characteristics of rice tissues. The arrow indicates the direction of IHg transportation and the percent represented the proportion of transportation.

3.4. MDF and MIF of MeHg in Rice Tissues. The temporal changes in δ^{202}Hg and Δ^{199}Hg of MeHg in rice tissues (roots, stalks, leaves, and grain) are shown in Figure 2c,d, respectively. Significant differences in δ^{202}Hg were found between MeHg and IHg in rice tissues, suggesting their different bioaccumulation pathways in rice plants. δ^{202}Hg of MeHg in roots, stalks, leaves, and grains averaged at −1.30 ± 0.95‰ (−1.74 to −0.72‰, n = 4), −1.18 ± 1.19‰ (−1.73 to −0.41‰, n = 4), −1.13 ± 1.08‰ (−1.61 to −0.54‰, n = 4), and −1.81‰ (n = 1), respectively (Table S3). Similar δ^{202}Hg values of MeHg were observed between roots, stalks, leaves, and grains at the same growth stage. δ^{202}Hg values of MeHg in the rice tissues decreased gradually with the growing season. Δ^{199}Hg of MeHg in roots, stalks, leaves, and grains averaged at 0.12 ± 0.10‰ (0.06 to 0.18‰, n = 4), 0.15 ± 0.09‰ (0.11 to 0.20‰, n = 4), 0.13 ± 0.07‰ (0.10 to 0.17‰, n = 3), and 0.17‰ (n = 1), respectively. These Δ^{199}Hg values were in the range of those of MeHg in rhizosphere soil and THg in paddy water but significantly different from those of TGM (Figure 2d).

δ^{202}Hg values of MeHg in rice tissues and soil gradually decreased with the rice-growing season, but Δ^{199}Hg signals remained stable (Figure 4). At the 15th day of growth, a negative shift of 0.66‰ in δ^{202}Hg for MeHg was observed between the rhizosphere soil and rice tissues. This shift became increasingly smaller and no significant difference was observed at the 75th days. The consistency of δ^{202}Hg of MeHg between rhizosphere soil and rice tissues after the heading stage

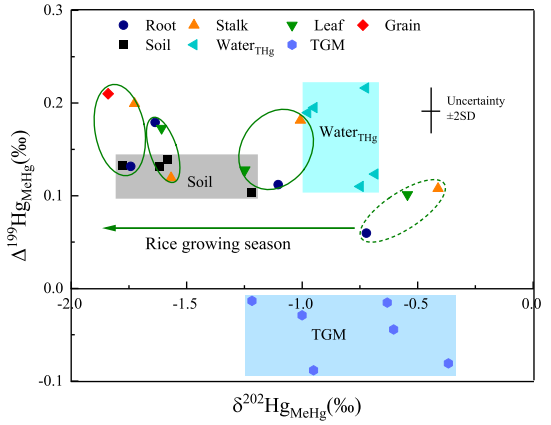

Figure 4. δ^{202}Hg and Δ^{199}Hg values in MeHg of the paddy ecosystem. The circle means each rice-growing season. The squares represented the isotopic characteristics of each end member. The green circles represented the isotopic characteristics of rice tissues in each growth period.

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suggested that MDF should not have occurred during the uptake of MeHg from the soil by rice plants. We speculated that the decreasing δ^{202}Hg of MeHg in rice tissues might be the result of mixing isotopic signatures with that of rhizosphere soil. The rice seedlings were first cultivated at different places using farmyard manure for 1 month, and then the rice plants were transplanted to the paddy. The isotope fractionation of MeHg in rice tissues at tillering stage might reflect the isotopic character of the initial cultivated soil. These MeHg in rice seedlings were later diluted by bioaccumulation of MeHg from rhizosphere soil with lower δ^{202}Hg values after transplant at the 15th day.

Δ^{199}Hg values can be used as an effective tracer to understand the influence of different Hg sources in biota because no MIF occurred during the metabolism process.[55,56] The consistency of positive Δ^{199}Hg values of MeHg between paddy soil (0.13 ± 0.03‰) and rice tissues (0.14 ± 0.08‰) indicated that MeHg in soil was the main source of MeHg in rice plants, as has also been confirmed previously in other rice fields.[7,19] We also found that Δ^{199}Hg values of MeHg in soil were in the range of those of irrigation water (0.17 ± 0.09‰). Higher MeHg concentrations were observed in the pore water than the surface water, indicating that IHg-methylation mainly occurred in the soil subsurface.[45] Hg with the positive signal of Δ^{199}Hg in paddy water might be methylated by microorganisms (sulfate reducing bacteria, iron reducing bacteria, and methanogens)[57−59] in the soil subsurface and then uptake by rice plants. Our isotope data with similar positive Δ^{199}Hg signatures among irrigation water, soil, and rice tissues indicated that Hg in irrigation water played an important role in Hg-methylation. Thus, the role of Hg in irrigation water on Hg-methylation processes might be underestimated previously, and Hg levels in irrigation water should be taken into consideration in remediation actions conducted in Hg contaminated regions. The Hg isotope in pore water was not measured in this study, resulting in a lack of direct evidence for supporting our speculations. More Hg isotope measurements are needed for investigating methylation and/or demethylation in paddy soil and water.

Feng et al.[60] found a specific enrichment in lighter δ^{202}Hg values of rice grains containing lower MeHg % (<30%) when compared to rice grains containing higher MeHg % (>50%), which reflected that disparate isotope fractionation existed between MeHg and IHg. An enriched isotope tracer study conducted by Strickman and Mitchell (2016) indicated that the accumulation and translocation of MeHg and IHg were disparate.[7] Our results supported that the different isotopic signatures between MeHg and IHg in rice plants were induced by the different uptake sources of Hg (water, soil, or atmosphere) (Figures 3 and 4). Based on X-ray absorption near-edge spectroscopy, previous studies showed that MeHg was associated with proteins, which was easily transported to the endosperm and accumulated in rice grains, and IHg was associated with phytochelatins, which was largely immobile and restricted to the grain.[41,61] Our results of MeHg and IHg isotope fractionation in rice tissues also suggested the different translocation pathways between MeHg and IHg in rice plants. The unique Hg isotope fractionation of IHg in different rice tissues suggested that limited translocation of IHg between roots, stalks, leaves, and grains might be because of immobile IHg-phytochelatins association (Figure 2a,b).[41] However, the similar signals of δ^{202}Hg and Δ^{199}Hg of MeHg in different rice tissues indicated that MeHg can be translocated among rice tissues maybe because of mobile MeHg-protein association (Figure 2c,d).[41] The application of stable isotope technique in sawgrass also showed that sawgrass preferred MeHg over THg in both uptake and upward translocation.[54]

In this study, MeHg and IHg in rice tissues showed distinct isotopic signals, indicating their different sources and pathways in rice plants. Δ^{199}Hg values of IHg in aboveground tissues of rice plants (leaves, stalks, and grain) reflected the mixing of IHg from the atmosphere, paddy water, and soil. We found that there was restricted transportation of IHg between tissues. IHg in tissues of upper water was controlled by the atmosphere and IHg in tissues of lower water was controlled by water and soil. The consistency of Δ^{199}Hg of MeHg between paddy soil and rice tissues suggested that rice tissues mainly received MeHg from paddy soil as no MIF occurred during the bioaccumulation of MeHg in rice plants. This is the first time for the application of MeHg compound-specific stable isotope analysis in the paddy ecosystem, which confirmed that no significant MDF and MIF occurred during the process of MeHg uptake by rice plants. Results from the present study suggest that controlling Hg emission sources in Hg-polluted areas and water management in paddy fields are crucial to reducing Hg bioaccumulation in rice grains and associated human health risks.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c03341.

Protocol of sampling; total Hg isotope composition in air, water, soil, and rice tissues; MeHg and IHg isotope composition in rice tissues and soil; map of the study area and sampling site; details of paddy and the location of sample; and variations of MeHg mass and biomass in rice plants during the rice-growing season (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Stein, E. D.; Cohen, Y.; Winer, A. M. Environmental distribution and transformation of mercury compounds. *Crit. Rev. Env. Sci. Technol.* **1996**, *26*, 1−43.

(2) Choi, A. L.; Grandjean, P. Methylmercury exposure and health effects in humans. *Environ. Chem.* **2008**, *5*, 112−120.

(3) Marvin-DiPasquale, M.; Windham-Myers, L.; Agee, J. L.; Kakouros, E.; Kieu, L. H.; Fleck, J. A.; Alpers, C. N.; Stricker, C. A. Methylmercury production in sediment from agricultural and non-agricultural wetlands in the Yolo Bypass, California, USA. *Sci. Total Environ.* **2014**, *484*, 288−299.

(4) Zhang, H.; Feng, X.; Larssen, T.; Qiu, G.; Vogt, R. D. In Inland China, Rice, Rather than Fish, Is the Major Pathway for Methylmercury Exposure. *Environ. Health Perspect.* **2010**, *118*, 1183−1188.

(5) Zhao, L.; Qiu, G.; Anderson, C. W. N.; Meng, B.; Wang, D.; Shang, L.; Yan, H.; Feng, X. Mercury methylation in rice paddies and its possible controlling factors in the Hg mining area, Guizhou province, Southwest China. *Environ. Pollut.* **2016**, *215*, 1−9.

(6) Ullrich, S. M.; Tanton, T. W.; Abdrashitova, S. A. Mercury in the aquatic environment: A review of factors affecting methylation. *Crit. Rev. Env. Sci. Technol.* **2001**, *31*, 241−293.

(7) Strickman, R.; Mitchell, C. P. J. Accumulation and translocation of methylmercury and inorganic mercury in Oryza sativa: An enriched isotope tracer study. *Sci. Total Environ.* **2017**, *574*, 1415−1423.

(8) Wang, Z.; Sun, T.; Driscoll, C. T.; Yin, Y.; Zhang, X. Mechanism of Accumulation of Methylmercury in Rice (*Oryza sativa L.*) in a Mercury Mining Area. *Environ. Sci. Technol.* **2018**, *52*, 9749−9757.

(9) Meng, B.; Feng, X.; Qiu, G.; Cai, Y.; Wang, D.; Li, P.; Shang, L.; Sommar, J. Distribution Patterns of Inorganic Mercury and Methylmercury in Tissues of Rice (*Oryza sativa L.*) Plants and Possible Bioaccumulation Pathways. *J. Agric. Food. Chem.* **2010**, *58*, 4951−4958.

(10) Meng, M.; Li, B.; Shao, J.-j.; Wang, T.; He, B.; Shi, J.-b.; Ye, Z.-h.; Jiang, G.-b. Accumulation of total mercury and methylmercury in rice plants collected from different mining areas in China. *Environ. Pollut.* **2014**, *184*, 179−186.

(11) Kritee, K.; Barkay, T.; Blum, J. D. Mass dependent stable isotope fractionation of mercury during mer mediated microbial degradation of monomethylmercury. *Geochim. Cosmochim. Acta.* **2009**, *73*, 1285−1296.

(12) Blum, J. D.; Sherman, L. S.; Johnson, M. W.; Jeanloz, R. Mercury Isotopes in Earth and Environmental Sciences. *Ann. Rev. Earth and Planet. Sci.* **2014**, *42*, 249−269.

(13) Bergquist, B. A.; Blum, J. D. Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems. *Science* **2007**, *318*, 417−420.

(14) Janssen, S. E.; Schaefer, J. K.; Barkay, T.; Reinfelder, J. R. Fractionation of Mercury Stable Isotopes during Microbial Methylmercury Production by Iron- and Sulfate-Reducing Bacteria. *Environ. Sci. Technol.* **2016**, *50*, 8077−8083.

(15) Jiménez-Moreno, M.; Perrot, V.; Epov, V. N.; Monperrus, M.; Amouroux, D. Chemical kinetic isotope fractionation of mercury during abiotic methylation of Hg(II) by methylcobalamin in aqueous chloride media. *Chem. Geol.* **2013**, *336*, 26−36.

(16) Yin, R.; Feng, X.; Meng, B. Stable Mercury Isotope Variation in Rice Plants (*Oryza sativa L.*) from the Wanshan Mercury Mining District, SW China. *Environ. Sci. Technol.* **2013**, *47*, 2238−2245.

(17) Yuan, W.; Sommar, J.; Lin, C.-J.; Wang, X.; Li, K.; Liu, Y.; Zhang, H.; Lu, Z.; Wu, C.; Feng, X. Stable Isotope Evidence Shows Re-emission of Elemental Mercury Vapor Occurring after Reductive Loss from Foliage. *Environ. Sci. Technol.* **2019**, *53*, 651−660.

(18) Estrade, N.; Carignan, J.; Sonke, J. E.; Donard, O. F. X. Mercury isotope fractionation during liquid−vapor evaporation experiments. *Geochim. Cosmochim. Acta.* **2009**, *73*, 2693−2711.

(19) Meng, B.; Feng, X.; Qiu, G.; Liang, P.; Li, P.; Chen, C.; Shang, L. The process of methylmercury accumulation in rice (*Oryza sativa L.*). *Environ. Sci. Technol.* **2011**, *45*, 2711−2717.

(20) Li, Y.; Zhao, J.; Zhang, B.; Liu, Y.; Xu, X.; Li, Y.-F.; Li, B.; Gao, Y.; Chai, Z. The influence of iron plaque on the absorption, translocation and transformation of mercury in rice (*Oryza sativa L.*) seedlings exposed to different mercury species. *Plant and Soil* **2016**, *398*, 87−97.

(21) Rothenberg, S. E.; Feng, X.; Dong, B.; Shang, L.; Yin, R.; Yuan, X. Characterization of mercury species in brown and white rice (*Oryza sativa L.*) grown in water-saving paddies. *Environ. Pollut.* **2011**, *159*, 1283−1289.

(22) Masbou, J.; Point, D.; Sonke, J. E. Application of a selective extraction method for methylmercury compound specific stable isotope analysis (MeHg-CSIA) in biological materials. *J.Anal. At. Spectrom.* **2013**, *28*, 1620−1628.

(23) Li, P.; Du, B.; Maurice, L.; Laffont, L.; Lagane, C.; Point, D.; Sonke, J. E.; Yin, R.; Lin, C.-J.; Feng, X. Mercury Isotope Signatures of Methylmercury in Rice Samples from the Wanshan Mercury Mining Area, China: Environmental Implications. *Environ. Sci. Technol.* **2017**, *51*, 12321−12328.

(24) Janssen, S. E.; Johnson, M. W.; Blum, J. D.; Barkay, T.; Reinfelder, J. R. Separation of monomethylmercury from estuarine sediments for mercury isotope analysis. *Chem. Geol.* **2015**, *411*, 19−25.

(25) Qin, C.; Chen, M.; Yan, H.; Shang, L.; Yao, H.; Li, P.; Feng, X. Compound specific stable isotope determination of methylmercury in contaminated soil. *Sci. Total. Environ.* **2018**, *644*, 406−412.

(26) Du, B.; Feng, X.; Li, P.; Yin, R.; Yu, B.; Sonke, J. E.; Guinot, B.; Anderson, C. W. N.; Maurice, L. Use of Mercury Isotopes to Quantify Mercury Exposure Sources in Inland Populations, China. *Environ. Sci. Technol.* **2018**, *52*, 5407−5416.

(27) Du, B.; Li, P.; Feng, X.; Qiu, G.; Zhou, J.; Maurice, L. Mercury Exposure in Children of the Wanshan Mercury Mining Area, Guizhou, China. *Inter. J. Env. Res. Pub. Heal.* **2016**, *13*, 1107.

(28) Fu, X.; Heimbürger, L.-E.; Sonke, J. E. Collection of atmospheric gaseous mercury for stable isotope analysis using iodine- and chlorine-impregnated activated carbon traps. *J. Anal. At. Spectrom.* **2014**, *29*, 841−852.

(29) Li, P.; Feng, X.; Shang, L.; Qiu, G.; Meng, B.; Liang, P.; Zhang, H. Mercury pollution from artisanal mercury mining in Tongren, Guizhou, China. *Appl. Geochem.* **2008**, *23*, 2055−2064.

(30) Yan, H.; Feng, X.; Shang, L.; Qiu, G.; Dai, Q.; Wang, S.; Hou, Y. The variations of mercury in sediment profiles from a historically mercury-contaminated reservoir, Guizhou province, China. *Sci. Total Environ.* **2008**, *407*, 497−506.
(31) Sun, R.; Enrico, M.; Heimbürger, L.-E.; Scott, C.; Sonke, J. E. A double-stage tube furnace–acid-trapping protocol for the pre-concentration of mercury from solid samples for isotopic analysis. *Anal. Bioanal. Chem.* **2013**, *405*, 6771−6781.
(32) Bloom, N. S. On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 1010−1017.
(33) Liang, L.; Horvat, M.; Cernichiari, E.; Gelein, B.; Balogh, S. Simple solvent extraction technique for elimination of matrix interferences in the determination of methylmercury in environmental and biological samples by ethylation-gas chromatography-cold vapor atomic fluorescence spectrometry. *Talanta* **1996**, *43*, 1883−1888.
(34) Liang, L.; Horvat, M.; Feng, X.; Shang, L.; Li, H.; Pang, P. Re-evaluation of distillation and comparison with HNO_3 leaching/solvent extraction for isolation of methylmercury compounds from sediment/soil samples. *Appl. Organomet. Chem.* **2004**, *18*, 264−270.
(35) Yin, R.; Feng, X.; Shi, W. Application of the stable-isotope system to the study of sources and fate of Hg in the environment: A review. *Appl. Geochem.* **2010**, *25*, 1467−1477.
(36) Blum, J. D.; Bergquist, B. A. Reporting of variations in the natural isotopic composition of mercury. *Anal. Bioanal. Chem.* **2007**, *388*, 353−359.
(37) Sun, G.; Sommar, J.; Feng, X.; Lin, C.-J.; Ge, M.; Wang, W.; Yin, R.; Fu, X.; Shang, L. Mass-Dependent and -Independent Fractionation of Mercury Isotope during Gas-Phase Oxidation of Elemental Mercury Vapor by Atomic Cl and Br. *Environ. Sci. Technol.* **2016**, *50*, 9232−9241.
(38) Estrade, N.; Carignan, J.; Sonke, J. E.; Donard, O. F. X. Measuring Hg Isotopes in Bio-Geo-Environmental Reference Materials. *Geostand. Geoanal. Res.* **2010**, *34*, 79−93.
(39) Wang, J.; Feng, X.; Anderson, C. W. N.; Qiu, G.; Ping, L.; Bao, Z. Ammonium thiosulphate enhanced phytoextraction from mercury contaminated soil - Results from a greenhouse study. *J. Hazard. Mater.* **2011**, *186*, 119−127.
(40) Xu, J.; Bravo, A. G.; Lagerkvist, A.; Bertilsson, S.; Sjöblom, R.; Kumpiene, J. Sources and remediation techniques for mercury contaminated soil. *Environ. Int.* **2015**, *74*, 42−53.
(41) Meng, B.; Feng, X.; Qiu, G.; Anderson, C. W. N.; Wang, J.; Zhao, L. Localization and speciation of mercury in brown rice with implications for Pan-Asian public health. *Environ. Sci. Technol.* **2014**, *48*, 7974−7981.
(42) Yin, D.; He, T.; Yin, R.; Zeng, L. Effects of soil properties on production and bioaccumulation of methylmercury in rice paddies at a mercury mining area, China. *J Environ Sci* **2018**, *68*, 194−205.
(43) Harris, R. C.; Rudd, J. W. M.; Amyot, M.; Babiarz, C. L.; Beaty, K. G.; Blanchfield, P. J.; Bodaly, R. A.; Branfireun, B. A.; Gilmour, C. C.; Graydon, J. A.; Heyes, A. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16586−16591.
(44) Zhao, L.; Anderson, C. W. N.; Qiu, G.; Meng, B.; Wang, D.; Feng, X. Mercury methylation in paddy soil: source and distribution of mercury species at a Hg mining area, Guizhou Province, China. *Biogeosciences* **2016**, *13*, 2429−2440.
(45) Rothenberg, S. E.; Feng, X. Mercury cycling in a flooded rice paddy. *J. Geophys. Res.: Biogeosci.*, **2012**, *117*(), DOI: 10.1029/2011jg001800
(46) Yuan, S.; Chen, J.; Cai, H.; Yuan, W.; Wang, Z.; Huang, Q.; Liu, Y.; Wu, X. Sequential samples reveal significant variation of mercury isotope ratios during single rainfall events. *Sci. Total. Environ.* **2018**, *624*, 133−144.
(47) Gray, J. E.; Pribil, M. J.; Higueras, P. L. Mercury isotope fractionation during ore retorting in the Almadén mining district, Spain. *Chem. Geol.* **2013**, *357*, 150−157.
(48) Kiczka, M.; Wiederhold, J. G.; Kraemer, S. M.; Bourdon, B.; Kretzschmar, R. Iron isotope fractionation during Fe uptake and translocation in alpine plants. *Environ. Sci. Technol.* **2010**, *44*, 6144−6150.
(49) Jouvin, D.; Weiss, D. J.; Mason, T. F. M.; Bravin, M. N.; Louvat, P.; Zhao, F.; Ferec, F.; Hinsinger, P.; Benedetti, M. F. Stable isotopes of Cu and Zn in higher plants: evidence for Cu reduction at the root surface and two conceptual models for isotopic fractionation processes. *Environ. Sci. Technol.* **2012**, *46*, 2652−2660.
(50) Demers, J. D.; Blum, J. D.; Zak, D. R. Mercury isotopes in a forested ecosystem: Implications for air-surface exchange dynamics and the global mercury cycle. *Global Biogeochem. Cycles.* **2013**, *27*, 222−238.
(51) Moeckel, C.; Thomas, G. O.; Barber, J. L.; Jones, K. C. Uptake anxd storage of PCBs by plant cuticles. *Environ. Sci. Technol.* **2008**, *42*, 100−105.
(52) Enrico, M.; Roux, G. L.; Marusczak, N.; Heimbürger, L.-E.; Claustres, A.; Fu, X.; Sun, R.; Sonke, J. E. Atmospheric Mercury Transfer to Peat Bogs Dominated by Gaseous Elemental Mercury Dry Deposition. *Environ. Sci. Technol.* **2016**, *50*, 2405−2412.
(53) Sonke, J. E. A global model of mass independent mercury stable isotope fractionation. *Geochim. Cosmochim. Acta.* **2011**, *75*, 4577−4590.
(54) Mao, Y.; Li, Y.; Richards, J.; Cai, Y. Investigating uptake and translocation of mercury species by sawgrass (*Cladium jamaicense*) using a stable isotope tracer technique. *Environ. Sci. Technol.* **2013**, *47*, 9678−9684.
(55) Gehrke, G. E.; Blum, J. D.; Slotton, D. G.; Greenfield, B. K. Mercury Isotopes Link Mercury in San Francisco Bay Forage Fish to Surface Sediments. *Environ. Sci. Technol.* **2011**, *45*, 1264−1270.
(56) Laffont, L.; Sonke, J. E.; Maurice, L.; Hintelmann, H.; Pouilly, M.; Sánchez Bacarreza, Y.; Perez, T.; Behra, P. Anomalous Mercury Isotopic Compositions of Fish and Human Hair in the Bolivian Amazon. *Environ. Sci. Technol.* **2009**, *43*, 8985−8990.
(57) Fleming, E. J.; Mack, E. E.; Green, P. G.; Nelson, D. C. Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Appl. Environ. Microbiol* **2006**, *72*, 457−464.
(58) Gilmour, C. C.; Podar, M.; Bullock, A. L.; Graham, A. M.; Brown, S. D.; Somenahally, A. C.; Johs, A.; Hurt, R. A., Jr.; Bailey, K. L.; Elias, D. A. Mercury methylation by novel microorganisms from new environments. *Environ Sci. Technol* **2013**, *47*, 11810−11820.
(59) Hu, H.; Lin, H.; Zheng, W.; Rao, B.; Feng, X.; Liang, L.; Elias, D. A.; Gu, B. Mercury reduction and cell-surface adsorption by Geobacter sulfurreducens PCA. *Environ Sci. Technol.* **2013**, *47*, 10922−10930.
(60) Feng, C.; Pedrero, Z.; Li, P.; Du, B.; Feng, X.; Monperrus, M.; Tessier, E.; Berail, S.; Amouroux, D. Investigation of Hg uptake and transport between paddy soil and rice seeds combining Hg isotopic composition and speciation. *Elem. Sci. Anth.* **2016**, *4*, 000087.
(61) Krishnan, S.; Dayanandan, P. Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa L.*). *J. Biosci.* **2003**, *28*, 455−469.

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