



Characterization of mercury species in brown and white rice (*Oryza sativa* L.) grown in water-saving paddies

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Methylmercury was more efficiently translocated to the endosperm than inorganic mercury.

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ABSTRACT

In China, total Hg (Hg_T) and methylmercury (MeHg) were quantified in rice grain grown in three sites using water-saving rice cultivation methods, and in one Hg-contaminated site, where rice was grown under flooded conditions. Polished white rice concentrations of Hg_T (water-saving: 3.3 ± 1.6 ng/g; flooded: 110 ± 9.2 ng/g) and MeHg (water-saving 1.3 ± 0.56 ng/g; flooded: 12 ± 2.4 ng/g) were positively correlated with root-soil Hg_T and MeHg contents (Hg_T : $r^2 = 0.97$, MeHg: $r^2 = 0.87$, $p < 0.05$ for both), which suggested a portion of Hg species in rice grain was derived from the soil, and translocation of Hg species from soil to rice grain was independent of irrigation practices and Hg levels, although other factors may be important. Concentrations of Hg_T and other trace elements were significantly higher in unmilled brown rice ($p < 0.05$), while MeHg content was similar ($p > 0.20$), indicating MeHg infiltrated the endosperm (i.e., white rice) more efficiently than inorganic Hg(II).

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1. Introduction

China is the world's highest emitter of atmospheric mercury (Hg) contributing 40% to the total global Hg emissions (Pacyna et al., 2010), and the largest producer of rice, responsible for 29% of the global paddy rice production (IRRI, 2010). In Guizhou province, southwestern China, a legacy of Hg contamination from industrial runoff, Hg mining, zinc smelting, and reliance on coal for power generation and residential heating has elevated Hg concentrations in air, soil, water and rice (reviewed by Feng and Qiu, 2008). In this region of China, rice is the primary methylmercury (MeHg) exposure pathway for some residents, not fish (Feng et al., 2008; Zhang et al., 2010a).

The conversion of inorganic Hg(II) to MeHg is primarily mediated by anaerobic sulfate-reducers (Compeau and Bartha, 1985; Gilmour et al., 1992) and iron-reducing microbes (Fleming et al., 2006; Kerin et al., 2006), which are both ubiquitous in the vicinity of rice roots (Scheid et al., 2004). Wetlands are considered important Hg methylation sites, where flooded conditions enhance anaerobic microbial activity and increase MeHg yields (Hurley et al.,

1995; Marvin-DiPasquale et al., 2003; St. Louis et al., 1994). Like wetlands, rice paddies are active Hg methylation sites. In eastern Guizhou province, average MeHg levels in unpolished brown rice were enriched 9.6–35 times compared to other agricultural products (corn, wild greens, cabbage, tobacco) (rice: 25.3 ng/g; other crops: 0.721–2.63 ng/g, from Qiu et al., 2008), and this disparity was likely due to cultivation of rice under standing water.

Rice is not an aquatic plant, although it has been grown under flooded conditions for over 5000 years (Bindraban et al., 2006). Historically, flooded paddies ensured water availability during periods of drought, increased nitrogen fixation, increased phosphorous levels, and minimized competition from weeds and soil-borne pathogens (Bindraban et al., 2006). However, water scarcity in Asia due to rapid urbanization and industrialization has prompted agricultural managers to develop rice cultivation methods utilizing less water (Bindraban et al., 2006; Li, 2006). The most widely employed method in China is safe alternating wetting and drying (AWD) (Li and Barker, 2004), where “safe” refers to a reduction in freshwater use without compromising rice yields (Bouman et al., 2007). Safe AWD is characterized by mid-season drainage 2–3 weeks before panicle initiation, then intermittent irrigation of the paddy soil through the milk ripening stage, each time allowing the water to dry below the soil surface before re-flooding (Bouman and Tuong, 2001; Bouman et al., 2007; Dong et al., 2004). Complete water drainage results in more aerobic conditions in the bulk soil,

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which likely reduces anaerobic microbial activity and may decrease MeHg levels in rice. In China, approximately 40% of rice farmers employ some version of AWD (Li and Barker, 2004).

In 2009, total mercury (Hg_T) and MeHg in water, soil, rice plants, and rice grain were compared between three neighboring sites in Hubei province, southern China, where AWD was applied. Two sites were located downwind from a factory that produced cooking oil, and one site bordered a constructed wetland (Fig. 1). The primary objective was to document the translocation of Hg species from the soil and roots to the aerial portions of the plant, including the endosperm (i.e., polished white rice). To the best of our knowledge, this is the first study to report Hg species in both brown and white rice grain. Results were compared with concentrations of other macronutrients (magnesium, Mg; and potassium, K) and micronutrients (manganese, Mn; zinc, Zn; copper, Cu; and nickel, Ni) in rice grain, and with Hg_T and MeHg concentrations in rice grain harvested from Guizhou province, China, where water was stored in the rice paddy during the entire rice-growing season.

2. Methods

2.1. Locations and sampling approach

Sites 1, 2, and 3 were located near the village of Tuanlin, within the Zhanghe Irrigation District, a major rice producing area in Hubei province, southern China, covering 160,000 ha (Fig. 1). AWD irrigation methods were employed at all three sites, but varied within each site. Site 1 was located at Tuanlin Experimental Station, managed by Wuhan University for field-testing water-saving irrigation methods in rice paddies (for detailed description see Dong et al., 2004). Site 2 was located

downstream from site 1 while site 3 was located upstream, both within a 15-min walking distance from site 1 (Fig. 1). In 2009, similar irrigation methods were practiced in sites 1 and 3 due to a drought; i.e., drier conditions between panicle initiation and milk ripening (notes for sites provided by Bin Dong, personal communication). Paddies in site 2 were located near a constructed wetland planted with cattails, reeds and water peanut, designed to filter agricultural water, which was pumped back into the rice fields through a series of channels. Paddies in the latter site were more difficult to drain due to their location near a year-around flooded wetland.

Samples from all three sites were collected on July 30–31, 2009, when rice plants were in the mid-reproductive stage (i.e., booting, when the hull was formed but not yet filled), and on September 5, 2009, when paddy fields were dried and mature rice grain was within 10 days of harvest. Within each site, two neighboring paddies were sampled in July and September. In site 3, a different pair of paddies was sampled in September, since rice grain from paddies sampled in July was already harvested. In each paddy field, the study area was limited to approximately 15×8 m, although each paddy was several times larger (e.g., site 1: 85×8 m; site 2: 38×15 m, site 3: 30×90 m). In July and September, two individual plants were collected and composited from each rice paddy ($n = 6$ paddies) for analysis of root-soil, root, stem, leaf, and rice grain. In July, one composite soil sample was collected from 4 points within the study area of each rice paddy, and in September, one composite sample of rice grain was harvested from 10 plants within each study area. Spatial variability for Hg_T and MeHg is likely within the rice paddy; therefore, composite samples were collected (for soil in July, and for rice grain in September) to compare with root-soil and rice grain from individual plants, collected in July and September, respectively. In September, a surface water sample (used for irrigation) was collected within each site near the paddies, and in the canal downstream from the cooking oil factory between sites 1 and 2 (Fig. 1).

In September 2009, one rice sample was collected near Wanshan, in eastern Guizhou province, China, where traditional flooded irrigation methods were practiced (Fig. 1).

2.2. Laboratory methods

2.2.1. Sample preparation and preservation

In July, rice samples (roots, stems, leaves, and seeds) were rinsed with Milli-Q H_2O , air-dried for 2 weeks, then freeze-dried and ground for analysis. In September, rinsed rice samples (roots, stems, and leaves) were air-dried for about 2 months and then ground for analysis. The stainless steel grinder was cleaned with ethanol after each sample to avoid cross-contamination. In September, aqueous samples were collected in 100 mL borosilicate bottles, which were acid-cleaned (20% HNO_3) and rinsed with Milli-Q H_2O , followed by heating in an oven at $550^\circ C$ to thermally desorb Hg. Filtered ($0.22 \mu m$) and unfiltered aqueous samples were immediately acidified to 0.5% HCl at Tuanlin Experimental Station, and stored in a dark refrigerator ($4^\circ C$). Soil samples were collected in acid-cleaned plastic tubes and frozen at Tuanlin Experimental Station. All samples were transported back to the Institute of Geochemistry in Guiyang (<1 day of travel), where soil samples (for Hg_T and MeHg analysis) and 45 mL aliquots of aqueous samples (for MeHg analysis) were stored frozen until analysis ($-20^\circ C$), while aqueous samples in borosilicate bottles (for Hg_T analysis) were refrigerated in the dark until analysis ($4^\circ C$). All analyses were completed within 6 months following sample collection.

2.2.2. Rice milling

Air-dried mature rice grain from Hubei province was milled at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (CAS) (Beijing, China), and air-dried mature rice samples from Wanshan, Guizhou province, were milled at the Institute of Geochemistry, CAS (Guiyang, China), following methods described by Sun et al. (2008) and using the same instruments at both locations. Briefly, the hull was removed exposing the edible rice (i.e., the caryopsis, referred to as unpolished brown rice) (Huller, JLGJ4.5, China); for half the sample, the bran was removed to produce polished white rice (Rice mill, JNMJ3, China); lastly, both brown and white rice samples were ground to a powder in a blender that was cleaned with ethanol after each sample to avoid cross-contamination. Approximately 7% of the bran was removed, which was slightly higher than the proportion of bran in commercial brown rice (6.3%, from Franz and Sampson, 2006).

2.2.3. Hg species

Hg_T was measured in aqueous samples following the addition of 0.5% (v/v) 0.2 N bromine monochloride, reduction with hydroxylamine hydrochloride, further reduction with tin chloride, purging of volatile Hg^0 onto gold traps, and detection of Hg^0 by cold vapor atomic fluorescence spectrophotometry (CVAFS, Brooks Rand Model III, Seattle, USA), using methods detailed in EPA Method 1631, Revision E (USEPA, 2002). MeHg in aqueous samples was analyzed following distillation, adjustment of pH using 2 M acetate buffer, ethylation with 1% sodium tetraethylborate, capture of gaseous methyl ethylmercury onto Tenax traps, thermal desorption from traps, separation by gas chromatography, and detection by CVAFS (Brooks Rand Model III, Seattle, USA), as described in EPA Method 1630 (USEPA, 2001a).

Solid-phase Hg_T concentrations in rice and soil samples were analyzed with a portable Hg vapor analyzer (Lumex, Model RA-915+/PYRO-915+, St. Petersburg, Russia), using methods described in EPA Method 7473 (USEPA, 1998). No pre-digestion

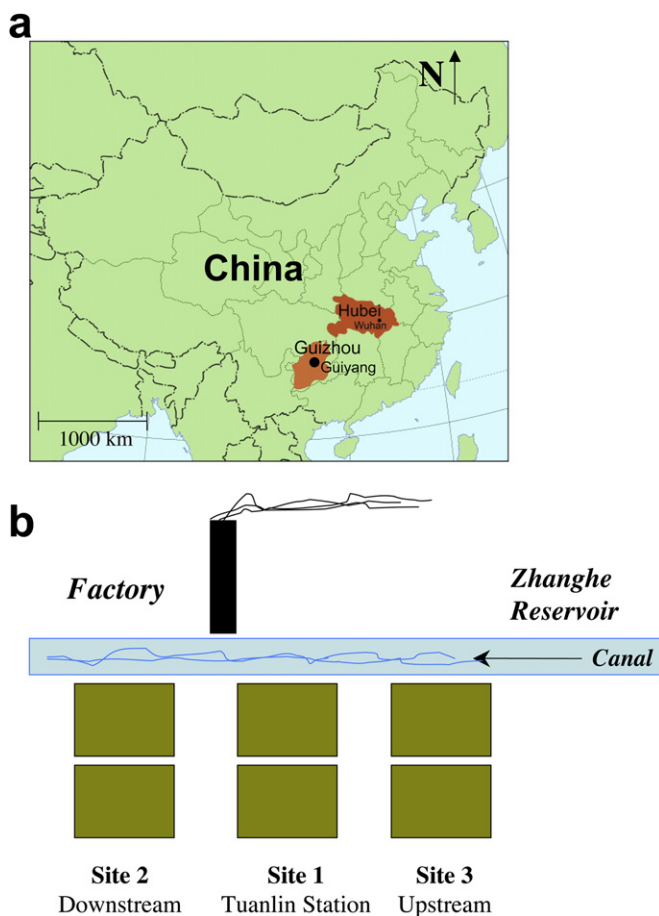


Fig. 1. a) Map of China, including Hubei province and Guizhou province; b) orientation for sites 1–3 in Hubei province, 2 paddies in each site. A constructed wetland bordered the rice paddies in site 2.

steps were necessary. MeHg concentrations in soil (1–2 g) and rice samples (~1 g) were analyzed using solvent extraction with dichloromethane (CH₂Cl₂). Soil samples were leached in 1.5 mL 2 M copper sulfate, 7.5 mL 25% HNO₃ and 10 mL CH₂Cl₂ (Liang et al., 2004). For rice samples, 5 mL of 25% KOH–water were added and samples were heated for 3 h at 75 °C in a water bath, then 5 mL concentrated HCl and 10 mL CH₂Cl₂ were added (based on Liang et al., 1996). After leaching, rice and soil samples were vigorously shaken (~1 h), centrifuged (25 min, 3500 RPM, 25 °C), the phases were separated by suction leaving the organic phase, about 30 mL Milli-Q-H₂O were added, and samples were heated in a 50 °C water bath for 1.5 h to evacuate CH₂Cl₂. MeHg was detected using CVAFS, as described for aqueous MeHg in distilled samples. Concentrations for all solid-phase samples are reported in dry weight.

2.2.4. Organic content

Hg_T and MeHg concentrations for wet soil samples were converted to dry weight following oven drying to constant weight at 60 °C. Organic carbon content was determined for dried samples by percent loss on ignition (LOI) at 550 °C for two hours.

2.2.5. Other elements

Mg, K, Mn, Zn, Cu, and Ni were determined in brown and white rice grains following EPA Method 3050b (USEPA, 1996). All elements were analyzed using flame atomic absorption spectrometry (AAS, Model 5100 PC, Perkin Elmer, Inc., Waltham, USA), aside from Ni, which was analyzed by inductively coupled plasma mass spectrometry (GV Instruments, Cheshire, UK).

2.2.6. QA/QC

Hg_T, MeHg and other trace elements were quantified using daily calibration curves with the coefficient of variation (r^2) > 0.99. The minimum detection levels (MDLs) for Hg_T and MeHg were estimated from the region of the standard curve where there was a significant change in sensitivity (40 CFR, Appendix B to Part 136, Definition and Procedure for Determination of the Method Detection Limit). MDLs for trace elements (Mg, K, Mn, Zn, Cu, and Ni) were based on instrument aqueous detection levels, corrected for the total volume (50 mL) and the mass of rice digested (1 g). MDLs, percent recovery of matrix spikes and standard reference material, and relative percent difference between replicate analyses (RPDs) are summarized in Table 1.

2.3. Statistics

The statistical program, Stata, was used for unpaired *t*-tests and one-way analysis of variance (ANOVA), and tests were considered significant at the 95% alpha-level. Due to the small sample size, interpretation of *p*-values is cautionary.

3. Results and discussion

3.1. Aqueous Hg_T and MeHg concentrations

Mean unfiltered and filtered aqueous Hg_T and MeHg levels in sites 1–3 (Table S1) were similar to mean values reported for

background sites in eastern Guizhou province (unfiltered Hg_T 2.2–18 ng/L, filtered Hg_T 0.89–5.3 ng/L from this study; unfiltered Hg_T 8.4–13 ng/L, filtered Hg_T 3.7–5.2 ng/L from Zhang et al., 2010b; unfiltered MeHg 0.47–0.50 ng/L, filtered MeHg 0.077–0.46 ng/L from this study; unfiltered MeHg 0.32 ng/L, filtered 0.14 ng/L from Zhang et al., 2010c). For the present study, highest unfiltered aqueous MeHg levels were observed in the canal (site 1: 0.47 ng/L; site 2: 0.50 ng/L; site 3: 0.50 ng/L and canal: 3.6 ng/L), located downstream from a cooking oil factory and between sites 1 and 2 (Fig. 1). Although water from the canal may be a source of MeHg to the rice paddies in site 2, unfiltered aqueous MeHg levels collected within each site were similar (see Section 3.4).

3.2. Hg_T and MeHg concentrations in rice plants

Hg_T and MeHg concentrations for rice plants harvested in July and September are shown in Figs. 2 and 3. In both months, mean leaf Hg_T levels were similar or higher than root Hg_T levels, which suggested stems were not the only source of Hg_T to the leaves. Findings were consistent with previous research that demonstrated leaf Hg_T was mainly accumulated from the atmosphere and not the roots (Ericksen et al., 2003; Rea et al., 2002; Stamenkovic and Gustin, 2009; Zhang et al., 2010d).

In July leaf Hg_T levels were significantly higher in site 1 compared to sites 2 and 3 (ANOVA, $p < 0.05$, $n = 6$) (site 1: 300 ± 47 ng/g, site 2: 42 ± 12 ng/g, site 3: 52 ± 24 ng/g). Site 1 was located downwind from a cooking oil factory emitting pollution from a smokestack at the same time rice plants were collected, although atmospheric Hg was not measured. Site 2 was located upwind from the factory and Site 3 was further downwind (Fig. 1). The mean Hg_T value for leaves at site 1 (300 ng/g) was derived from rice plants collected in 2 paddies (leaf Hg_T paddy 1: 270 ± 14 ng/g, $n = 2$ replicates; leaf Hg_T paddy 2: 330 ± 90 ng/g, $n = 4$ replicates). In contrast to July, leaf Hg_T levels in September were similar at all 3 sites (one-way ANOVA, $p > 0.05$, $n = 6$), which suggested Hg_T was not stored between July and September in the leaves. Instead, inorganic Hg(II) was possibly reemitted to the atmosphere from the leaves (Graydon et al., 2006; Stamenkovic and Gustin, 2009), or transported to other plant components, or exuded from the roots to the soil (Marschner, 1995).

Table 1

QA/QC data for Hg_T, MeHg and other trace elements. The minimum detection levels (MDLs) for Hg_T and MeHg were estimated from the region of the standard curve where there was a significant change in sensitivity. MDLs for other trace elements were based on instrument aqueous detection levels, which were corrected for the total volume (50 mL) and the mass of rice digested (1 g). The relative percent difference (RPD) was defined as the percent difference between replicate analyses (i.e., 100 × the standard deviation divided by the mean).

Parameter	Matrix	Average recovery ± 1sd (%)	Reference material	MDL	Average RPD (%) ($n = \#$ of samples)
Hg _T	Surface water	100 ± 2.9	Matrix spike	0.71 ng/L	4.8 ^a ($n = 76$) ^a
	Rice and plants	85 ± 7.6	Cabbage: GBW10014 (GSB-5)	1.2 ng/g	12 ($n = 66$)
		92 ± 11	Lobster: Tort-2		
	Soil	85 ± 0.56	Soil: GBW (e) 070009	2.5 ng/g	18 ($n = 18$)
		90 ± 11	Sediment: GBW07305 (GSD-5)		
		100 ± 16	Soil: GBW07405 (GSS-5)		
MeHg	Surface water	92 ± 18	Matrix spike	0.056 ng/L	32 ^a ($n = 8$) ^a
	Rice and plants	99 ± 34	Matrix spike	0.075 ng/g	24 ($n = 30$)
	Soil	95 ± 20	Sediment: BCR 580	0.075 ng/g	25 ($n = 3$)
Mn	Rice	110 ± 2.1	Rice: GBW10010 (GSB-1)	0.075 µg/g	5.3 ($n = 5$)
Mg	Rice	86 ± 5.7	Rice: GBW10010 (GSB-1)	0.0075 µg/g	7.8 ($n = 5$)
K	Rice	120 ± 1.4	Rice: GBW10010 (GSB-1)	0.15 µg/g	3.8 ($n = 5$)
Cu	Rice	88 ± 0.83	Rice: GBW10010 (GSB-1)	0.075 µg/g	7.2 ($n = 5$)
Zn	Rice	110 ± 7.1	Rice: GBW10010 (GSB-1)	0.075 µg/g	4.0 ($n = 5$)
Ni	Rice	97 ± 13	Rice: GBW10010 (GSB-1)	0.025 µg/g	27 ($n = 5$)

^a RPD for aqueous Hg_T and MeHg represented values for samples run at the same time, since there was insufficient volume for duplicate analyses for samples collected for this study.

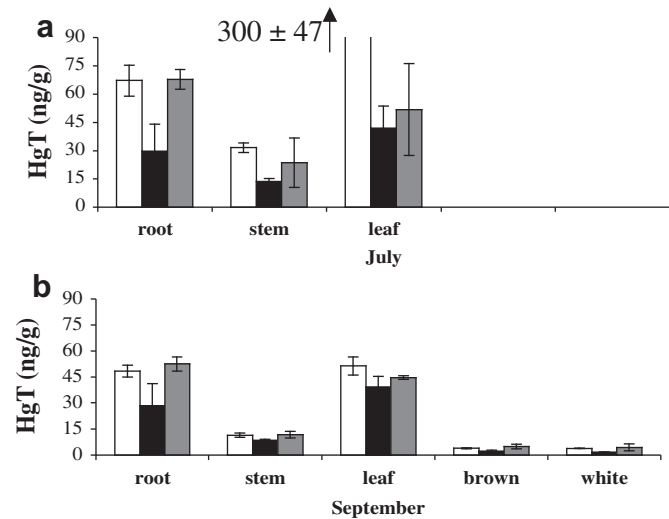


Fig. 2. Hg_T levels in plant components collected in a) July (root, stems, and leaves) and b) September (roots, stems, leaves and grain) for site 1 Tuanlin (white), site 2 Downstream (black) and site 3 Upstream (gray). Results represent the mean (± 1 sd) from rice plants collected from 2 paddies within each site.

MeHg levels were highest in rice grain (Fig. 3) and %MeHg (of Hg_T) was also highest in rice grain (white: 40–43%; brown: 26–36%) compared to roots, stems and leaves (range: 1.0–4.7%) (Table 2, Table S1). Hg_T and MeHg bioaccumulation factors (BAFs) were calculated as the ratio between the concentrations of Hg_T and MeHg in rice grain and root-soil (Table 2). For brown and white rice grain, Hg_T BAFs ranged from 0.018–0.072 and 0.016–0.053, respectively, while MeHg BAFs ranged from 1.4–2.0 and 1.5–1.8, respectively. There were no differences between Hg_T and MeHg BAFs for brown and white rice (paired *t*-test, $p > 0.10$, $n = 6$ pairs). The ratios of MeHg BAF and Hg_T BAF for brown and white rice were 26–76 and 29–110, respectively, which were comparable to the ratio for MeHg and inorganic $Hg(II)$ BAFs reported for brown rice grain in the Wanshan Hg mining area, Guizhou province (0.71–50, from Zhang et al., 2010d).

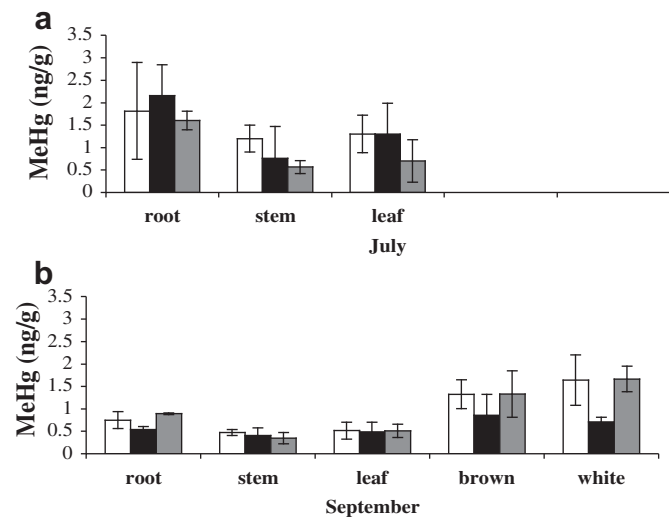


Fig. 3. MeHg levels in root, stems, and leaves collected in a) July and b) roots, stems, leaves and grain collected in September for site 1 Tuanlin (white), site 2 Downstream (black) and site 3 Upstream (gray). Results represent the mean (± 1 sd) from rice plants collected from 2 paddies within each site.

Table 2

Concentrations of trace elements from composite and individual plants at sites 1–3, including Hg_T , MeHg, %MeHg (of Hg_T) and other trace elements (Cu, K, Mg, Mn, Ni and Zn). Bioaccumulation factors (BAFs, unitless) were calculated as the ratio between rice grain and root-soil concentrations of Hg species ($n = 4$ per site).

Rice fraction	Trace element	Site 1	Site 2	Site 3
		Tuanlin Station	Downstream	Upstream
White rice	Hg_T (ng/g)	3.6 ± 0.33	1.8 ± 0.15	4.6 ± 1.9
	MeHg (ng/g)	1.3 ± 0.52	0.67 ± 0.028	1.8 ± 0.91
	% MeHg of Hg_T	43 ± 13	42 ± 5.6	40 ± 12
	Hg_T BAF	0.024 ± 0.0035	0.053 ± 0.0021	0.016 ± 0.0051
	MeHg BAF	1.8 ± 0.27	1.5 ± 0.84	1.7 ± 0.18
	MeHg BAF/ Hg_T BAF	75 ± 0.11	29 ± 15	110 ± 26
	Cu ($\mu\text{g/g}$)	3.0 ± 0.78	2.4 ± 0.25	2.3 ± 0.40
	K (mg/g)	1.9 ± 0.21	1.6 ± 0.039	1.9 ± 0.26
	Mg (mg/g)	0.73 ± 0.13	0.57 ± 0.034	0.71 ± 0.17
	Mn ($\mu\text{g/g}$)	19 ± 3.1	14 ± 0.95	15 ± 2.8
	Ni ($\mu\text{g/g}$)	0.29 ± 0.088	0.33 ± 0.038	0.35 ± 0.16
	Zn ($\mu\text{g/g}$)	19 ± 4.2	14 ± 1.6	15 ± 2.1
Brown rice	Hg_T (ng/g)	3.5 ± 0.50	2.5 ± 0.42	5.0 ± 1.6
	MeHg (ng/g)	1.2 ± 0.36	0.69 ± 0.35	1.5 ± 1.2
	% MeHg of Hg_T	34 ± 5.6	36 ± 11	26 ± 3.8
	Hg_T BAF	0.024 ± 0.0023	0.072 ± 0.020	0.018 ± 0.0020
	MeHg BAF	1.5 ± 0.037	2.0 ± 1.7	1.4 ± 0.44
	MeHg BAF/ Hg_T BAF	61 ± 9.4	26 ± 17	76 ± 17
	Cu ($\mu\text{g/g}$)	2.9 ± 0.42	2.7 ± 0.24	2.6 ± 0.19
	K (mg/g)	2.9 ± 0.12	3.0 ± 0.27	3.1 ± 0.30
	Mg (mg/g)	1.3 ± 0.019	1.2 ± 0.17	1.4 ± 0.19
	Mn ($\mu\text{g/g}$)	27 ± 1.3	26 ± 2.7	25 ± 3.7
	Ni ($\mu\text{g/g}$)	0.43 ± 0.19	0.41 ± 0.11	0.28 ± 0.12
	Zn ($\mu\text{g/g}$)	21 ± 4.1	18 ± 2.3	19 ± 0.85

Higher accumulation of MeHg compared to Hg_T in rice grain was consistent with earlier studies that reported inorganic $Hg(II)$ sorbed by roots was not readily translocated to the aerial portions of the plants (Beauford et al., 1977; Cavallini et al., 1999; Greger et al., 2005; Lindberg et al., 1979), and the proportion of MeHg was higher compared to inorganic $Hg(II)$ in the aboveground plant biomass (Schwesig and Krebs, 2003). One hypothesis is that glutathione-derived peptides present in the roots, called phytochelatin (Cobbett and Goldsbrough, 2002), chelate inorganic $Hg(II)$ more efficiently than MeHg, which was suggested by Krupp et al. (2009). Limitation of inorganic $Hg(II)$ translocation may be a protective mechanism since inorganic $Hg(II)$ may hinder transpiration, affect plant growth, or cause oxidative stress (e.g., Zhou et al., 2007), and should be further tested.

3.3. Calculation of daily MeHg ingestion rate

The average rice ingestion rate in 2008 in China was 270 g/d, which was based on the annual total rice consumption ($130,778 \times 10^3$ t, from IRRI, 2010) and the population of China ($1,324,655 \times 10^3$, from PRB, 2010). Using the mean MeHg levels in white rice (average: 1.2 ng/g, $n = 12$), and assuming mean body weight is 60 kg, the mean daily MeHg intake rate from rice ingestion was $0.0054 \mu\text{g kg}^{-1} \text{d}^{-1}$, which was 17 times lower than the U.S. EPA recommended level ($0.1 \mu\text{g kg}^{-1} \text{d}^{-1}$, USEPA, 2001b). Higher MeHg exposure levels were reported for brown rice in the Hg mining area in China, assuming the daily rice ingestion rate was 620 g/d (e.g., $0.26 \mu\text{g kg}^{-1} \text{d}^{-1}$ from Qiu et al., 2008; 0.012 – $0.44 \mu\text{g kg}^{-1} \text{d}^{-1}$ from Zhang et al., 2010c). Using the higher ingestion rate, the daily MeHg exposure level for this study was $0.012 \mu\text{g kg}^{-1} \text{d}^{-1}$ (i.e., 8.1 times lower than the U.S. EPA reference dose, USEPA, 2001b), which indicated rice grain harvested from these paddies near Tuanlin village, Hubei province, China was not a significant source of MeHg to humans.

3.4. The effect of AWD on the uptake rate of Hg_T and MeHg in rice grain

For brown and white rice grains, Hg_T and MeHg levels for composite rice samples and individual plants collected within the same paddy field did not differ (ANOVA, $p > 0.95$, $n = 12$), indicating spatial variability within these rice paddies did not significantly affect results. Hg_T and MeHg in rice grain (from both composite and individual plants) were ranked as follows: site 2 < site 1 < site 3 (Fig. 4). For both brown and white rice, Hg_T content was significantly higher in site 3 compared to site 2 (Hg_T log-transformed, ANOVA, $p < 0.05$, $n = 6$), but MeHg content was not significantly higher in either rice fraction between sites (MeHg log-transformed, ANOVA, $p > 0.05$, $n = 6$), which was not unexpected, since aqueous MeHg levels in irrigation water were similar between all three sites (see Section 3.1, also Table S1).

Hg_T and MeHg concentrations in white rice were most correlated with Hg_T and MeHg levels in root-soil compared to all other parameters, including organic content (Fig. 5, Table S2, $n = 6$). For comparison, one paired rice grain and soil sample was included from the Wanshan Hg mining area (soil data from Meng et al., 2010), where the rice paddy was continuously flooded under 4–5 cm of standing water. The coefficients of variation (r^2) between polished rice grain and root-soil for Hg_T and MeHg were 0.97 and 0.87, respectively (all variables were log-transformed, $n = 7$, $p < 0.05$ for both). However, the slopes between white rice and root-soil for Hg_T and MeHg differed; for Hg_T the slope was 0.64, while the slope for MeHg was 1.3 (Fig. 5). When the Wanshan observation was excluded, polished white rice and root-soil Hg_T and MeHg were still highly correlated: $r^2 = 0.75$ and 0.79, respectively (all variables were not log-transformed, $n = 6$, $p < 0.05$ for both, figures not shown).

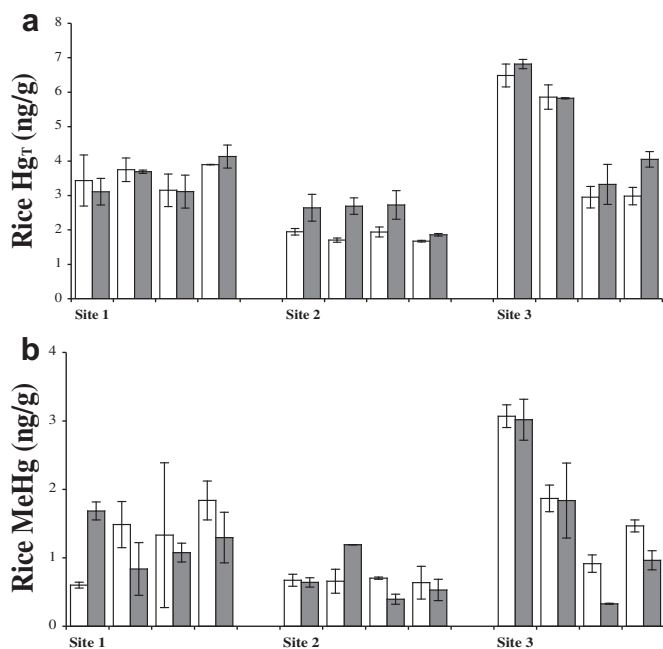


Fig. 4. Hg_T (ng/g) (a) and MeHg (ng/g) (b) concentrations in brown rice grain (gray) and white rice grain (white) (± 1 standard deviation for replicate analyses) for site 1 (Tuanlin), site 2 (downstream) and site 3 (upstream). Within each site, rice samples were collected within 2 paddies, including one composite sample ($n =$ rice grain from 10 plants) and one individual sample ($n =$ rice grain from 2 plants). The four bars for each site represent the composite sample for paddy 1, the individual sample for paddy 1, the composite sample for paddy 2 and the individual sample for paddy 2. For brown and white rice grains, Hg_T and MeHg levels for composite rice samples and individual plants collected within the same paddy did not differ (ANOVA, $p > 0.95$, $n = 12$), indicating spatial variability within these paddies did not significantly affect results.

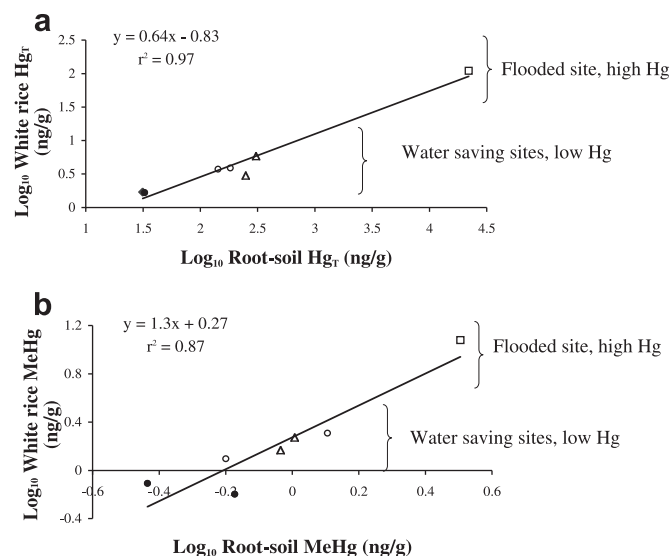


Fig. 5. White rice versus root-soil for a) Hg_T and b) MeHg, collected from water-saving sites in Hubei province (site 1 (Tuanlin): open circles, site 2 (downstream): black circles, and site 3 (upstream): open triangles), and one paddy field near Wanshan, Guizhou province (open square). For the latter site, the soil sample was collected within the rice paddy, not necessarily in contact with the rice root.

Although this data set is small, results suggested at least a portion of Hg_T and MeHg in rice grain likely originated from the soil, which was consistent with observations by Meng et al. (2010). However, the slope for MeHg was twice as high as the slope for Hg_T (1.3 versus 0.64), which indicated MeHg was more efficiently translocated from the soil to the rice grain, or MeHg was not recycled in the plant once transferred to the rice grain. Additionally, uptake and translocation of Hg species from the soil into the grain may be independent of irrigation practices and soil Hg concentrations. AWD practices result in more aerobic rice paddies; however, drier conditions practiced in these AWD sites did not inhibit the uptake of Hg species from the soil and loading into the grain. This may reflect the “safe” AWD approach, where paddies are intermittently flooded, water savings are minimal (about 15%), and rice yield is not compromised (Bouman et al., 2007). There are other factors that may influence the uptake of Hg_T and MeHg, including soil pH (Overesch et al., 2007), interaction with other metals (Du et al., 2005) and/or sulfur species (Hu et al., 2007), and differences between cultivars (Liu et al., 2004), which were not examined in this analysis. More research is needed to calibrate the effect of AWD on uptake of nutrients and trace elements into the rice grain, to optimize nutritional quality and minimize exposure to toxic heavy metals.

3.5. Hg speciation in brown and white rice

This is the first study, to the best of our knowledge, to report Hg species in both brown and white rice fractions. Earlier studies from Guizhou province reported MeHg levels in unpolished brown rice (e.g., Horvat et al., 2003; Meng et al., 2010; Qiu et al., 2006, 2008; Zhang et al., 2010a,d). Since milling may reduce micronutrient levels (Heinemann et al., 2005; Sellappan et al., 2009; Villareal et al., 1991), Hg species and other elements were compared between unpolished brown rice grain and polished white rice grain, and between sites (Table 2).

Using combined results from both composite and individual rice grain samples grown in 3 water-saving sites, Hg_T levels were significantly higher in brown rice grain (paired t -test, $p < 0.05$,

$n = 12$), while MeHg levels did not differ between the two fractions (paired t -test, $p > 0.20$, $n = 12$) (Fig. 4). Concentrations of other trace elements (Mg, K, Mn, Zn, and Ni) were also significantly higher in brown rice compared to white rice ($p < 0.05$, $n = 12$), and borderline significant for Cu ($p < 0.054$, $n = 12$). Between sites, there were no significant differences for MeHg and other trace elements (Mg, K, Mn, Zn, Cu, and Ni) in brown and white rice grain; however, Hg_T levels in brown rice were significantly higher in site 3 compared to site 2 (ANOVA, $p < 0.05$, $n = 12$) (Table 2).

The concentration of Hg species in brown rice is comprised of the weighted contributions from bran and polished white rice. The proportion of brown rice in white rice may be derived as follows, where 7% is the percentage of bran milled (see Section 2.2.2 methods for rice milling).

$$[\text{Brown}] = (0.07 \times [\text{bran}]) + (0.93 \times [\text{white}]) \quad (1)$$

Dividing by the concentration in brown rice in equation (1) gives the following result:

$$1 = (0.07 \times [\text{bran}]/[\text{brown}]) + (0.93 \times [\text{white}]/[\text{brown}]) \quad (2)$$

The term on the right (i.e., $0.93 \times [\text{white}]/[\text{brown}]$) represents the proportion of trace element transferred from brown rice to white rice (from Villareal et al., 1991). The authors reported the following percentages for rice with 10% bran removed: Cu 99%, Zn 66%, K 48%, Mn 45%, and Mg 40%, which indicated Cu was transported more efficiently into the endosperm (i.e., white rice) compared to Mg (Villareal et al., 1991). From this study, average percentages for the same trace elements differed but the rankings were the same (Cu $86 \pm 13\%$, Zn $77 \pm 7.4\%$, K $57 \pm 7.9\%$, Mn $57 \pm 8.3\%$, and Mg $48 \pm 8.5\%$). Percentages for Ni and Hg species were: Ni $85 \pm 24\%$, Hg_T $82 \pm 14\%$, and MeHg $120 \pm 59\%$. When one rice sample from Wanshan was included, which was grown under flooded conditions (white rice Hg_T: 110 ± 9.2 , MeHg 12 ± 2.4 ; brown rice: Hg_T: 200 ± 16 , MeHg 17 ± 8.9), percentages for Hg species did not significantly change (Hg_T $80 \pm 16\%$, and MeHg $120 \pm 58\%$).

Percentage differences reflect where elements reside in the rice grain. Using synchrotron X-ray fluorescence (XRF) and particle induced X-ray emission (PIXE) to map the locations of nutrients in the rice grain, Lombi et al. (2009) reported both Zn and Cu decreased in concentration from the pericarp to the interior of the endosperm, while K and Mn were localized in the aleurone/pericarp region. During the milling process, the embryo, pericarp and most of the aleurone layer are removed, leaving polished white rice. Nutrients more concentrated in these layers are reduced during the milling process.

Unlike most other trace elements, milled white rice did not result in a reduction in MeHg levels. Variability for MeHg was also higher compared to other micronutrients, which possibly reflected higher RPD between replicate digestions (MeHg: 24%; Hg_T: 12%; Ni: 27%, others: <8%) (Table 1). Similar MeHg levels in white and brown rice grain suggested more efficient unloading of this species into the endosperm. In rice grain, the filial tissue (endosperm, germ, and aleurone) is isolated from the maternal tissue (pericarp, placenta, vascular tissue, chalaza, and nucellus) (Krishnan and Dayanandan, 2003). The ovular vascular trace, which is part of the maternal tissue, is the only source of nutrients for the developing grain (Krishnan and Dayanandan, 2003), and is considered a bottleneck for nutrient unloading (Lombi et al., 2009). It is possible organic nutrients, including MeHg, utilize membrane transport pathways into the grain more efficiently, which was reported in rice grain for organic dimethylarsinic acid (Carey et al., 2010; Lombi et al., 2009; Meharg et al., 2008) and for organic selenium species (Li et al., 2010; Sun et al., 2010; Williams et al., 2009).

4. Conclusions

AWD water-saving methods did not affect the uptake rate (i.e., slope of the regression line) of Hg_T and MeHg between the soil and rice grain, although other factors may be important. Despite drier, more aerobic conditions in the water-saving paddies, the uptake and translocation rates of Hg_T and MeHg from the soil to the rice grain were similar between three water-saving sites and one non-water-saving site. Unlike other trace elements, MeHg concentrations were not significantly reduced in milled white rice, which indicated MeHg infiltrated the endosperm more easily than inorganic Hg(II). The latter is important, since polished white rice is the fraction ingested throughout Asia. This was an observational study, and more research is needed to understand the effect of water-saving irrigation methods on the bioavailability and uptake of trace elements, including toxic elements such as MeHg, from paddy soil.

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Appendix A. Supporting information

Supplementary data associated with this article can be found, in the on-line version, at doi:10.1016/j.envpol.2011.01.027.

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