



1 Mercury Methylation in paddy soil: Source and distribution of 2 mercury species at a Hg mining area, Guizhou Province, China

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13 **Abstract.**

14 Rice paddy plantation for human consumption is dominant land uses throughout Asia. Rice paddy fields
15 have been identified as important sites for methylmercury (MeHg) production in the terrestrial
16 ecosystem, and a primary pathway of MeHg exposure to human in mercury (Hg) mining areas. We
17 compared the source and distribution of Hg species in different compartments of the rice paddy during a
18 complete rice-growing season at two different typical Hg-contaminated mining sites: an abandoned site
19 with high Hg concentration in soil but low concentration in atmosphere, and a current-day artisanal
20 with low concentration in soil but high concentration in atmosphere. The contribution of new Hg to the
21 ecosystem from irrigation and atmospheric deposition was insignificant relative to the pool of old Hg;
22 the dominant source of MeHg to paddy soil is in situ methylation of inorganic Hg. Elevated MeHg



1 concentrations jointly with the high proportion of Hg as MeHg in paddy water and the surface soil layer
2 at the artisanal site demonstrated active Hg methylation at this site only. We propose that the in situ
3 production of MeHg is dependent on elevated IHg in the atmosphere, and the deposition of new Hg into
4 a low pH anoxic geochemical system. In contrast, the absence of depth-dependent variability in the
5 MeHg concentration in soil cores collected from abandoned Hg mining site, consistent with the low
6 concentration of Hg in atmospheric deposition and high pH of the paddy water/irrigation water,
7 suggested that the net production of MeHg was limited. We also propose that the concentration of Hg in
8 ambient air is an indicator for the risk of MeHg accumulation in paddy rice.


9 1 Introduction

10 Reports that rice (*Oryza sativa*) is a bioaccumulator species of MeHg have recently focused scientific
11 attention on this important agricultural crop (Qiu et al., 2008; Zhang et al., 2010a; Meng et al., 2010,
12 2011, 2014). Numerous studies have reported high MeHg concentrations in rice grain collected from
13 Indonesia (Krisnayanti et al., 2012) and different parts of China (Qiu et al., 2008; Hui et al., 2003;
14 Meng et al., 2014) and the MeHg concentrations in rice grain can be enhanced even in cases where soil
15 is not significantly elevated in Hg (Zhang et al., 2010a; Horvat et al., 2003). A common theme to these
16 studies is a high flux of Hg into the environment through mining or other industrial contamination that
17 discharges into the atmosphere.

18 Rice paddy plantation is one of the most prevalent land uses in South and East Asia where rice is the
19 dominant foodstuff (FAO, 2002). Rice throughout Asia is generally cultivated in paddy soil and this
20 land use represents an ephemeral wetland, which is known to be a significant environment for Hg
21 methylation. MeHg accumulated throughout a rice plant during the growing season can be readily
22 translocated to grain during rice-seed ripening (Meng et al., 2011). Rice paddy fields have therefore
23 been identified as important sources of MeHg in the terrestrial ecosystem (Meng et al., 2010, 2011), and
24 a primary vector for human exposure to MeHg in Hg mining areas (Feng et al., 2008; Zhang et al.,
25 2010b).

26 The general consensus among Hg researchers is that soil is the principle source of MeHg in rice plants,
27 whereas Hg from the ambient air is the principal source of IHg in rice grain (Meng et al., 2010, 2011,



1 2012, 2014). Recently, Meng et al. (2010, 2011) suggested that newly deposited Hg is more readily
2 transformed to MeHg and accumulated in rice plants than Hg forms with an extended residence time in
3 mining-contaminated soil. Meng et al. (2010) focused on the Wanshan area of China, a region of both
4 historical large scale  and current small-scale mercury mining. ~~The concentration of MeHg~~ in rice grain
5 collected from artisanal Hg mining areas was shown to be significantly higher than ~~that~~ in rice grain
6 collected from abandoned Hg mining areas (Meng et al., 2010). Despite these ~~reported~~ observations, the
7 biochemical processes that control the cycling of Hg in paddy soils impacted by Hg mining are poorly
8 understood.

9 ~~All reports~~ on MeHg and rice emphasize that the **concentration and distribution of Hg in ambient air,**
10 **wet/dry deposition, irrigation water, and solid and liquid phases of soil** are important factors that control
11 the biochemical cycling of Hg within rice paddy ecosystems ~~impacting~~ by mining activities. These
12 factors in turn impact the absorption, transportation, and accumulation of Hg in rice plants (Meng et al.,
13 2010, 2011, 2012, 2014; Rothenberg and Feng, 2012; Liu et al., 2012; Wang et al., 2014; Peng et al.,
14 2012). Past studies have focused on the source, distribution, and accumulation of IHg and MeHg in rice
15 plant, but, no study has presented results from a systemic survey of the concentration of Hg fractions in
16 the rice paddy ecosystem. Such a study is necessary to mitigate ~~against~~ the risk ~~for~~ MeHg formation in
17 paddy soil ~~where a rice crop is being grown in a~~ Hg contaminated area. Better understanding of the
18 distribution of Hg species in paddy soils within a Hg mining area is necessary to underpin more reliable
19 risk assessment and appropriate strategies to remediate contaminated soil.

20 To expand our knowledge of the biochemical processes that affect Hg **we assessed the status of Hg**
21 **species** in different compartments of the rice paddy ecosystem. We measured the concentration of Hg
22 species in ambient air, atmospheric deposition, irrigation water, paddy water, and soil cores at two
23 contrasting Hg mining sites during a complete rice-growing season ~~to quantify the distribution and~~
24 ~~origin of Hg species in the rice paddy~~. The relative contribution of different Hg vectors to the surface
25 pool of soil Hg was subsequently estimated. The primary objectives of our study were ~~to 1)~~ investigate
26 ~~the~~ speciation and distribution ~~of Hg~~ in the paddy soil, and 2) to assess the primary source and
27 mechanism for **transformation of Hg species** within paddy soil at a Hg mining area. Documenting Hg
28 cycling in rice paddy ecosystems within Hg mining areas is an important step towards better assessing



1 potential health threats that may be associated with ~~the cultivation of rice~~ in a Hg-contaminated
2 environment.

3 **2 Materials and methods**

4 **2.1 Site description**

5 This study was conducted in the Wanshan Hg mining district, Guizhou province, Southwest China,
6 where historical large-scale Hg smelting combined with current artisanal Hg smelting activities have
7 resulted in Hg contamination of ambient air, water, soil, sediment, and biota (Qiu et al., 2005; Li et al.,
8 2008, 2009). Two Hg contaminated sites within the Wanshan Hg mining district were selected for this
9 study: an artisanal Hg mining site (Gouxu); and an abandoned Hg mining site (Wukeng) (Fig. 1). The
10 sampling sites of Gouxu and Wukeng are situated within the Wanshan district which experiences
11 subtropical monsoon-type ~~weather~~ with an average annual rainfall of 1200-1400 mm and a perennial
12 mean temperature of 17 °C. Historical Hg mining activities in the Wanshan area can be dated back to
13 the Qin Dynasty (221 B.C.) but large-scale mining activities officially ceased in 2001. Mining activity
14 across Wanshan generated an estimated cumulative 1.0×10^8 tons of calcine and waste rock between
15 1949 and the 1990s. Recently, illegal artisanal Hg and small-scale mining activities have been revived
16 due to an increase in the global Hg price and domestic demand.

17 The Gouxu artisanal Hg mining site is located to the north of Wanshan town (Fig. 1). Small-scale
18 artisanal smelting was ongoing during the rice growing seasons of 2012 when the samples for the
19 current study were collected. Mercury is released into the atmosphere during artisanal smelting and is
20 subsequently deposited onto nearby rice paddy fields through wet and dry deposition. The Wukeng
21 sampling site is located north east of Wanshan town at an abandoned Hg mining area where large
22 quantities of calcines were deposited along the river.

23 Paddy field ~~rice~~ is the primary agricultural land use at both Gouxu and Wukeng. Field sampling for the
24 current research focused on two 10×10 m plots (one at each site) within rice paddies that were
25 established according to the following methodology: The rice paddies were flooded on 10th May; rice
26 seedlings (hybrid rice) widely grown throughout Guizhou province were transplanted into the



1 submerged soil 20 days after flooding (1 plant each 25×25 cm area on 1st June, defined as Day 0).
2 Thereafter, the two experimental plots were cultivated during the period 1st June through 10th September
3 (100 days) 2012. Standing water (2-8 cm) was maintained above the soil surface (flooded condition)
4 throughout the growing period, from Day 0 to Day 80. The paddy fields were thereafter drained from
5 Day 80, prior to harvest between Days 90 to 100. During the 10 days draining period, approximately 2-
6 4 cm depth of water was maintained above the soil surface. The paddy plots received water through
7 precipitation and stream water irrigation, while evaporation and **seepage** were the primary vectors for
8 water loss. There was no **outflow** from either paddy.

9 **2.2 Sample collection and preparation**

10 Five consecutive sampling campaigns were conducted during the rice growing season (1st June-10th
11 September, 2012). The first sampling was initiated 20 days after the plants were planted out (20th June,
12 2012; Day 20), and thereafter samples were collected on Days 40, 60, 80, and 100 (Day 100 was 10th
13 September, 2012; final harvest). The Hg concentration in ambient air was measured ~~at~~ each sampling
14 time, and ~~a sample~~ of cumulative precipitation, irrigation water, paddy water, and soil cores were
15 collected ~~at each time~~. The paddy fields were dry from Day 90, and therefore irrigation water, paddy
16 water, and soil pore water samples were not collected on Day 100.

17 **2.2.1 Mercury in ambient air and wet/dry deposition**

18 The concentration of total gaseous mercury (TGM, Hg⁰) in ambient air at both Gouxu and Wukeng was
19 measured in the field at each sampling time using an automated Hg vapor analyzer (LUMEX, RA-915⁺,
20 Ohio Lumex Co., Twinsburg, OH) with detection limit of 2 ng m⁻³. The average Hg⁰ concentration
21 during a 10 s interval was quantified and stored in a portable computer. ~~At each sampling time,~~
22 ~~measurements~~ were carried out continuously for at least one hour period; therefore, ~~360 data points (10~~
23 ~~s reading)~~ were collected at each location ~~at each sampling time~~.

24 An integrated bulk precipitation sampler based on the design of Guo et al. (2008) was used in the field
25 to quantify the concentration of Hg in cumulative precipitation (Oslo and Paris, 1998). Both dry and wet
26 atmospheric deposition were collected concurrently with the TGM measurement once every 20 days



1 using this sampling method. Samples collected at each site were poured into two 100 mL pre-cleaned
2 borosilicate glass bottles for direct and unfiltered total Hg (HgT_{unf}) and total MeHg (MeHg_{unf}) analysis.

3 **2.2.2 Irrigation water and paddy water**

4 Samples of irrigation water at both Gouxi and Wukeng were collected at rice paddy inlets on Days 20,
5 40, 60, and 80. All water samples were collected by hand using ultra-clean handling protocols and
6 stored in acid-cleaned borosilicate glass bottles. Each bottle was rinsed three times with irrigation water
7 on site before sample collection. Filtered samples were collected on site using a 0.45 μm disposable
8 polycarbonate filter unit (Millipore), and subsequently analyzed for dissolved total Hg concentrations
9 (HgT_{f}) and dissolved MeHg concentration (MeHg_{f}). In addition, unfiltered irrigation water samples
10 were siphoned into pre-cleaned borosilicate glass bottles using a disposable syringe and analyzed for
11 total Hg (HgT_{unf}) and MeHg (MeHg_{unf}).

12 Paddy water (overlying water) and corresponding soil pore water samples at both Wukeng and Gouxi
13 were collected at the centre of the two plots on Days 20, 40, 60, and 80, simultaneously with the
14 irrigation water collection. Firstly, an undisturbed soil core was collected at each sampling site by
15 pushing a pre-cleaned 6-cm diameter polycarbonate core tube into the soil to approximately 20 cm
16 depth. The paddy water (0-8 cm above the soil surface) in the core tube was siphoned into a 200 ml pre-
17 cleaned borosilicate glass bottles. One aliquot of the paddy water was then filtered into a 100 ml pre-
18 cleaned borosilicate glass bottle using a 0.45 μm disposable polycarbonate filter unit (Millipore), and
19 subsequently analyzed for HgT_{f} and MeHg_{f} . A second aliquot of paddy water for HgT_{unf} and MeHg_{unf}
20 analysis was immediately transferred into another 100 ml pre-cleaned borosilicate glass bottle.

21 General Water Quality Characteristics of irrigation water and paddy water including pH, dissolved
22 oxygen (DO) concentration, and temperature (T) were measured *in situ* using a portable analyzer. All
23 water samples were promptly acidified on site to 0.5% (v/v) using adequate volumes of ultra-pure
24 concentrated hydrochloric acid (HCl). The sample bottles were then capped, sealed with Parafilm[®],
25 double-bagged, transported to the laboratory in an ice-cooled container to the lab within 24 h. Prior to
26 Hg analysis, samples were stored in a refrigerator at +4 °C in the dark.



1 **2.2.3 Soil pore water (liquid phase) and soil core (liquid phase+solid phase)**

2 The soil cores were immediately sliced on site into 2 cm intervals using a plastic cutter in an oxygen-
3 free glove box under argon. The soil samples were placed in acid-cleaned 50-ml plastic centrifuge tubes,
4 capped and sealed with Parafilm[®]. All samples were transported in an ice-cooled container to the lab
5 within 24 h and stored at 3-4 °C for further laboratory processes. Following centrifugation (30 min,
6 3000 r min⁻¹, and 5 °C), the samples were returned to the glove box where the pore water was then
7 filtered through 0.45 µm disposable nitrocellulose filter unit. The filtrate was stored in borosilicate glass
8 bottles and divided for HgT_f and MeHg_f analysis. The water content of soil cores was estimated by
9 weight loss.

10 ~~At each sampling site and at each sampling time~~, a second soil core was collected, and immediately
11 placed into liquid nitrogen. This second set of soil cores was transported in a liquid nitrogen-iced
12 container to the lab within 24 h and then sliced at 2 cm intervals. The sliced soil cores were then freeze-
13 dried, prior to homogenisation to 200 mesh with a mortar and pestle for analysis of total Hg (THg) and
14 MeHg. The concentration of each Hg species in this second set of soil cores is therefore the sum of both
15 liquid and solid phase. Precautions were taken to avoid cross-contamination during sample processing;
16 the mortar and pestle were thoroughly cleaned after each sample with drinking water followed by
17 deionized water rinses. The powdered samples were subsequently packed into plastic dishes, sealed in
18 polyethylene bags and stored in a refrigerator within desiccators for subsequent laboratory analysis.

19 **2.3 Sample analysis**

20 All reagents used in this study were of guaranteed quality purchased from Shanghai Chemicals Co.
21 (Shanghai, China).

22 **2.3.1 Total Hg and MeHg in soil samples**

23 For THg analysis, a soil sample (0.1-0.2 g) was digested using a fresh mixture of HCl and HNO₃ (1:3,
24 v/v). THg was determined by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran 2500,



1 Tekran Instruments) preceded by BrCl oxidation, SnCl₂ reduction, pre-concentration, and thermo-
2 reduction to Hg⁰ (U.S. EPA, 2002).

3 For MeHg analysis, a soil sample (0.3-0.4 g) was prepared using the CuSO₄-methanol/solvent
4 extraction (Liang et al., 1996). MeHg in samples was extracted with methylene chloride, then back-
5 extracted from the solvent phase into an aqueous ethyl phase. The ethyl analog of MeHg,
6 methylethylHg (CH₃CH₂CH₂Hg), was separated from solution by purging with N₂ onto a Tenax trap.
7 The trapped CH₃CH₂CH₂Hg was then thermally desorbed, separated from other Hg species by an
8 isothermal gas chromatography (GC) column, decomposed to Hg⁰ in a pyrolytic decomposition column
9 (800°C), and analyzed by CVAFS (Brooks Rand Model III, Brooks Rand Labs, U.S.A.) following
10 method 1630 (U.S. EPA, 2001).

11 **2.3.2 Total Hg and MeHg in water samples**

12 The analysis of Hg species in water samples was conducted within three weeks of sampling. The HgT_{unf}
13 and HgT_f concentration in water samples was quantified using dual amalgamation CVAFS (Tekran
14 2500, Tekran Inc., Toronto, Ontario, Canada) following approved methodology (U.S. EPA, 2002).
15 Samples for HgT_{unf} and HgT_f analysis were oxidized with 0.5% (v/v) BrCl (bromine chloride). Excess
16 BrCl was reduced with hydroxyl-ammonium chloride before adding SnCl₂ (stannous chloride) to
17 convert Hg²⁺ to volatile Hg⁰. The Hg⁰ was trapped by gold amalgamation (U.S. EPA, 2002). Water
18 samples were analyzed for MeHg using CVAFS (Brooks Rand Model III, Brooks Rand Labs, Seattle,
19 WA, USA) following distillation, aqueous phase ethylation, and isothermal GC separation (U.S. EPA,
20 2001).

21 **2.4 Quality control**

22 Quantification for THg and MeHg in soil and water samples was conducted using daily calibration
23 curves with the coefficient of variation (r^2) ≥ 0.99. Quality control and assurance measurements for all
24 analytes were performed using triplicates, method detection limits, field blanks, matrix spike recoveries,
25 and certified reference materials. Field blanks of water samples were 0.12 ng L⁻¹ and 0.011 ng L⁻¹ for
26 THg and MeHg. The method detection limits (3×σ) were 0.02 µg kg⁻¹ for THg and 0.002 µg kg⁻¹ for



1 MeHg in soil samples; 0.02 ng L⁻¹ for THg and 0.01 ng L⁻¹ for MeHg in water samples. The precision
2 and bias for triplicate samples were less than 7.5% for THg and MeHg analysis for both water and soil
3 samples. Recoveries for matrix spikes in water samples ranged from 88 to 108 % for THg analysis, and
4 from 86 to 113% for MeHg. The following certified reference materials were employed: Montana soil
5 (SRM-2710, National Institute of Standards and Technology), Loamy Sand 1 (CRM024-050, Resource
6 Technology Corporation), Sandy Loam 3 (CRM021-100, Resource Technology Corporation), and
7 Sediment (BCR-580, Institute for Reference Materials and Measurements). The results of the certified
8 reference material analysis are shown in Table 1.

9 Statistical analysis was performed using SPSS 13.0 software (SPSS). Mercury concentrations in
10 samples are described by the analysed mean ± standard deviation (SD) unless otherwise stated.
11 Relationships between covariant sets of data were subjected to regression analysis. Correlation
12 coefficients (*r*) and significance probabilities (*p*) were computed for the linear regression fits.
13 Differences are declared as significant for *p* < 0.05. Kolmogorov-Smirnov test (K-S) and Kruskal-Wallis
14 test (K-W) were processed for comparing the ~~significant~~ difference between ~~the~~ two or more
15 independent datasets.

16 3 Results and Discussion

17 3.1 Mercury in ambient air and precipitation

18 The average TGM concentration in ambient air over the 100 days rice season at Gouxi (403±399 ng m⁻³)
19 was significantly higher than that at Wukeng (28±13 ng m⁻³) and the regional background (6.2±3.0 ng
20 m⁻³) (Table 2). Serious Hg contamination of air was therefore observed at Gouxi during the monitoring
21 period. The elevated TGM concentration in ambient air at Gouxi compared to Wukeng and the regional
22 background area (Huaxi) is attributed to the emission of TGM from nearby artisanal Hg smelters (Meng
23 et al., 2010; Li et al., 2008, 2009).

24 During the rice growing season, the HgT_{unf} concentration in precipitation at Gouxi was highly elevated
25 (mean=2599±1874 ng L⁻¹), and 1-3 orders of magnitude higher than that recorded for Wukeng
26 (mean=445±296 ng L⁻¹) and the regional background concentration at Huaxi (mean=27±17 ng L⁻¹)
27 (Table 2). The relative concentration of Hg in precipitation between the three sites was comparable to



1 the concentration of Hg in the ambient air suggesting that elevated Hg in precipitation at Gouxi can be
 2 linked to the ongoing Hg smelting activities. Mercury in precipitation is therefore a function of the
 3 enhanced flux of both dry and wet deposition of Hg from the atmosphere.

4 The MeHg_{unf} concentration in precipitation collected from the two sites (Gouxi; $0.48 \pm 0.20 \text{ ng L}^{-1}$;
 5 Wukeng: $0.30 \pm 0.15 \text{ ng L}^{-1}$) was similar to the regional background concentration of MeHg (0.28 ± 0.14
 6 ng L^{-1}) ~~in these compartments~~ (Table 2). Furthermore, there was no difference in MeHg_{unf} concentration
 7 between the two sampling sites during the rice growing season (*K-S* test, $p > 0.05$). These results
 8 confirm previous suggestions that atmospheric deposition is responsible for the flux of inorganic Hg but
 9 not MeHg to mining areas where artisanal Hg mining is ongoing (Meng et al., 2011).

10 3.2 Mercury in irrigation water and paddy water

11 The concentration of Hg in irrigation water and paddy water across the two sampling sites is presented
 12 in Table 2. Paddy fields selected in this study were irrigated with local stream water with a high
 13 concentration of Hg due to the ~~contaminated of the streams~~ with calcines and tailings. During the rice
 14 growing season, irrigation water at Wukeng had a significantly higher HgT_{unf} (~~mean=~~ $513 \pm 215 \text{ ng L}^{-1}$)
 15 and MeHg_{unf} ($1.1 \pm 1.7 \text{ ng L}^{-1}$) concentration than at Gouxi (~~mean-~~ $\text{HgT}_{\text{unf}} = 159 \pm 67 \text{ ng L}^{-1}$; ~~mean~~
 16 $\text{MeHg}_{\text{unf}} = 0.75 \pm 0.65 \text{ ng L}^{-1}$). Mercury concentrations in irrigation water at both sites were significantly
 17 higher than the regional background ($p < 0.05$).

18 Clear differences were observed between the two sites with regard to MeHg concentration and the ratio
 19 of $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ in paddy water. The highest values of MeHg_{unf} in paddy water were all observed at
 20 Gouxi (~~mean=~~ 5.9 ± 4.4), whereas sampling at Wukeng (~~mean=~~ 0.48 ± 0.63) maintained a relatively low
 21 MeHg concentration in paddy water throughout the rice growing season ~~when compared with the~~
 22 ~~corresponding data from Gouxi~~. The ratio of MeHg to total Hg is recognized as a measure of Hg
 23 methylation efficiency (Sunderland et al., 2006). In our study, the $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ ratio was up to 11 %
 24 ($\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$) for paddy water at Gouxi and the ~~mean-~~ $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ ratio for this water
 25 compartment was significantly higher than ~~the corresponding data~~ for irrigation water (~~mean=~~ 0.71 ± 0.93)
 26 and precipitation (~~mean=~~ 0.031 ± 0.028) (Table 2). However, there was no significant difference between
 27 the $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ ratios for the various water compartments at Wukeng (*K-W* test, $p > 0.05$). These



1 results imply active net Hg methylation paddy fields at Gouxi but not at Wukeng.

2 3.3 Mercury in soil profiles

3 3.3.1 Hg in soil pore water

4 The vertical distribution of HgT_f and MeHg_f in pore water was monitored over four successive time
5 intervals during the rice growing season (Fig. 2). The distribution of pore water HgT_f as a function of
6 depth was different to pore water MeHg_f at both sampling sites. The mean concentration of HgT_f in
7 pore water samples over the 100 days rice growing season was $142 \pm 111 \text{ ng L}^{-1}$ (range: $15\text{--}460 \text{ ng L}^{-1}$) at
8 Gouxi and $180 \pm 160 \text{ ng L}^{-1}$ (range: $38\text{--}916 \text{ ng L}^{-1}$) at Wukeng. The highest concentration of HgT_f in pore
9 water was observed at the soil surface layer (top 2cm), and decreased with depth at both sampling sites.
10 The HgT_f concentration in pore water at Gouxi was consistent with respect to time, and showed no
11 significant difference between the different sampling dates (K-W test, $p > 0.05$). However, the
12 concentration of HgT_f in pore water at Wukeng was time dependent, with the highest concentration in
13 surface layer recorded at Day 20, and the lowest at Day 80.

14 The maximum concentration of MeHg_f in soil pore water (15 ng L^{-1}) was observed at sampling site of
15 Gouxi, which was approximately 2.3 times higher than the corresponding data (6.6 ng L^{-1}) at Wukeng.
16 The MeHg concentration in soil pore water collected from Gouxi was significantly higher than that for
17 Wukeng throughout the 100 days monitoring (K-S test, $p < 0.01$) suggesting different rates of net Hg
18 methylation between the Gouxi and Wukeng sites. The concentration of MeHg_f in pore water was
19 general highest in the surface soil at Gouxi, and then sharply declined from a depth of 4 cm. However,
20 the vertical distributions of MeHgT_f in soil pore water of Wukeng showed little variation, with the
21 exception of small (unexplained) peaks at 10 cm for Day 20 and at 6 cm for Day 60. The proportion of
22 pore water HgT_f that was MeHg_f ($\text{MeHg}_f/\text{HgT}_f$) ranged from 0.50 to 8.7 % (mean value of $2.6 \pm 1.7\%$)
23 and from 0.089 to 4.8 % (mean value of $1.6 \pm 1.1\%$) at Gouxi and Wukeng respectively. Regression
24 analysis revealed a significant and positive correlation between HgT_f and MeHg_f at Gouxi ($r = 0.75$,
25 $p < 0.001$, $n = 40$) but not for Wukeng ($r = 0.22$, $p = 0.17$, $n = 40$) (Fig. 3), suggesting a mechanistic
26 relationship between these two Hg species at the artisanal mining site only.



1 3.3.2 Mercury in soil cores

2 The concentration and distribution of THg as a function of depth in soil cores at Gouxi and Wukeng is
3 shown in Fig. 4. Over the rice growing season, the mean concentrations of THg in soil was 3.2 ± 0.75 mg
4 kg^{-1} (0.88 – 4.4 mg kg^{-1}) and 38 ± 4.8 mg kg^{-1} (27 – 48 mg kg^{-1}) at Gouxi and Wukeng, respectively. The
5 THg concentration in paddy soil collected from both Wukeng and Gouxi was higher than the domestic
6 environmental quality standard for paddy fields in China (0.5 mg kg^{-1}) (GB15618-2008), and
7 considered non-suitable for agricultural or residential use according to the level III criterion (1.5 mg kg^{-1})
8 in the Chinese national standard for soil environmental quality. The THg concentration in soil cores
9 showed no significant difference with depth although there was a nominal trend towards decreasing
10 concentration at Gouxi. For all depths the THg concentration in soil was elevated at Wukeng relative to
11 Gouxi, indicating a greater degree of historic contamination at Wukeng as a function of a long period of
12 commercial mining activities.

13 In contrast, the MeHg concentration in soil cores showed significant variation with depth and time (Fig.
14 4). The MeHg concentration in soil cores at Gouxi showed a maximum value at the water-soil interface
15 and decreased with depth for sampling from Day 20 to Day 80. For Day 100, however, there was no
16 recorded increase in surface MeHg concentration. We believe this observation reflects active net Hg
17 methylation in the soil surface layer at Gouxi throughout the flooded period (from Day 20 to Day 80).
18 The MeHg concentration in Wukeng soil cores showed very little variation with depth, and the MeHg
19 concentration at this site, for all depths, was significantly lower than at Gouxi (K-S test, $p < 0.001$).
20 Recorded MeHg concentrations at the top of the Gouxi soil profile varied from 0.76 ng g^{-1} to 6.2 ng g^{-1} ,
21 but remained relatively stable at Wukeng (range: 0.80 – 3.8 ng g^{-1}). Comparison of the MeHg
22 concentration and distribution patterns between the two sites supports the hypothesis of active Hg
23 methylation in the Gouxi soil only.

24 Changing redox parameters over the rice growing season may affect the process of Hg methylation.
25 Previous studies have observed that in artificially Hg-polluted soil, the bioavailability of Hg to
26 methylation can be significantly affected by the level of water saturation (Rothenberg and Feng, 2012;
27 Wang et al., 2014; Peng et al., 2012). Peng et al. (2012) specified that intermittent flooding, as opposed



1 to continuous flooding, could reduce soluble Hg concentrations and inhibit Hg methylation in the rice
2 rhizosphere, subsequently decreasing the accumulation of MeHg in rice grain. Flooded conditions
3 enhance anaerobic microbial activities and increase MeHg yields. Drying of a paddy field is an
4 important cultivation step to control rice plant tillering and increase yield. Therefore, one possible
5 reason for the considerably elevated MeHg concentrations in soil at Gouxi between Day 20 and Day 80
6 relative to Day 100 is an enhancement of Hg bioavailability under flooded conditions that stimulated
7 Hg methylation, and increased the soil MeHg concentration (Wang et al., 2014). As the paddy field
8 dried from Day 80, some degree of net MeHg degradation may have occurred. This likely contributed to
9 a decreasing trend in soil MeHg concentration during the harvest period.

10 The profile of MeHg concentration with depth for the Wukeng site indicates limited MeHg production
11 in this soil despite a significantly higher THg concentration when compared with Gouxi. The average
12 concentration of THg in soil cores collected from Gouxi was 1 order of magnitude lower than that at
13 Wukeng, whereas the MeHg concentration in soil cores at Gouxi was significantly higher than at
14 Wukeng (K-S test, $p < 0.001$) during the rice growing season. Further comparison reveals that the
15 average MeHg concentration in the surface soil layer (2 cm) at Gouxi was approximately 3 times higher
16 than that at Wukeng. To explain this apparent anomaly, differences in the source and pool of Hg at each
17 site need to be considered.

18 3.4 The relative contribution of different vectors to the surface pool of soil Hg

19 Using enriched stable isotopes, Hintelmann et al. (2002) examined the fate and relative importance of
20 newly deposited Hg in a forest ecosystem. They showed that newly deposited Hg was mainly stored in
21 the uppermost soil layer (<3 cm) after three months of simulative deposition, suggesting that a large
22 fraction of newly deposited Hg is not immediately mobile. Munthe et al. (2001) observed that Hg
23 deposited in the dissolved form is incorporated into the uppermost soil layer and is retained there.
24 Citing this evidence we propose that Hg derived from atmospheric deposition and irrigation water (with
25 a greater flux for Gouxi) was restricted to the surface soil layer throughout the rice growing season.



1 In order to access the proportional input of the various sources of Hg to paddy fields, we estimated the
 2 relative contribution of different Hg vectors (atmospheric- and irrigation-derived Hg, and **native Hg**) to
 3 the surface soil. We chose to select the uppermost 2 cm as the principle sink for new soil Hg
 4 (Hintelmann et al., 2002; Munthe et al., 2001). To avoid confusion in the naming of the various Hg
 5 pools, we refer to deposited Hg as ‘new Hg’. Mercury already present in the soil is termed ‘old Hg’,
 6 which can be either of geogenic and anthropogenic origin. The current study did not attempt to
 7 distinguish between these two sources of old Hg.

8 We assume 1) that during the flooded period of the rice growing season, the depth of overlying water
 9 remained the same, i.e. that an equilibrium existed between irrigation and water loss; and 2) that all Hg
 10 species derived from deposition or irrigation entered into the surface layer of paddy soil and there was
 11 no loss. Using these assumptions we derived the following equations to quantify the relative
 12 contribution of different sources of Hg to the surface soil layer based on the measured Hg concentration
 13 in atmospheric deposition, irrigation water, and soil:

$$14 \quad R_p = \frac{M_p \times A \times C_p \times 10^{-3}}{C_s \times W} \quad (1)$$

$$15 \quad R_w = \frac{M_w \times A \times C_w \times 10^{-3}}{C_s \times W} \quad (2)$$

$$16 \quad R_n = 1 - R_p + R_w \quad (3)$$

17 where, R_p , R_w , and R_n are the relative contribution of deposition, irrigation, and **native sources** to the Hg
 18 content of the surface soil layer, respectively; M_p is cumulative rainfall during the rice growing season
 19 (17.76 cm); M_w is the amount of irrigation water during the rice growing season (cm); C_p and C_w are the
 20 concentration of Hg (e.g. HgT_{unf} and MeHg_{unf}) in deposition and irrigation water (ng L^{-1}); C_s is the
 21 concentration of Hg species (e.g. THg and MeHg) in the surface soil layer (ng g^{-1}); W is the mass of the
 22 specific surface soil layer (g); and A is the cross-sectional area of the soil core (cm^2).

23 The amount of the irrigation water during the rice growing season can be calculated using the following
 24 equation (Lan et al., 2010):

$$25 \quad M_w + M_p = M_d + M_e + M_i + M_t + M_o \quad (4)$$



1 where, M_d is water demand by rice during the rice growing season; M_e is amount of water lost by
2 evaporation; M_i is amount of water lost by infiltration; M_t is amount of water lost water by transpiration;
3 M_o is amount of water lost by other pathways. According to published literature, values for M_d , M_e , M_i ,
4 M_t , and M_o specific to the study area are 34 cm, 40 cm, 0.45 cm, 1.4 cm, and 0.38 cm, respectively (Lan
5 et al., 2010).

6 Using equations 1-3, the relative contribution of the different sources of Hg in the soil surface layer
7 were calculated and are presented in Table 3. The MeHg content of the surface soil attributable to
8 atmospheric deposition (Gouxi=3.32 mg ha⁻¹; Wukeng=2.07 mg ha⁻¹) and irrigation (Gouxi=1.33 mg
9 ha⁻¹; Wukeng=3.02 mg ha⁻¹) was orders of magnitude smaller than the amount of native MeHg already
10 present in the paddy soil (Gouxi=508 mg ha⁻¹, Wukeng=382 mg ha⁻¹). Only 0.65% of total MeHg in the
11 surface soil layer at Gouxi and 0.54% at Wukeng was assessed as being from atmospheric deposition,
12 while 0.79 % of total MeHg at Wukeng and 0.26 % at Gouxi was attributed to irrigation. A similar low
13 contribution of atmospheric deposition at irrigation water was apparent for THg. Our calculations
14 therefore show that despite the highly elevated Hg concentration in atmospheric deposition and
15 irrigation water, the contribution of new Hg (MeHg and THg) from external sources was small because
16 of the relatively large pool of old Hg in soil (Dai et al., 2013). Therefore, we propose that the dominant
17 source of MeHg to the surface soil layer is *in situ* methylation of inorganic Hg.

18 3.5 Source and mechanism for Hg transformation in paddy field soils

19 The mean concentration of HgT_{unf} in paddy water at Wukeng was 1-2 orders of magnitude higher than
20 that at Gouxi, whereas the $MeHg_{unf}$ concentration in paddy water at Gouxi was approximately 12 times
21 higher than that at Wukeng. Furthermore, the concentration of MeHg in paddy water at Wukeng was
22 significantly higher than that in precipitation, but slightly lower than in irrigation water. The sampling
23 site for the Wukeng paddy was located next to calcine piles and the proximity of this waste had a major
24 impact on water chemistry. Both the irrigation water (pH=11±0.45) and paddy water (pH=8.6±1.3)
25 were alkaline during the rice growing season (Table 4). We propose that the alkaline conditions of the
26 irrigation and paddy water at Wukeng could restrain Hg methylation and/or stimulate MeHg
27 demethylation (Rothenberg and Feng, 2012; Ullrich et al., 2001). The concentration of $MeHg_{unf}$ in



1 paddy water at Gouxi was significantly higher than the concentration in either the precipitation or the
2 irrigation water and this implies active Hg methylation within the Gouxi rice paddy. Both paddy water
3 and irrigation water at Gouxi exhibit a pH that can be considered optimal for Hg methylation (Table 4),
4 favouring net methylation in the paddy fields (Ullrich et al., 2001).

5 During the rice growing season, HgT_{unf} in paddy water exceeded the EPA water-quality criterion of 50
6 ng L^{-1} (U.S. EPA, 2000). No regulatory criterion for MeHg exists, but Rudd (1995) suggested that
7 MeHg above a concentration of 0.1 ng L^{-1} is elevated and is likely to lead to significant MeHg
8 bioaccumulation. During the rice growing season, photo demethylation can reduce paddy water MeHg.
9 However, the MeHg concentration in both filtered and unfiltered paddy water samples at both sites
10 exceeded 0.1 ng L^{-1} (Table 2), confirming that rice paddies across the Hg mining area are an exposure
11 pathway for MeHg and may have direct implications for human and wildlife health. Previous studies
12 have indicated that vertebrates and fish cultivated in flooded rice paddies will accumulate MeHg to
13 critical threshold levels within 30 days (Ackerman et al., 2010a, 2010b). In rice paddy fields that
14 combine rice and fish cultivation, potential co-exposure of MeHg through rice and fish consumption
15 should receive more attention (Qiu et al., 2008; Feng et al., 2008; Lansing and Kremer, 2011).


16 Our finding that MeHg concentrations in surface soil at Gouxi were much higher than those at Wukeng
17 indicate that newly deposited mercury can be expected to rapidly methylate after deposition. The peak
18 concentration of MeHg in paddy soil at Gouxi, was at the soil-water interface and decreased with depth.
19 We believe that restricted supply of newly deposited Hg to depths below the soil-water interface is a
20 plausible explanation for the sharply reducing concentration of MeHg with depth at Gouxi; newly
21 deposited Hg is constrained to surface soil and cannot be transferred to lower depth. However,
22 stimulated MeHg production due to favorable geochemical conditions (pH) at Gouxi, while likely of
23 lesser importance, cannot be excluded.

24 The Wukeng site has received significant historic Hg deposition as a function of large scale mining, but
25 is not currently receiving significant inputs of fresh Hg. Atmosphere-derived mercury is physically
26 unstable and bioavailable when it first enters the rice paddy (Hintelmann et al., 2002; Schuster, 2011).
27 Immediate reactions of this new Hg with soil constituents are governed by adsorption-desorption
28 interactions with soil surfaces (Schuster, 1991) which favour the retention of Hg in the surface layers of



1 the soil profile. Over time this newly deposited Hg will be transformed into more stable, less available
2 forms (Schuster, 1991), and the net methylation potential of this Hg will consequently decrease. The
3 relatively low MeHg concentration in soil at Wukeng is indicative of old Hg which has become tightly
4 bound to soil complexes over time, and is unavailable for methylation (Hintelmann et al., 2002). Our
5 data indicates that the ~~concentration of THg~~ concentration of Hg in soil is not a reliable indicator of Hg methylation
6 potential in soil. Instead the concentration of bioavailable or new Hg must be considered, in agreement
7 with the findings of Meng et al. (2010, 2011)

8 **3.6 Implications of this work to environmental risk assessment**

9 Elevated MeHg concentrations combined with an elevated MeHg% in surface soil ~~in this work infer~~
10 active Hg methylation processes are occurring in Gouxu rice paddy soil. The ~~rate of~~ Hg methylation is a
11 function of an elevated ~~concentration of Hg~~ concentration of Hg in atmosphere and the low pH of irrigation water/paddy
12 water. The absence of depth-dependent variability in the MeHg concentration in soil cores at Wukeng is
13 consistent with the low concentration of Hg in ambient air and corresponding atmospheric deposition,
14 and high pH of the paddy water/irrigation water. The *in situ* production of MeHg in Wukeng soil,
15 despite the elevated concentration of THg  low. Our results demonstrate that soil is the primary
16 source of MeHg for paddy rice, and elevated MeHg in rice pose a potential threat to wildlife and local
17 residents. Mercury in surface soil that has been derived from atmospheric deposition is susceptible to
18 methylation in the rice paddy ecosystem immediately after deposition. Consequently, net MeHg
19 production is principally governed by the supply of fresh deposited Hg to soil and the pH conditions
20 therein. The relationship between MeHg and fresh deposited Hg implies that the ~~concentration of Hg~~
21 concentration of Hg in ambient air could be used as a monitoring tool to assess the relative risk of MeHg production in the rice
22 paddy environment, and the possible risk to human health that may be associated with the accumulation
23 of this MeHg in rice grain.

24 **Acknowledgements**



1 This research was financed by the National Key Basic Research Program of China (973 Program
2 2013CB430004), financed by natural science foundation of China (41173126, 41573135, 41203091,
3 and 41473123), and also financed by natural science foundation of Guizhou (2012-2333).

4



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11



1 Table 1. List of certified reference materials used in the present study and corresponding analytical
2 result.

Producer	CRM	<i>n</i>	Hg speciation	Obtained value	Certified value
NIST	SRM-2710	10	THg (mg kg ⁻¹)	32.4±0.7	32.6±1.8
RTC	CRM024-050	10	THg (mg kg ⁻¹)	0.70±0.02	0.71
RTC	CRM021-100	10	THg (mg kg ⁻¹)	4.73±0.15	4.7
IRMM*	BCR-580	20	MeHg (mg kg ⁻¹)	0.070±0.007	0.075±0.004

3 IRMM: Institute for Reference Materials and Measurements

4 NIST: National Institute of Standards and Technology.

5 RTC: Resource Technology Corporation

6



1 **Table 2.** Hg in ambient air, precipitation, irrigation water, and overlying water at artisanal Hg mining
 2 site, abandoned Hg mining site, and regional background of Huaxi (mean±SD)

Sample matrix	Hg species	Artisanal Hg mining site (Gouxu)	Abandoned Hg mining Site (Wukeng)	Regional background area of Huaxi ²
Ambient air ¹	Hg ⁰ (ng m ⁻³)	403±388	28±13	6.2±3.0
Precipitation ¹	HgT _{unf} (ng L ⁻¹) ³	2599±1874	445±296	27±17
	MeHg _{unf} (ng L ⁻¹) ³	0.48±0.20	0.30±0.15	0.28±0.14
	MeHg _{unf} /HgT _{unf} (%)	0.031±0.028	0.16±0.20	0.76±0.41
Irrigation ¹ water	HgT _{unf} (ng L ⁻¹)	159±67	513±215	7.1±4.0
	HgT _f (ng L ⁻¹) ³	39±9.4	195±45	
	MeHg _{unf} (ng L ⁻¹)	0.75±0.65	1.7±1.1	0.14±0.044
	MeHg _f (ng L ⁻¹) ³	0.31±0.30	0.96±0.50	
Paddy water ¹	MeHg _{unf} /HgT _{unf} (%)	0.71±0.93	0.45±0.53	2.2±0.98
	HgT _{unf} (ng L ⁻¹)	189±117	430±279	7.5±4.3
	HgT _f (ng L ⁻¹)	105±58	196±78	
	MeHg _{unf} (ng L ⁻¹)	13±16	1.1±0.52	0.71±0.66
	MeHg _f (ng L ⁻¹)	4.7±4.2	0.62±0.29	
	MeHg _{unf} /HgT _{unf} (%)	5.9±4.4	0.48±0.63	10±7.9

3 ¹ Hg species concentrations in ambient air and precipitation were averaged with data sets of five
 4 sampling campaigns at Days 20, 40, 60, 80 100; Hg species concentrations in irrigation water and
 5 paddy water were averaged with data sets of four sampling campaigns at Days 20, 40, 60, 80.

6 ² data were obtained from Zheng, (207), Meng et al. (2010) and Meng (2011).

7 ³HgT_{unf}, unfiltered total mercury; HgT_f, filtered total mercury; MeHg_{unf}, unfiltered methylmercury; MeHg_f,
 8 filtered methylmercury;

9



- 1 **Table 3.** Contribution of Hg species derived from deposition and irrigation water to the surface soil
 2 layer at an artisanal Hg mining site and abandoned Hg mining site.

Sampling sites	Hg species in surface soil layer (0-2 cm)	Irrigation water (%)	Atmospheric deposition (%)	Native soil pool (%)
Gouxi	THg	0.06	3.7	96.24
	MeHg	0.26	0.65	99.1
Wukeng	THg	0.014	0.049	99.9
	MeHg	0.79	0.54	98.67

3

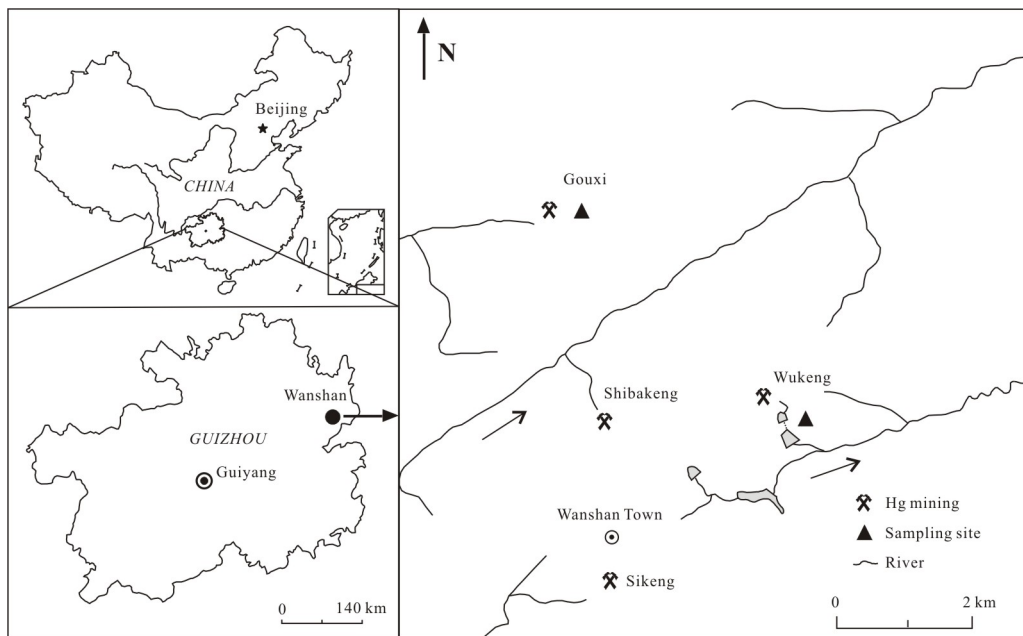
4



- 1 Table 4. ~~General Water Quality Characteristics of irrigation water and overlying~~ water at the artisanal
 2 Hg mining site and abandoned Hg mining site (mean±SD, range)

Sample matrix	Parameters	Artisanal Hg mining site (Gouxu)	Abandoned Hg mining Site (Wukeng)
Irrigation water (ng L ⁻¹)	T (°C)	24±1.7 (23-26)	25±2.1 (23-27)
	pH	8.3±0.24 (8.1-8.6)	11±0.45 (11-12)
	DO (mg L ⁻¹)	7.4±0.43 (6.9-8.0)	7.4±0.56 (6.8-8.1)
Paddy water	T (°C)	28±4.4 (24-33)	25±2.7 (23-29)
	pH	7.2±0.24 (6.9-7.4)	8.6±1.3 (7.3-9.8)
	DO (mg L ⁻¹)	3.0±0.95 (1.8-3.9)	4.4±0.73 (3.6-5.2)

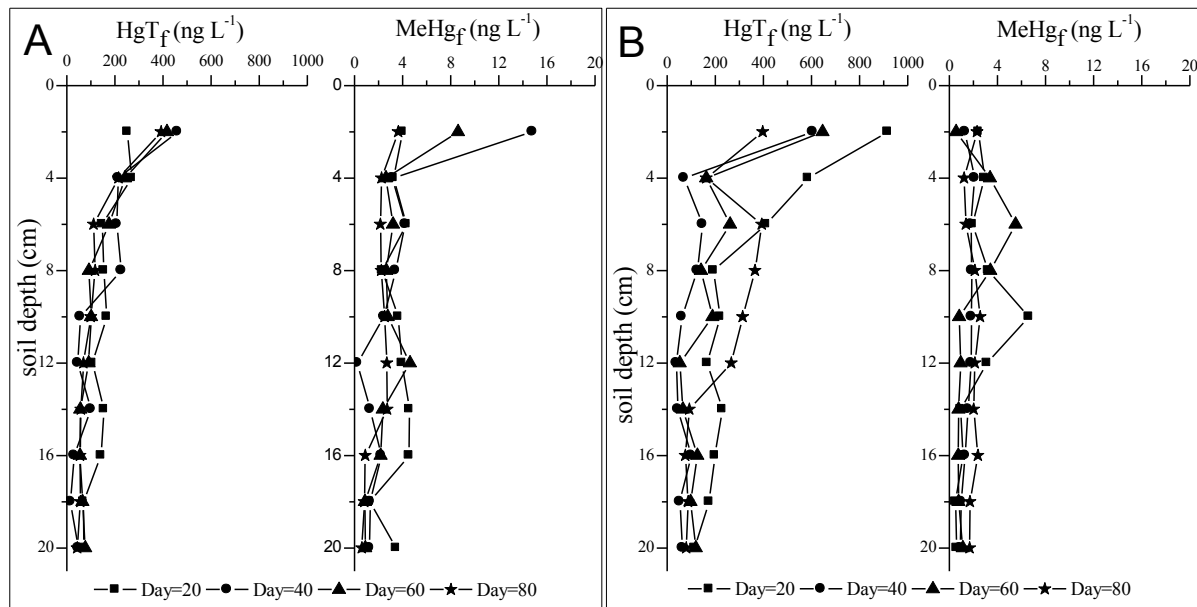
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1

2 Figure 1. Map of the study area and sampling sites including abandoned Hg mining site (Wukeng) and
3 artisanal Hg mining site (Gouxi).

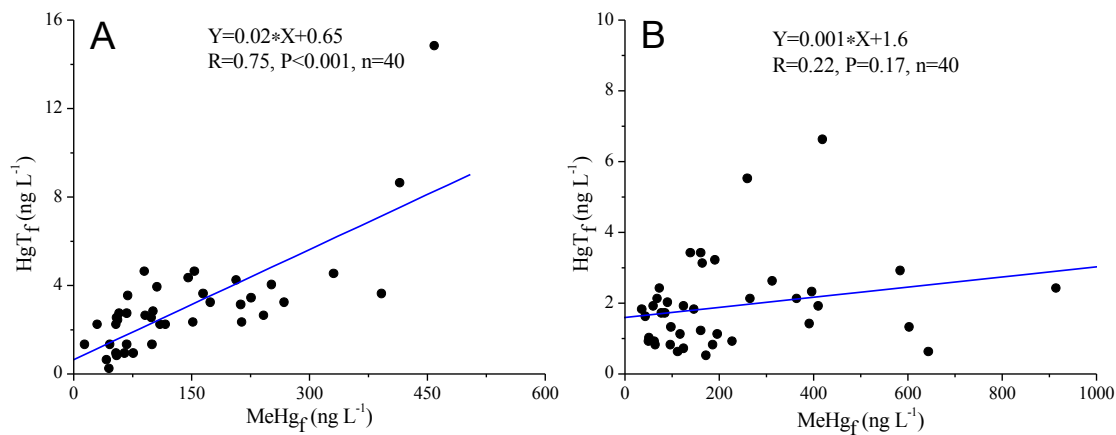
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1

2 Fig 2. Concentration of HgT_f and $MeHg_f$ in pore water during the rice growing season on Days 20,
 3 40, 60, and 80 (A: artisanal Hg mining site of Gouxu; B: abandoned Hg mining site of Wukeng).

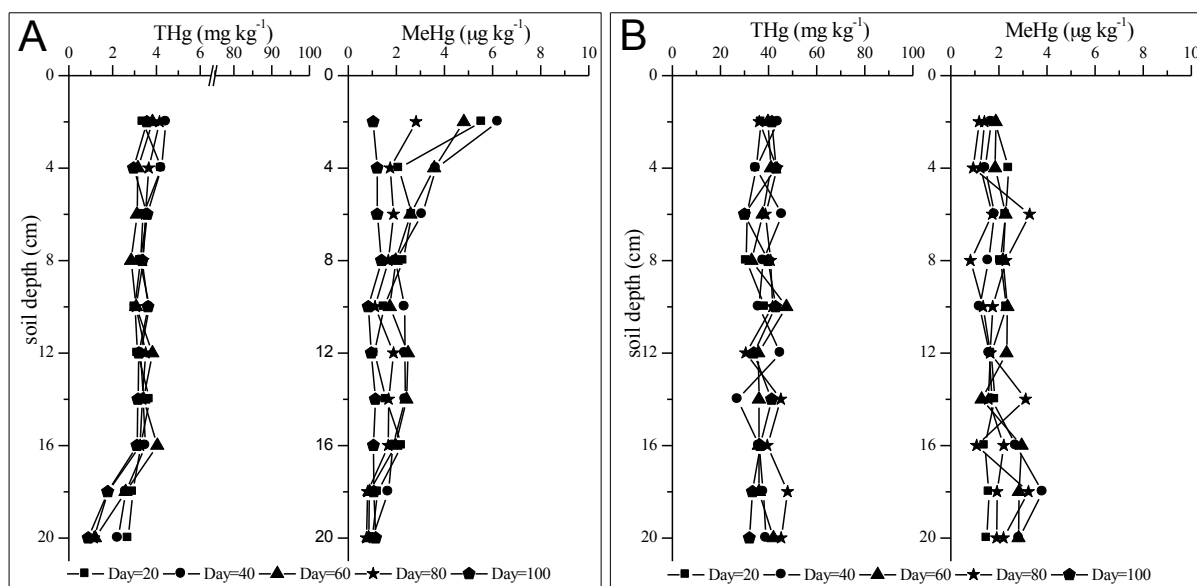
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1

2 Figure 3. Correlation between HgT_f and MeHg_f concentrations in soil pore water during the rice
3 growing season on Days 20, 40, 60, and 80 (A: artisanal Hg mining site of Gouxi; B: abandoned Hg
4 mining site of Wukeng).

5



1
2 Figure 4. Concentration of THg and MeHg in soil cores during the rice growing season on Days 20, 40,
3 60, 80, and 100 (A: artisanal Hg mining site of Gouxi; B: abandoned Hg mining site of Wukeng).