

Response to comments from reviewers 1

General comment:

The authors have done an excellent job responding to the critiques. The addition of microbial and biogeochemical explanations have greatly improved this manuscript.

On behalf of my co-authors, I sincerely thank the anonymous reviewer for dedicating their time to provide further comments and criticism. My co-authors and I have made appropriate changes to the manuscript. Revisions are shown in red color in the revised manuscript, with our point-by-point response presented as follows.

My general concern is that the model presented in Section 3.4 is still unnecessary. The authors' response to my comments and Reviewer #2 comments did not adequately justify its need and only present a simplified model. I argue it is not needed at all. The authors should consider removing the model or describe how Section 3.4 provides insight not provided by other sections.

Yes, the authors agree with the reviewer's comment here. After carefully considering the comments herein in combination with those of Reviewer 2, the model presented in Section 3.4 (The relative mercury flux of different vectors to the soil Hg pool) was deleted in the revised manuscript.

Lastly, I believe the authors should end Section 3.6 with a few concluding sentences about future work that may/should be done.

Yes, we re-organized the Section 3.6 and a briefly concluding sentences concerning future work was shown in the revised manuscript as follows:

The relationship between MeHg and fresh deposited Hg implies that the concentration of Hg in ambient air could be used as a monitoring tool to assess the relative risk of MeHg production in the rice paddy environment, and the possible risk to human health that may be associated with the

1 accumulation of this MeHg in rice grain. However, we cannot distinguish between newly deposited
2 Hg and old Hg stored in paddy soil over decades and ongoing research is necessary to continue to
3 develop an improved understanding of Hg dynamics in rice paddy soils. When comparing relative
4 risk between different vectors for Hg contamination (i.e. small-scale or historic large-scale mining),
5 quantification of the pool of Hg available for methylation is critical to estimating reliable
6 methylation rates. Ongoing work is urgently needed to further ascertain the relative importance of
7 newly deposited Hg versus in situ Hg to the bioavailable pool of Hg that can be methylated in rice
8 paddy ecosystems. (see page18 lines 9-18)

9

10 Technical corrections:

11 Page 2, Line 22: Change "a" to "an".

12

13 **Yes, "a" was changed to "an" in the revised manuscript. (page 2 line 22)**

14

15 Page 14, Line 12: Change "As the paddy field dried from Day 80," to "As the paddy field dried
16 beginning on Day 80,". The phrase "from Day 80" is ambiguous.

17

18 **Yes, we re-organized this sentence as follows: "As the paddy field dried beginning on Day 80..."**
19 **in the revised manuscript. (page 14 line 12)**

20

Response to comments from reviewers 2

The authors have made a substantial effort in the revision and the manuscript has greatly improved. I have only a few minor comments.

The authors are most thankful the reviewer for dedicating time to review this manuscript and provide further comments and criticism, which will definitely improve this manuscript. We have completed a substantial revision and our changes are highlighted in red color in the revised manuscript. Our point-by-point response to the reviewer's comments is as follows:

p.14, l.12: "from Day 80 on"

Yes, we re-worked this sentence in the revised manuscript as follows: "As the paddy field dried beginning on Day 80....." (see page 14 line 12)

p.15, l.13-17: Fp, Fw in the equations but Rp, Rw in the text.

p.16, l.5: cf. previous comment on the fraction of transpiration vs. evaporation in total ET. For example, Hossen et al. (2012) reported 64-70% of transpiration in total ET for two rice species in Bangladesh. It is very difficult for me to agree on the numbers $M_e = 40$ cm and $M_t = 1.4$ cm (even though the influence on the model output is null because these values are added.) Please clarify, or update the values. Lan et al. 2010 is in Chinese, which I cannot read, so I cannot refer to their paper to understand more how this water balance was obtained.

We agree with the reviewer's comments here. After carefully considering Reviewer 1's comments in combination with those of Reviewer 2, the model presented in Section 3.4 (The relative mercury flux of different vectors to the soil Hg pool) was deleted in the revised manuscript.

1 p.18, l.6: rephrase for ex. "Active Hg methylation within the Gouxi rice paddy is implied in the present
2 study, even though data directly supporting this hypothesis are lacking. Further work may help to bring
3 more confidence on that particular point."
4

5 **Yes, we re-organized these sentences as follows: "Active Hg methylation within the Gouxi rice**
6 **paddy is implied in the present study, even though data directly supporting this hypothesis are**
7 **lacking. Further work may help to bring more confidence on that particular point" (page 16 line 1-3)**
8

9 Figure 4 caption "season on" (space)
10

11 **Yes, a space was added in the revised manuscript (page 32 line 2)**
12

13 Table 4 and various places in the text: I suggest to change everywhere "native Hg" to "old Hg" to be
14 consistent throughout the manuscript.
15

16 **Yes, "native Hg" was changed to "old Hg" throughout the manuscript. (page 1 line 22, page 17**
17 **line 4, page 17 line 24)**

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

3

4 L. Zhao^{1,2}, C.W.N. Anderson³, G.L. Qiu², B. Meng², D.Y. Wang¹, and X. B. Feng²

5

6 ¹College of Resources Environment, Southwest University, Chongqing 400716, P. R. China

7 ²State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of
8 Sciences, Guiyang 550002, P.R. China

9 ³Soil and Earth Sciences, Institute of Agriculture and Environment, Massey University, Palmerston
10 North, New Zealand

11 Correspondence to: X. B. Feng (fengxinbin@vip.skleg.cn) and B. Meng (mengbo@vip.skleg.cn)

12

13 **Abstract.**

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

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

10 **1 Introduction**

11 Reports of methyl mercury (MeHg) contamination of rice grain (*Oryza sativa*) have recently focussed
12 scientific attention on this important agricultural crop (Qiu et al., 2008; Zhang et al., 2010a; Meng et al.,
13 2010, 2011, 2014). Numerous studies have reported high MeHg concentrations in rice grain collected
14 from Indonesia (Krisnayanti et al., 2012) and different parts of China (Horvat et al., 2003; Qiu et al.,
15 2008; Meng et al., 2014). The MeHg concentration in rice grain (brown rice) can be enhanced even in
16 cases where soil is not significantly elevated in Hg (Zhang et al., 2010a; Horvat et al., 2003). Meng et al.
17 (2014) specified that the majority (~80%) of MeHg was found in edible white rice. A common theme to
18 these studies is the presence of a high Hg flux into the environment through mining or other industrial
19 contamination that discharges into the atmosphere.

20 Rice paddy plantation is one of the most prevalent land uses in South and East Asia where rice is the
21 dominant foodstuff (FAO, 2002). Rice throughout Asia is generally cultivated in paddy soil and this
22 ephemeral wetland is known to be an environment for Hg methylation. Current understanding is that the
23 mobility and methylation of Hg in ephemeral flooded soil is determined by a range of factors, such as
24 redox potential, pH, dissolved organic carbon, sulfur, iron, and dissolved Hg content (e.g., Ullrich et al.,
25 2001; Benoit et al., 2001). Mercury methylation is largely facilitated by a subset of sulfate-reducing
26 bacteria (SRB) (Gilmour et al., 1992) and/or iron-reducing bacteria (Fleming et al., 2006) in anoxic
27 conditions. Specially, the methylation of inorganic Hg (IHg) in paddy soil primarily occurs through a

1 process mediated by sulfate-reducing bacteria (Peng et al., 2012; Rothenberg and Feng, 2012; Wang et
2 al., 2014; Liu et al., 2014; Liu et al., 2009; Somenahally et al., 2011). MeHg accumulated throughout a
3 rice plant during the growing season can be readily translocated to grain during rice-seed ripening
4 (Meng et al., 2011). Rice paddy fields have therefore been identified as important sources of MeHg in
5 the terrestrial ecosystem (Meng et al., 2010, 2011), and a primary vector for human exposure to MeHg
6 in Hg mining areas (Feng et al., 2008; Zhang et al., 2010b).

7 The general consensus among Hg researchers is that soil is the principle source of MeHg in rice plants,
8 whereas Hg from the ambient air is the principal source of IHg in rice grain (Meng et al., 2010, 2011,
9 2012, 2014; Qiu et al., 2012; Yin et al., 2013). Recently, Meng et al. (2010, 2011) suggested that newly
10 deposited Hg is more readily transformed to MeHg and accumulated in rice plants than Hg forms with
11 an extended residence time in mining-contaminated soil. Meng et al. (2010) focused on the Wanshan
12 area of China, a region of both historical large-scale and current small-scale mercury mining and showed
13 that the MeHg concentration in rice grain collected from an active artisanal Hg mining areas (32 ± 14 ng
14 g^{-1}) was significantly higher than in rice grain collected from an abandoned Hg mining area (7.0 ± 3.2 ng
15 g^{-1}). Such studies on MeHg and rice emphasize that factors which control the biochemical cycling of
16 Hg within rice paddy ecosystems are very complex, and include the concentration and distribution of
17 Hg in ambient air, wet/dry deposition, irrigation water, and the solid and liquid phases of soil. These
18 factors in turn impact the absorption, transportation, and accumulation of Hg in rice plants (Meng et al.,
19 2010, 2011, 2012, 2014; Rothenberg and Feng, 2012; Liu et al., 2012; Wang et al., 2014; Peng et al.,
20 2012).

21 While the source, distribution, and accumulation of IHg and MeHg in rice plants has been reported, no
22 study has presented results from a systemic survey of the concentration of Hg in the various
23 physiochemical fractions of the rice paddy ecosystem. The biochemical processes that control the
24 cycling of Hg in paddy soils impacted by Hg mining are poorly understood. The objectives of the
25 current study were therefore to 1) investigate the speciation and distribution of Hg in paddy soil, and 2)
26 assess the primary source and mechanism for Hg methylation within a Hg mining area. Documenting
27 Hg cycling in rice paddy ecosystems within Hg mining areas is an important step towards better
28 assessing potential health threats that may be associated with rice cultivation in a Hg-contaminated

1 environment and is necessary to mitigate the risk of MeHg formation in paddy soils used for rice
2 cultivation in Hg contaminated areas. Better understanding of the distribution of Hg species in paddy
3 soils within a Hg mining area is necessary to underpin more reliable risk assessment and appropriate
4 strategies to remediate contaminated soil.

5 **2 Materials and methods**

6 **2.1 Site description**

7 This study was conducted in the Wanshan Hg mining district (E: 109°07'~109°24', N: 27°24'~27°38'),
8 Guizhou province, Southwest China, where historical large-scale Hg smelting combined with current
9 artisanal Hg smelting activities have resulted in Hg contamination of ambient air, water, soil, sediment,
10 and biota (Qiu et al., 2005; Li et al., 2008, 2009). Two typical Hg contaminated sites within the
11 Wanshan Hg mining district were selected for this study: an artisanal Hg mining site (Gouxu); and an
12 abandoned Hg mining site (Wukeng) (Fig. 1). The sampling sites of Gouxu and Wukeng are situated
13 within the Wanshan district which experiences subtropical monsoon-type climate with an average
14 annual rainfall of 1200-1400 mm y⁻¹ and a perennial mean temperature of 17 °C. Historical Hg mining
15 activities in the Wanshan area can be dated back to the Qin Dynasty (221 B.C.) but large-scale mining
16 activities officially ceased in 2001. Mining activity across Wanshan generated an estimated cumulative
17 1.0×10⁸ tons of calcine and waste rock between 1949 and the 1990s. Recently, illegal artisanal Hg and
18 small-scale mining activities have been revived due to an increase in the global Hg price and domestic
19 demand.

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

1 Paddy field is the primary agricultural land use at both Gouxu and Wukeng. Field sampling for the
2 current research focused on two 10×10 m plots (one at each site) within rice paddies that were
3 established according to the following methodology: The rice paddies were flooded on 10th May; rice
4 seedlings (hybrid rice) widely grown throughout Guizhou province were transplanted into the
5 submerged soil 20 days after flooding (1 plant each 25×25 cm area on 1st June, defined as Day 0).
6 Thereafter, the two experimental plots were cultivated during the period 1st June through 10th September
7 (100 days) 2012. Standing water (2-8 cm) was maintained above the soil surface (flooded condition)
8 throughout the growing period, from Day 0 to Day 80. The paddy fields were thereafter drained from
9 Day 80, prior to harvest between Days 90 to 100. During the 10 day draining period, approximately 2-4
10 cm depth of water was maintained above the soil surface. The paddy plots received water through
11 precipitation and stream water irrigation, while evaporation to air and seepage to the subsoil were the
12 primary vectors for water loss. There was no direct runoff from either paddy.

13 **2.2 Sample collection and preparation**

14 Five consecutive sampling campaigns were conducted during the rice growing season (1st June-10th
15 September, 2012). The first sampling was initiated 20 days after the plants were planted out (20th June,
16 2012; Day 20), and thereafter samples were collected on Days 40, 60, 80, and 100 (Day 100 was 10th
17 September, 2012; final harvest). The Hg concentration in ambient air was measured at each sampling
18 time, and samples of cumulative deposition (wet and dry), irrigation water, paddy water, and soil cores
19 were also collected each time. It should be noted that current study focused on the speciation
20 and distribution of Hg in the paddy soil during the rice growing season. Rice plant samples were not,
21 however, collected as part of this study. The paddy fields were dry from Day 90, and therefore irrigation
22 water, paddy water, and soil pore water samples were not collected on Day 100.

23 **2.2.1 Mercury in ambient air and wet/dry deposition**

24 The concentration of total gaseous mercury (TGM, Hg⁰) in ambient air at both Gouxu and Wukeng was
25 measured in the field at each sampling time using an automated Hg vapor analyzer (LUMEX, RA-915⁺,
26 Ohio Lumex Co., Twinsburg, OH) with detection limit of 2 ng m⁻³. The average Hg⁰ concentration

1 during a 10 s interval was quantified and stored in a portable computer. Measurements were carried out
2 continuously for at least one hour. For each sampling interval 360 data points were collected at each
3 location.

4 An integrated bulk precipitation sampler based on the design of Guo et al. (2008) was used in the field
5 to quantify the concentration of Hg in cumulative precipitation (Oslo and Paris, 1998). Both dry and wet
6 atmospheric deposition were collected concurrently with the TGM measurement once every 20 days
7 using this sampling method. Samples collected at each site were poured into two 100 mL pre-cleaned
8 borosilicate glass bottles for direct and unfiltered total Hg (HgT_{unf}) and total MeHg (MeHg_{unf})
9 analysis. Filtered samples were collected on site using a 0.45 μm disposable polycarbonate filter unit
10 (Millipore), and subsequently analyzed for dissolved total Hg concentrations (HgT_{f}) and dissolved
11 MeHg concentration (MeHg_{f})

12 **2.2.2 Irrigation water and paddy water**

13 Samples of irrigation water at both Gouxu and Wukeng were collected at rice paddy inlets on Days 20,
14 40, 60, and 80. All water samples were collected by hand using ultra-clean handling protocols and
15 stored in acid-cleaned borosilicate glass bottles. Each bottle was rinsed three times with irrigation water
16 on site before sample collection. Filtered samples were collected on site through a 0.45 μm disposable
17 nitrocellulose filter unit (Millipore) HgT_{f} and MeHg_{f} analysis. In addition, unfiltered irrigation water
18 samples were siphoned into pre-cleaned borosilicate glass bottles using a disposable syringe and
19 analyzed for total Hg (HgT_{unf}) and total MeHg (MeHg_{unf}).

20 Paddy water (overlying water) and corresponding soil pore water samples at both Wukeng and Gouxu
21 were collected at the centre of the two plots on Days 20, 40, 60, and 80, simultaneously to the irrigation
22 water collection. Firstly, an undisturbed soil core was collected at each sampling site by pushing a pre-
23 cleaned 6cm diameter polycarbonate core tube into the soil to approximately 20 cm depth. The paddy
24 water (0-8 cm above the soil surface) in the core tube was siphoned into a 200 ml pre-cleaned
25 borosilicate glass bottles. One aliquot of the paddy water was then filtered into a 100 ml pre-cleaned
26 borosilicate glass bottle using a 0.45 μm disposable polycarbonate filter unit (Millipore), and

1 subsequently analyzed for HgT_f and MeHg_f . A second aliquot of paddy water for HgT_{unf} and MeHg_{unf}
2 analysis was immediately transferred into another 100 ml pre-cleaned borosilicate glass bottle.
3 General Water Quality Characteristics of irrigation water and paddy water including pH, dissolved
4 oxygen (DO) concentration, and temperature (T) were measured *in situ* using a portable analyzer. All
5 water samples were promptly acidified on site to 0.5% (v/v) using adequate volumes of ultra-pure
6 concentrated hydrochloric acid (HCl). The sample bottles were then capped, sealed with Parafilm[®],
7 double-bagged, transported to the laboratory in an ice-cooled container to the lab within 24 h. Prior to
8 Hg analysis, samples were stored in a refrigerator at +4 °C in the dark.

9 **2.2.3 Soil pore water (liquid phase) and soil core (liquid phase+solid phase)**

10 The soil cores were immediately sliced on site into 2 cm intervals using a plastic cutter in an oxygen-
11 free glove box under argon. Firstly, the air (oxygen) in the glove bag was eliminated manually. Then, the
12 pure argon from a portable argon tank was injected into the glove bag through a Teflon tubing. The soil
13 samples were placed in acid-cleaned 50-ml plastic centrifuge tubes, capped and sealed with Parafilm[®].
14 All samples were transported in an ice-cooled container to the lab within 24 h and stored at 3-4 °C for
15 further laboratory processes. Following centrifugation (30 min, 3000 r min⁻¹, and 5 °C), the samples
16 were returned to the glove box where the pore water was then filtered through 0.45 µm disposable
17 nitrocellulose filter unit (Millipore). The filtrate was stored in borosilicate glass bottles and divided for
18 HgT_f and MeHg_f analysis. The water content of soil cores was estimated by weight loss.

19 At each sampling time (Days 0, 20, 40, 60, and 80) a second soil core was collected and immediately
20 placed into liquid nitrogen. This second set of soil cores was transported in a liquid nitrogen-iced
21 container to the lab within 24 h and then sliced at 2 cm intervals. The sliced soil cores were then freeze-
22 dried, prior to homogenisation to 200 mesh with a mortar and pestle for analysis of total Hg (THg) and
23 MeHg. The concentration of each Hg species in this second set of soil cores is therefore the sum of both
24 liquid and solid phase. Precautions were taken to avoid cross-contamination during sample processing;
25 the mortar and pestle were thoroughly cleaned after each sample with drinking water followed by
26 deionized water rinses. The powdered samples were subsequently packed into plastic dishes, sealed in
27 polyethylene bags and stored in a refrigerator within desiccators for subsequent laboratory analysis.

1 **2.3 Sample analysis**

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

4 **2.3.1 Total Hg and MeHg in soil samples**

5 For THg analysis, a soil sample (0.1-0.2 g) was digested using a fresh mixture of HCl and HNO₃ (1:3,
6 v/v). THg was determined by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran 2500,
7 Tekran Instruments) preceded by BrCl oxidation, SnCl₂ reduction, pre-concentration, and thermo-
8 reduction to Hg⁰ (U.S. EPA, 2002).

9 For MeHg analysis, a soil sample (0.3-0.4 g) was prepared using the CuSO₄-methanol/solvent
10 extraction (Liang et al., 1996). MeHg in samples was extracted with methylene chloride, then back-
11 extracted from the solvent phase into an aqueous ethyl phase. The ethyl analog of MeHg,
12 methylethylHg (CH₃CH₃CH₂Hg), was separated from solution by purging with N₂ onto a Tenax trap.
13 The trapped CH₃CH₃CH₂Hg was then thermally desorbed, separated from other Hg species by an
14 isothermal gas chromatography (GC) column, decomposed to Hg⁰ in a pyrolytic decomposition column
15 (800°C), and analyzed by CVAFS (Brooks Rand Model III, Brooks Rand Labs, U.S.A.) following
16 EPAMethod 1630 (U.S. EPA, 2001).

17 **2.3.2 Total Hg and MeHg in water samples**

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

1 **2.4 Quality control**

2 Quantification for THg and MeHg in soil and water samples was conducted using daily calibration
3 curves with the coefficient of variation (r^2) ≥ 0.99 . Quality control and assurance measurements for all
4 analytes were performed using triplicates, method detection limits, field blanks, matrix spike recoveries,
5 and certified reference materials. Field blanks of water samples were 0.12 ng L^{-1} and 0.011 ng L^{-1} for
6 THg and MeHg, respectively. The method detection limits ($3 \times \sigma$) were $0.02 \text{ } \mu\text{g kg}^{-1}$ for THg and 0.002
7 $\mu\text{g kg}^{-1}$ for MeHg in soil samples; 0.02 ng L^{-1} for THg and 0.01 ng L^{-1} for MeHg in water samples,
8 respectively. The variability between the triplicate samples was less than 7.5% for THg and MeHg
9 analysis for both water and soil samples. Recoveries for matrix spikes in water samples ranged from 88
10 to 108 % for THg analysis, and from 86 to 113% for MeHg. The following certified reference materials
11 were employed: Montana soil (SRM-2710, National Institute of Standards and Technology), Loamy
12 Sand 1 (CRM024-050, Resource Technology Corporation), Sandy Loam 3 (CRM021-100, Resource
13 Technology Corporation), and Sediment (BCR-580, Institute for Reference Materials and
14 Measurements). The results of the certified reference material analysis are shown in Table 1.
15 Statistical analysis was performed using SPSS 13.0 software (SPSS). Mercury concentrations in
16 samples are described by the analysed mean \pm standard deviation (SD) unless otherwise stated.
17 Relationships between covariant sets of data were subjected to regression analysis. Correlation
18 coefficients (r) and significance probabilities (p) were computed for the linear regression fits.
19 Differences are declared significant for $p < 0.05$. Kolmogorov-Smirnov (K-S) and Kruskal-Wallis (K-W)
20 tests were processed for comparing the differences between the two or more independent datasets (non-
21 parametric tests).

22 **3 Results and Discussion**

23 **3.1 Mercury in ambient air and precipitation**

24 The average TGM concentration in ambient air over the 100 day rice season at Gouxi ($403 \pm 399 \text{ ng m}^{-3}$)
25 was significantly higher than that at Wukeng ($28 \pm 13 \text{ ng m}^{-3}$) and the regional background ($6.2 \pm 3.0 \text{ ng}$
26 m^{-3}) (Table 2). Serious Hg contamination of air was therefore observed at Gouxi during the monitoring

1 period. The elevated TGM concentration in ambient air at Gouxi compared to Wukeng and the regional
2 background area (Huaxi) is attributed to the emission of gaseous Hg^0 from nearby artisanal Hg smelters
3 (Meng et al., 2010; Li et al., 2008, 2009).

4 During the rice growing season, the HgT_{unf} concentration in precipitation (wet and dry deposition) at
5 Gouxi was elevated (mean= 2599 ± 1874 ng L^{-1}), and 1-3 orders of magnitude higher than that recorded
6 for Wukeng (mean= 445 ± 296 ng L^{-1}) and the regional background measured at Huaxi (mean= 27 ± 17 ng
7 L^{-1}) (Table 2). The relative concentration of Hg in precipitation between the three sites was comparable
8 to the concentration of Hg in the ambient air suggesting that elevated Hg in precipitation at Gouxi can
9 be linked to the ongoing Hg smelting activities. Mercury in precipitation is therefore a function of the
10 enhanced flux of both dry and wet deposition of Hg from the atmosphere.

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

17 **3.2 Mercury in irrigation water and paddy water**

18 The concentration of Hg in irrigation water and paddy water across the two sampling sites is presented
19 in Table 3. Paddy fields selected in this study were irrigated with local stream water with a high
20 concentration of Hg due to contamination of streams with calcines and tailings. During the rice growing
21 season, irrigation water at Wukeng had a significantly higher HgT_{unf} (513 ± 215 ng L^{-1}) and MeHg_{unf}
22 (1.7 ± 1.1 ng L^{-1}) concentration than at Gouxi ($\text{HgT}_{\text{unf}} = 159 \pm 67$ ng L^{-1} ; $\text{MeHg}_{\text{unf}} = 0.75 \pm 0.65$ ng L^{-1}).
23 Mercury concentrations in irrigation water at both sites were significantly higher than the regional
24 background ($p < 0.05$).

25 Clear differences were observed between the two sites with regard to MeHg concentration and the ratio
26 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
27 Gouxi (13 ± 16 ng L^{-1}), whereas samples from Wukeng (1.1 ± 0.52 ng L^{-1}) maintained a relatively low

1 MeHg concentration in paddy water throughout the rice growing season. The ratio of MeHg to total Hg
2 is recognized as a measure of Hg methylation efficiency (Sunderland et al., 2006). In our study, the
3 $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ ratio was up to 11 % ($\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$) for paddy water at Gouxi and the mean ratio for
4 this water compartment was significantly higher than for irrigation water ($0.71\pm 0.93\%$) and
5 precipitation ($0.031\pm 0.028\%$) (Table 2 and Table 3). However, there was no significant difference
6 between the $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ ratios for the various water compartments at Wukeng (K-W test, $p>0.05$).
7 These results imply active net Hg methylation in paddy fields at Gouxi but not at Wukeng. The
8 $\text{MeHg}_{\text{unf}}/\text{HgT}_{\text{unf}}$ ratios for precipitation ($0.76\pm 0.41\%$), irrigation water ($2.2\pm 0.98\%$), and paddy water
9 ($10\pm 7.9\%$) for both mining sites were elevated relative to the regional background, and we believe this is
10 due to the lower HgT_{unf} concentration reported for the regional background (Table 2).

11 3.3 Mercury in soil profiles

12 3.3.1 Hg in soil pore water

13 The vertical distribution of HgT_f and MeHg_f in pore water was monitored over four successive time
14 intervals during the rice growing season (Fig. 2). The distribution of HgT_f in pore water as a function of
15 depth was different to that for MeHg_f at both sampling sites. The mean HgT_f concentration in pore water
16 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 Gouxi and
17 $180\pm 160\text{ ng L}^{-1}$ (range: $38\text{-}916\text{ ng L}^{-1}$) at Wukeng. The highest concentration of HgT_f in pore water
18 was measured in the soil surface layer (top 2cm), and decreased with depth at both sampling sites. The
19 HgT_f concentration in pore water at Gouxi was relatively constant over time with no significant
20 difference between the different sampling dates (K-W test, $p>0.05$). At Wukeng, the HgT_f concentration
21 in pore water was time-dependent, with the highest concentration in the surface layer recorded on Day 20,
22 and the lowest on Day 80 (K-W test, $p<0.01$).

23 The maximum concentration of MeHg_f in soil pore water (15 ng L^{-1}) was observed at Gouxi, and was
24 approximately double that at Wukeng (6.6 ng L^{-1}). The MeHg concentration in soil pore water
25 collected at Gouxi was significantly higher than at Wukeng throughout the monitoring periods (K-S test,

1 $p < 0.01$), suggesting different rates of net Hg methylation between the Gouxi and Wukeng sites. The
2 MeHg_f concentration in pore water was generally highest in the surface soil at Gouxi, and then sharply
3 declined from a depth of 4 cm. In contrast, the vertical distributions of MeHgT_f in soil pore water of
4 Wukeng showed little variation, with the exception of small (unexplained) peaks at 10 cm on Day 20
5 and at 6 cm on Day 60. The proportion of pore water HgT_f that was MeHg_f (MeHg_f/HgT_f) ranged from
6 0.50 to 8.7 % (mean value of $2.6 \pm 1.7\%$) and from 0.089 to 4.8 % (mean value of $1.6 \pm 1.1\%$) at Gouxi
7 and Wukeng, respectively. Regression analysis revealed a significant and positive correlation between
8 HgT_f and MeHg_f at Gouxi ($r = 0.75$, $p < 0.001$, $n = 40$) but not at Wukeng ($r = 0.22$, $p = 0.17$, $n = 40$) (Fig.3),
9 suggesting a mechanistic relationship between these two Hg species at the artisanal mining site only.
10 In order to better understand the factors controlling Hg methylation in rice paddy soil, the concentration
11 of Fe²⁺, Fe³⁺, S²⁻, and SO₄²⁻ in soil pore water was determined and this data is described, in detail, in a
12 companion paper (Zhao et al., 2016). Briefly, no discernible vertical trend in Fe³⁺ distribution was
13 observed in the soil pore water across the two sampling sites during the sampling period. The Fe²⁺
14 concentrations in soil pore water at Gouxi exhibited a narrow range (41~417 μM), relative to that at
15 Wukeng (2.3~843 μM). The S²⁻ concentration in the soil pore water showed limited variation with depth
16 at Wukeng (mean = $0.70 \pm 0.36 \mu\text{M}$, range = $0.07 \sim 1.2 \mu\text{M}$) relative to Gouxi (mean = $1.8 \pm 0.79 \mu\text{M}$,
17 range = $0.69 \sim 3.8 \mu\text{M}$), with the highest value recorded in the surface soil layer at both sites. Temporal
18 variation of sulfide concentrations at Wukeng and Gouxi was insignificant (K-W test, $p = 0.73$ and $p = 0.33$
19 for Wukeng and Gouxi, respectively). The highest SO₄²⁻ concentrations were recorded in the surface
20 soil layer and decreased with depth across the two sampling sites. As described in the companion
21 paper (Zhao et al., 2016), SO₄²⁻ stimulation of SRB activity was a potentially important metabolic
22 pathway for Hg methylation in the rice paddy soil at the two Hg mining sites, while iron cycling in the
23 rice paddies could impact the availability of Hg in pore water for methylation.

24 **3.3.2 Mercury in soil cores**

25 The concentration and distribution of THg as a function of depth in soil cores at Gouxi and Wukeng is
26 shown in Fig.4. Over the rice growing season, the mean concentration of THg in soil was $3.2 \pm 0.75 \text{ mg}$
27 kg^{-1} ($0.88 \sim 4.4 \text{ mg kg}^{-1}$) and $38 \pm 4.8 \text{ mg kg}^{-1}$ ($27 \sim 48 \text{ mg kg}^{-1}$) at Gouxi and Wukeng, respectively. The

1 THg concentration in paddy soil collected from both Wukeng and Gouxi was higher than the domestic
2 environmental quality standard for paddy fields in China (0.5 mg kg^{-1}) (GB15618-2008), and
3 considered non-suitable for agricultural or residential use according to the level III criterion (1.5 mg kg^{-1})
4 in the Chinese national standard for soil environmental quality. The THg concentration in soil cores
5 showed no significant difference with depth although there was a nominal trend towards decreasing
6 concentration at Gouxi. For all depths the THg concentration in soil was elevated at Wukeng relative to
7 Gouxi, reflecting a greater degree of historical contamination at Wukeng due to a long period of
8 commercial mining activities.

9 In contrast to THg, the MeHg concentration in soil cores showed significant variation with depth and
10 time (Fig. 4). The MeHg concentration in soil cores at Gouxi showed a maximum value at the water-soil
11 interface and decreased with depth on sampling Days 20 to 80. On Day 100, however, there was no
12 increased MeHg concentration at the surface. The MeHg concentration in Wukeng soil cores showed
13 very little variation with depth, and the MeHg concentration at this site, for all depths, was significantly
14 lower than at Gouxi (K-S test, $p < 0.001$). Measured MeHg concentrations at the top of the Gouxi soil
15 profile varied from 0.76 ng g^{-1} to 6.2 ng g^{-1} , but remained relatively stable at Wukeng (range: $0.80\text{--}3.8$
16 ng g^{-1}). Comparison of the MeHg concentration and distribution patterns between the two sites supports
17 the hypothesis of active Hg methylation in the Gouxi soil only.

18 Methylation can be affected by the pH and organic matter content of soil, and an analysis of soil
19 physiochemical parameters in the soil cores of this study is reported in a companion paper (Zhao et al.,
20 2016). Briefly, the mean organic matter in soil cores was $4.8 \pm 0.75\%$ and $3.5 \pm 0.59\%$ at Gouxi and
21 Wukeng, respectively. The mean soil pH was the same for both sites (6.7 ± 0.10 at Gouxi and 6.6 ± 0.14 at
22 Wukeng) and did not change as a function of sampling time, despite the variation reported for irrigation
23 water and paddy water at Wukeng in the current study (Table 4). The consistency of soil pH throughout
24 the sampling period indicates that irrigation water and paddy water have little influence on bulk soil pH.
25 Statistical analysis revealed that there is no direct impact of pH and organic matter content on the MeHg
26 concentration in soil across the two sampling sites, indicating that absolute pH and organic matter might
27 not be the most important factors regulating Hg methylation activity (Zhao et al., 2016).

1 Changing redox parameters over the rice growing season may affect the process of Hg methylation.
2 Previous studies have observed that in artificially Hg-polluted soil, Hg bioavailability for methylation
3 can be significantly affected by the level of water saturation (Rothenberg and Feng, 2012; Wang et al.,
4 2014; Peng et al., 2012). Peng et al. (2012) specified that intermittent flooding, as opposed to
5 continuous flooding, could reduce soluble Hg concentrations and inhibit Hg methylation in the rice
6 rhizosphere, subsequently decreasing the accumulation of MeHg in rice grain. Flooded conditions
7 enhance anaerobic microbial activities and increase MeHg yields. The drying of a paddy field is an
8 important cultivation step to control rice plant tillering and increase yield. Therefore, one possible
9 reason for the considerably elevated MeHg concentrations in soil at Gouxu between Day 20 and Day 80
10 relative to Day 100 is an enhancement of Hg bioavailability and numbers of SRB under flooded
11 conditions that stimulated Hg methylation, and increased the soil MeHg concentration (Wang et al.,
12 2014). **As the paddy field dried beginning on Day 80**, some degree of net MeHg degradation may have
13 occurred, which could be attributed to the decreased SRB numbers and proportion of Hg methylators in
14 the rhizosphere under aerobic conditions (Wang et al., 2014). This could have contributed to a
15 decreasing trend in soil MeHg concentration during the harvest period.

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

24 **3.4 Source and mechanism for Hg transformation in paddy field**

25 The mean concentration of HgT_f in paddy water at Wukeng ($197 \pm 78 \text{ ng L}^{-1}$) was proximately 2 times
26 higher than that at Gouxu ($105 \pm 58 \text{ ng L}^{-1}$), whereas the MeHg_f concentration in paddy water at Gouxu
27 ($4.7 \pm 4.2 \text{ ng L}^{-1}$) was approximately 8 times higher than that at Wukeng ($0.62 \pm 0.29 \text{ ng L}^{-1}$) (Table 3).

1 Furthermore, the concentration of MeHg_f in paddy water at Wukeng (0.62 ± 0.29 ng L⁻¹) was
2 significantly higher than that in precipitation (0.14 ± 0.07 ng L⁻¹), but significantly lower than in
3 irrigation water (0.96 ± 0.50 ng L⁻¹) and soil pore water (1.7 ± 0.88 ng L⁻¹) in the soil surface layer during
4 the rice growing season (K-S test, $p < 0.001$) (Table 2 and Table 3). Generally, there are three possible
5 sources of MeHg in the paddy water: 1) in situ production being controlled by chemistry condition (e.g.
6 redox and pH), 2) diffusion of MeHg from underlying soil, and 3) MeHg flux of atmospheric deposition
7 and irrigation. We propose that baseline MeHg_f in paddy water at Wukeng is primarily due to the
8 diffusion of MeHg from the surface layer of sediment and MeHg flux from atmospheric deposition and
9 irrigation.

10 The sampling site for the Wukeng paddy was located next to a calcine pile and the proximity of this
11 waste had a major impact on water chemistry. Both the irrigation water ($\text{pH} = 11\pm 0.45$) and paddy water
12 ($\text{pH} = 8.6\pm 1.3$) were alkaline during the rice growing season (Table 4). We suggest that the alkaline
13 conditions of the irrigation at Wukeng could restrain Hg methylation and/or stimulate MeHg
14 demethylation in paddy water (Ullrich et al., 2001). Rothenberg et al. (2012) reported that alkaline
15 paddy water ($\text{pH} > 11$) at highly-contaminated mining sites can restrain the bioavailability of Hg²⁺ for Hg
16 methylation, resulting in lower pore water and soil MeHg concentrations despite higher total Hg
17 concentrations. The findings of our study are in agreement with those of Rothenberg et al. (2012).

18 In contrast, the MeHg_f concentration in paddy water at Gouxu (4.7 ± 4.2 ng L⁻¹) was significantly higher
19 than in precipitation (0.33 ± 0.17 ng L⁻¹) and irrigation water (0.31 ± 0.30 ng L⁻¹), but significantly lower
20 than in soil pore water (7.8 ± 5.2 ng L⁻¹) in the soil surface layer during the rice growing season (K-S test,
21 $p < 0.001$) (Table 2 and Table 3), with the data at Day 80 as an exception. The maximum
22 MeHg_f concentration was not recorded for the surface soil pore water (3.6 ng L⁻¹) but for the paddy
23 water (4.7 ng L⁻¹) at Day 80. The implication is that MeHg in this region is not only due to MeHg
24 diffusion from surface soil and/or the MeHg flux of atmospheric precipitation and irrigation, but also
25 from in situ methylation in anoxic water with relatively low pH ($\text{pH} = 6.9$ on Day 80) (Table 4). Gilmour
26 and Henry (1991) specified that low pH and anaerobic condition not only increase methylation rates but
27 also decrease demethylation rates, resulting in net production of MeHg. Both paddy water and irrigation
28 water at Gouxu exhibit pH and redox conditions that can be considered optimal for Hg methylation

1 (Table 4), favouring net methylation in the paddy water (Ullrich et al., 2001). **Active Hg methylation**
2 **within the Gouxi rice paddy is implied in the present study, even though data directly supporting this**
3 **hypothesis are lacking. Further work may help to bring more confidence on that particular point.**

4 During the rice growing season, HgT_{unf} in paddy water exceeded the EPA water-quality criterion of 50
5 ng L^{-1} (U.S. EPA, 2000). No regulatory criterion for MeHg exists, but Rudd (1995) suggested that
6 MeHg above a concentration of 0.1 ng L^{-1} is elevated and is likely to lead to significant MeHg
7 bioaccumulation. During the rice growing season, photo demethylation can reduce paddy water MeHg.
8 However, the MeHg concentration in both filtered and unfiltered paddy water samples at both sites
9 exceeded 0.1 ng L^{-1} (Table 3), confirming that rice paddies across the Hg mining area are an exposure
10 pathway for MeHg and may have direct implications for human and wildlife health. Previous studies
11 have indicated that vertebrates and fish cultivated in flooded rice paddies will accumulate MeHg to
12 critical threshold levels within 30 days (Ackerman et al., 2010a, 2010b). In rice paddy fields that
13 combine rice and fish cultivation, potential co-exposure of MeHg through rice and fish consumption
14 should receive more attention (Qiu et al., 2008; Feng et al., 2008; Lansing and Kremer, 2011).

15 Our finding that MeHg concentrations in surface soil at Gouxi were much higher than those at Wukeng
16 indicate that newly deposited mercury can be expected to rapidly methylate after deposition. The peak
17 concentration of MeHg in paddy soil at Gouxi, was at the soil-water interface and decreased with depth.
18 As concluded in a companion paper (Zhao et al., 2016), absolute pH and organic matter might not be
19 the most important factors regulating Hg methylation activity in rice paddy soil. Therefore, we believe
20 that a restricted supply of newly deposited Hg to depths below the soil-water interface is a plausible
21 explanation for the sharply reducing concentration of MeHg with depth at Gouxi; newly deposited Hg is
22 constrained to surface soil and cannot be transferred to lower depth. Therefore, a direct positive
23 relationship between HgT_f and MeHg_f concentrations in soil pore water was observed at Gouxi during the
24 rice growing season (see section 3.3.1).

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

1 interactions with soil surfaces (Schuster, 1991), which favour the retention of Hg in the surface layers of
2 the soil profile. Over time this newly deposited Hg will be transformed into more stable, less available
3 forms (Schuster, 1991), and the net methylation potential of this Hg will consequently decrease. The
4 relatively low MeHg concentration in soil at Wukeng is indicative of **old Hg** which has become tightly
5 bound to soil complexes over time, and is unavailable for methylation (Hintelmann et al., 2002).
6 Consequently, there is no correlation between HgT_f and $MeHg_f$ in soil pore water at Wukeng (see
7 section 3.3.1). Our data indicates that the THg concentration in soil is not a reliable indicator of Hg
8 methylation potential in soil. Instead, the concentration of bioavailable or new Hg must be considered,
9 in agreement with the findings of Meng et al. (2010, 2011).

10 **3.5 Implications of this work to environmental risk assessment**

11 Elevated MeHg concentrations combined with an elevated MeHg% in surface soil active Hg
12 methylation processes are occurring in Gouxi rice paddy soil. The Hg methylation rate is a function of
13 an elevated Hg concentration in atmosphere. The absence of depth-dependent variability in the MeHg
14 concentration in soil cores at Wukeng is consistent with the low concentration of Hg in ambient air and
15 corresponding atmospheric deposition. The in situ production of MeHg in Wukeng soil, despite the
16 elevated concentration of THg, is low. Our results demonstrate that soil is the primary source of MeHg
17 for paddy rice, and we believe that elevated MeHg in rice poses a potential threat to wildlife and local
18 residents. Mercury in surface soil that has been derived from atmospheric deposition is susceptible to
19 methylation in the rice paddy ecosystem immediately after deposition. Consequently, net MeHg
20 production is principally governed by the supply of fresh deposited Hg to soil.

21 Because MeHg can be demethylated to IHg biotically and abiotically in soil or paddy water, rapid
22 cycling occurs between the IHg and MeHg pools. The current study was limited to the rice growing
23 season, not the entire year or an period of time. Therefore our results define the initial rather than long-
24 term influence of newly deposited Hg on MeHg production. The overall contribution of **old** versus
25 newly-deposited Hg to the pool of Hg in paddy soil that is available for methylation will likely depend
26 on the balance of Hg deposition and the rate at which this deposited Hg binds to soil constituents, and
27 the magnitude of the IHg flux in the atmosphere. Our study provides no information on the extent to

1 which the MeHg concentration in rice paddies will respond to Hg emission controls which seek to
2 reduce the flux of atmospheric Hg. The response of the paddy ecosystem to reductions in Hg emissions
3 will depend on how long previously deposited Hg has been stored in paddy soil and its availability to
4 SRB. This issue is poorly understood, but previously reported declines in Hg loading suggest that MeHg
5 levels in soil at abandoned Hg mining areas begin to respond within a few years of Hg
6 reductions(Rothenberg et al., 2012). This provides hope that environmental risk mitigation strategies
7 based on a more detailed understanding of the rice-paddy ecosystem at mining contaminated sites can
8 be effectively enacted to protect human health.

9 **The relationship between MeHg and fresh deposited Hg implies that the concentration of Hg in ambient**
10 **air could be used as a monitoring tool to assess the relative risk of MeHg production in the rice paddy**
11 **environment, and the possible risk to human health that may be associated with the accumulation of this**
12 **MeHg in rice grain. However, we cannot distinguish between newly deposited Hg and old Hg stored in**
13 **paddy soil over decades and ongoing research is necessary to continue to develop an improved**
14 **understanding of Hg dynamics in rice paddy soils. When comparing relative risk between different**
15 **vectors for Hg contamination (i.e. small-scale or historic large-scale mining), quantification of the pool**
16 **of Hg available for methylation is critical to estimating reliable methylation rates. Ongoing work is**
17 **urgently needed to further ascertain the relative importance of newly deposited Hg versus in situ Hg to**
18 **the bioavailable pool of Hg that can be methylated in rice paddy ecosystems.**

19 **Acknowledgements**

20 This research was financed by the National Key Basic Research Program of China (973 Program
21 2013CB430004), financed by natural science foundation of China (41173126, 41573135, 41203091,
22 and 41473123), and also financed by natural science foundation of Guizhou (2012-2333).

23

1 **References**

- 2 Ackerman, J. T. and Eagles-Smith, C. A.: Agricultural wetlands as potential hotspots for
3 bioaccumulation: experimental evidence using caged fish, *Environ. Sci. Technol.*, 44, 1451-1457, 2010a.
- 4 Ackerman, J. T., Miles, A. K., and Eagles-Smith, C. A.: Invertebrate mercury bioaccumulation in
5 permanent, seasonal, and flooded rice wetlands within California's Central Valley, *Sci. Total Environ.*,
6 408, 666-671, 2010b.
- 7 Benoit, J.M., Gilmour, C.C., Mason, R.P.: The influence of sulfide on solid-phase
8 mercurybioavailability for methylation by pure cultures of *Desulfobulbus propionicus* (1pr3).
9 *Environ.Sci. Technol.*, 35, 127-132, 2001.
- 10 Columbus, C.: *How I Discovered America*. Hispanic Press, Barcelona, 1492.
- 11 FAO.: *Rice Information*, Rome, Italy, 2002.
- 12 Feng, X. B., Li, P., Qiu, G. L., Wang, S., Li, G. H., Shang, L. H., Meng, B., Jiang, H. M., Bai, W. Y., Li,
13 Z. G., and Fu, X. W.: Human exposure to methylmercury through rice intake in mercury mining areas,
14 guizhou province, china, *Environ. Sci. Technol.*, 42, 326-332, 2008.
- 15 Fleming, E.J., Mack, E. E., Green, P.G., Nelson, D.C.: Mercury methylation from unexpectedsources:
16 Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Appl.Environ. Microbiol.*,
17 72, 457-464, 2006.
- 18 Gilmour, C.C., E.A. Henry.: 1991. Mercury methylation in aquatic systems affected by acid deposition.
19 *Environ. Pollut.*, 71, 131-169, 1991.
- 20 Gilmour, C.C., Henry, E.A., Mitchell, R.: Sulfate stimulation of mercury methylation infresh-water
21 sediments. *Environ. Sci. Technol.*, 26, 2281-2287, 1992.
- 22 Green, R. J., Fred, U. P., and Norbert, W. P.: Things that go bump in the night, *Psych. Today*, 46, 345-
23 678, 1900.

- 1 Guo, Y. N., Feng, X. B., Li, Z. G., He, T. R., Yan, H. Y., Meng, B., Zhang, J. F., and Qiu, G. L.:
2 Distribution and wet deposition fluxes of total and methyl mercury in Wujiang River Basin, Guizhou,
3 China, *Atmos. Environ.*, 42, 7096-7103, 2008.
- 4 Hintelmann, H., Harris, R., Heyes, A., Hurley, J. P., Kelly, C. A., Krabbenhoft, D. P., Lindberg, S., Rudd,
5 J. W. M., Scott, K. J., and St. Louis, V. L.: Reactivity and Mobility of New and Old Mercury
6 Deposition in a Boreal Forest Ecosystem during the First Year of the METAALICUS Study, *Environ.*
7 *Sci. Technol.*, 36, 5034-5040, 2002.
- 8 Horvat, M., Nolde, N., Fajon, V., Jereb, V., Logar, M., Lojen, S., Jacimovic, R., Falnoga, I., Qu, L. Y.,
9 Faganeli, J., and Drobne, D.: Total mercury, methylmercury and selenium in mercury polluted areas in
10 the province Guizhou, China, *Sci. Total Environ.*, 304, 231-256, 2003.
- 11 James, K., Harris, Jr., G., and Wollops, W.: American independence and magnetism, *Revol. Tracts*, 32,
12 34-55, 1776.
- 13 Krisnayanti, B. D., Anderson, C. W. N., Utomo, W. H., Feng, X. B., Handayanto, E., Mudarisna, N.,
14 Ikram, H., and Khususiah.: Assessment of environmental mercury discharge at a four-year-old artisanal
15 gold mining area on Lombok Island, Indonesia. *J. Environ. Monitor.*, 14, 2598-2670, 2012.
- 16 Lansing, J. S. and Kremer, J. N.: Rice, fish, and the planet. *PNAS.*, 108, 19841-19842, 2011.
- 17 Li, P., Feng, X. B., Qiu, G. L., Shang, L. H., Wang, S. F., and Meng, B.: Atmospheric mercury emission
18 from artisanal mercury mining in Guizhou Province, Southwestern China, *Atmos. Environ.*, 43, 2247-
19 2251, 2009.
- 20 Li, P., Feng, X. B., Shang, L. H., Qiu, G. L., Meng, B., Liang, P., and Zhang, H.: Mercury pollution from
21 artisanal mercury mining in Tongren, Guizhou, China, *Appl. Geochem.*, 23, 2055-2064, 2008.
- 22 Liang, L., Horvat, M., Cernichiari, E., Gelein, B., and Balogh, S.: Simple solvent extraction technique
23 for elimination of matrix interferences in the determination of methylmercury in environmental and
24 biological samples by ethylation gas chromatography cold vapor atomic fluorescence spectrometry.
25 *Talanta.*, 43, 1883-1888, 1996.

- 1 Liu, J. L., Feng, X. B., Qiu, G. L.; Anderson, C. W. N., and Yao, H.: Prediction of Methyl Mercury
2 Uptake by Rice Plants (*Oryza sativa* L.) Using the Diffusive Gradient in Thin Films Technique, *Environ.*
3 *Sci. Technol.*, 46, 11013-11020, 2012.
- 4 Liu, X.Z., Zhang, L.M., Prosser, J.I., He, J.Z.: Abundance and community structure of sulfatereducing
5 prokaryotes in a paddy soil of southern China under different fertilization regimes. *Soil Biol.*
6 *Biochem.*, 41, 687-694, 2009.
- 7 Liu, Y.R., Zheng, Y.M., Zhang, L.M., He, J.Z.: Linkage between community diversity of sulfate-
8 reducing microorganisms and methylmercury concentration in paddy soil. *Environ. Sci. Pollut. Res.*, 21,
9 1339-1348, 2014.
- 10 Meng, B., Feng, X. B., Qiu G. L., Liang, P., Li, P., Chen, C. X., Shang, L. H. The process of
11 methylmercury accumulation in rice (*Oryza sativa* L.), *Environ. Sci. Technol.*, 45, 2711-2717, 2011.
- 12 Meng, B., Feng, X. B., Qiu, G. L., Cai, Y., Wang, D. Y., Li, P., Shang, L. H., Sommar, J.: Distribution
13 patterns of inorganic mercury and methylmercury in tissues of rice (*Oryza sativa* L.) plants and possible
14 bioaccumulation pathways, *J. Agric. Food Chem.*, 58, 4951-4958, 2010.
- 15 Meng, B., Feng, X. B., Qiu, G. L., Wang, D. Y., Liang, P., Li, P., and Shang, L. H.: Inorganic mercury
16 accumulation in rice (*Oryza sativa* L.), *Environ. Toxicol. Chem.*, 31, 2093-2098, 2012.
- 17 Meng, B., Feng, X. B.; Qiu, G. L., Anderson, C. W. N., Wang, J. X., and Zhao, L.: Localization and
18 Speciation of Mercury in Brown Rice with Implications for Pan-Asian Public Health, *Environ. Sci.*
19 *Technol.*, 48, 7974-7981, 2014.
- 20 Meng, B.: Biogeochemistry Process of Mercury in Sensitive Ecosystem and Health Risk Assessment,
21 Southwest China. Ph.D. Dissertation, The Graduate School of the Chinese Academy of Sciences (in
22 Chinese with English abstract), Beijing, China, 2011.
- 23 Meng, M., Li, B., Shao, J. J., Wang, T., He, B., Shi, J. B., Ye, Z. H., and Jiang, G. B. Accumulation of
24 total mercury and methylmercury in rice plants collected from different mining areas in China, *Environ.*
25 *Pollut.*, 184, 179-186, 2014.

- 1 Oslo and Paris C.: JAMP guidelines for the sampling and analysis of mercury in air and precipitation;
2 Joint Assessment and Monitoring Programme, 1-20, 1998.
- 3 Peng, X. Y., Liu, F. J., Wang, W. X., and Ye, Z. H.: Reducing total mercury and methylmercury
4 accumulation in rice grains through water management and deliberate selection of rice cultivars,
5 Environ. Pollut., 162, 202-208, 2012.
- 6 Phillips, T. P.: Possible influence of the magnetosphere on American history, [www.sma-](http://www.sma-london.ac.uk/~phillips/magnet_infl.htm)
7 [london.ac.uk/~phillips/magnet_infl.htm](http://www.sma-london.ac.uk/~phillips/magnet_infl.htm), 1999.
- 8 Qiu, G. L., Feng, X. B., Li, P., Wang, S. F., Li, G. H., Shang, L. H., and Fu, X. W.: Methylmercury
9 accumulation in rice (*Oryza sativa* L.) grown at abandoned mercury mines in Guizhou, China, J. Agric.
10 Food Chem., 56, 2465-2468, 2008.
- 11 Qiu, G. L., Feng, X. B., Meng, B., Wang, X.: Methylmercury in rice (*Oryza sativa* L.) grown from the
12 Xunyang Hg mining area, Shaanxi. Pure Appl. Chem., 84, 281-289, 2012.
- 13 Qiu, G. L., Feng, X. B., Wang, S. F., and Shang, L. H.: Mercury and methylmercury in riparian soil,
14 sediments, mine-waste calcines, and moss from abandoned Hg mines in east Guizhou province,
15 southwestern China, Appl. Geochem., 20, 627-638, 2005.
- 16 Rothenberg, S. E. and Feng, X. B.: Mercury cycling in a flooded rice paddy, J. Geophys. Res., 117,
17 2012.
- 18 Rothenberg, S. E., Feng, X. B., Zhou, W. J., Tu, M., Jin, B. W., You, J. M.: Environment and genotype
19 controls on mercury accumulation in rice (*Oryza sativa* L.) cultivated along a contamination gradient in
20 Guizhou, China, Sci. Total Environ., 426, 272-280, 2012.
- 21 Rudd, J. W. M.: Source of methyl mercury to fresh-water ecosystems- a review, Water air soil Pollut.,
22 80, 697-713, 1995.
- 23 Schuster, E.: The behavior of mercury in the soil with special emphasis on complexation and adsorption
24 processes - A review of the literature, Water air soil pollut., 56, 667-680, 1991.
- 25 Smith, J. G. and Weston, H. K.: Nothing particular in this year's history, J. Oddball Res., 2, 14-15, 1954.

1 Sunderland, E. M., Gobas, F. A. P. C., Branfireun, B. A., and Heyes, A.: Environmental controls on the
2 speciation and distribution of mercury in coastal sediments, *Mar. Chem.*, 102, 111-123, 2006.

3 U.S. EPA.: Method 1630: Methylmercury in Water by Distillation, Aqueous Ethylation, Purge and Trap,
4 and CVAFS, EPA-821-R-01-020, Washington, DC, 2001.

5 U.S. EPA.: Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic
6 Fluorescence Spectrometry (Revision E), EPA-821-R-02-019. Washington, DC, 2002.

7 U.S. EPA.: Water quality standards - establishment of numeric criteria for priority toxic pollutants for
8 the State of California, 40 CFR part 131. [http://www.epa.gov/fedrgstr/EPA-WATER/2000/May/Day-](http://www.epa.gov/fedrgstr/EPA-WATER/2000/May/Day-18/w11106.pdf)
9 [18/w11106.pdf](http://www.epa.gov/fedrgstr/EPA-WATER/2000/May/Day-18/w11106.pdf). 2000.

10 Ullrich, S. M., Tanton, T. W., and Abdrashitova, S. A.: Mercury in the aquatic environment: A review of
11 factors affecting methylation, *Crit. Rev. Environ. Sci. Technol.*, 31, 241-293, 2001.

12 Wang, X., Ye, Z. H., Li, B., Huang, L. A., Meng, M., Shi, J. B., and Jiang, G. B.: Growing Rice
13 Aerobically Markedly Decreases Mercury Accumulation by Reducing Both Hg Bioavailability and the
14 Production of MeHg, *Environ. Sci. Technol.*, 48, 1878-1885, 2014.

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

17 Zhang, H., Feng, X. B., Larssen, T., Qiu, G. L., and Vogt, R. D.: In inland China, rice, rather than fish is
18 the major pathway for methylmercury exposure, *Environ. Health Perspect.*, 118, 1183-1188, 2010b.

19 Zhang, H., Feng, X. B., Larssen, T., Shang, L. H., Li, P.: Bioaccumulation of methylmercury versus
20 inorganic mercury in rice (*Oryza sativa* L.) grain, *Environ. Sci. Technol.*, 44, 4499-4504, 2010a.

21 Zhao, L., Qiu, G. L., Anderson, C. W. N., Meng, B., Wang, D. Y., Shang, L. H., Yan, H. Y., Feng, X. B.:
22 Mercury methylation in rice paddies and its possible controlling factors in the Hg mining area, Guizhou
23 Province, Southwest China. *Environ. Pollut.*, Under review.

24 Zheng, W.: Mercury Species in the urban air of Guiyang. Ph.D. Dissertation, The Graduate School of
25 the Chinese Academy of Sciences (in Chinese with English abstract), Beijing, China, 2007.

26

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 and precipitation at artisanal Hg mining site (Gouxi), abandoned Hg mining
 2 site (Wukeng), and regional background of Huaxi (mean±SD)

Sampling sites	Ambient air ¹	Precipitation ¹				
	Hg ⁰ (ng m ⁻³)	HgT _{unf} (ng L ⁻¹) ³	HgT _f (ng L ⁻¹) ³	MeHg _{unf} (ng L ⁻¹) ³	MeHg _f (ng L ⁻¹) ³	MeHg _{unf} /HgT _{unf} (%)
Gouxi	403±388	2599±1874	648±672	0.48±0.20	0.33±0.17	0.031±0.028
Wukeng	28±13	445±296	164±166	0.30±0.15	0.14±0.07	0.16±0.20
Huaxi ²	6.2±3.0	27±17		0.28±0.14		0.76±0.41

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

5 ² data were obtained from Zheng, (2007), Meng et al. (2010) and Meng (2011).

6 ³HgT_{unf}, unfiltered total mercury; MeHg_{unf}, unfiltered methylmercury;

7

1 Table 3. Hg irrigation water and paddy water at artisanal Hg mining site (Gouxi), abandoned Hg mining site (Wukeng),
 2 and regional background of Huaxi (mean±SD)

Sampling sites	Irrigation water ¹					Paddy water ¹				
	HgT _{unf} (ng L ⁻¹)	HgT _f (ng L ⁻¹) ³	MeHg _{unf} (ng L ⁻¹)	MeHg _f (ng L ⁻¹) ³	MeHg _{unf} /HgT _{unf} (%)	HgT _{unf} (ng L ⁻¹)	HgT _f (ng L ⁻¹) ¹	MeHg _{unf} (ng L ⁻¹)	MeHg _f (ng L ⁻¹)	MeHg _{unf} /HgT _{unf} (%)
Gouxi	159±67	39±9.4	0.75±0.65	0.31±0.30	0.71±0.93	189±117	105±58	13±16	4.7±4.2	5.9±4.4
Wukeng	513±215	195±45	1.7±1.1	0.96±0.50	0.45±0.53	430±279	196±78	1.1±0.52	0.62±0.29	0.48±0.63
Huaxi ²	7.1±4.0		0.14±0.044		2.2±0.98	7.5±4.3		0.71±0.66		10±7.9

3 ¹ Hg species concentrations in irrigation water and paddy water were averaged with data sets of four sampling campaigns at Days 20, 40, 60, 80.

4 ² data were obtained from Zheng, (2007), Meng et al. (2010) and Meng (2011).

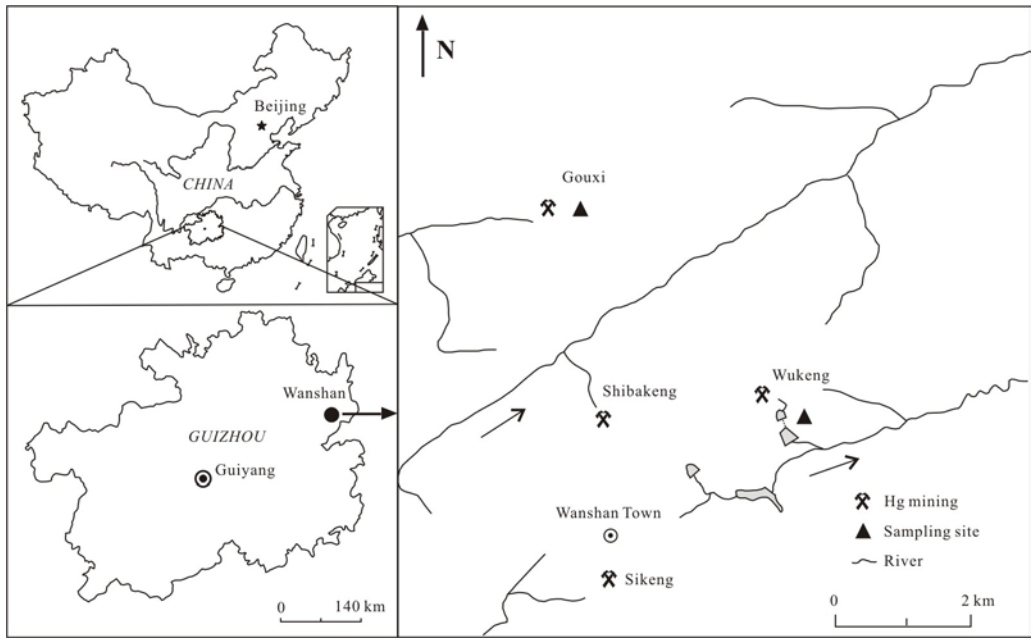
5 ³HgT_{unf}, unfiltered total mercury; HgT_f, filtered total mercury; MeHg_{unf}, unfiltered methylmercury; MeHg_f, filtered methylmercury;

6

1 Table 4. Temperature (T), pH, and dissolved oxygen (DO) in irrigation water and paddy water at the
 2 artisanal Hg mining site (Gouxi) and abandoned Hg mining site (Wukeng) (mean±SD, range)

Sampling sites	Irrigation water			Paddy water		
	T (°C)	pH	DO (mg L ⁻¹)	T (°C)	pH	DO (mg L ⁻¹)
Gouxi	24±1.7 (23-26)	8.3±0.24 (8.1-8.6)	7.4±0.43 (6.9-8.0)	28±4.4 (24-33)	7.2±0.24 (6.9-7.4)	3.0±0.95 (1.8-3.9)
Wukeng	25±2.1 (23-27)	11±0.45 (11-12)	7.4±0.56 (6.8-8.1)	25±2.7 (23-29)	8.6±1.3 (7.3-9.8)	4.4±0.73 (3.6-5.2)

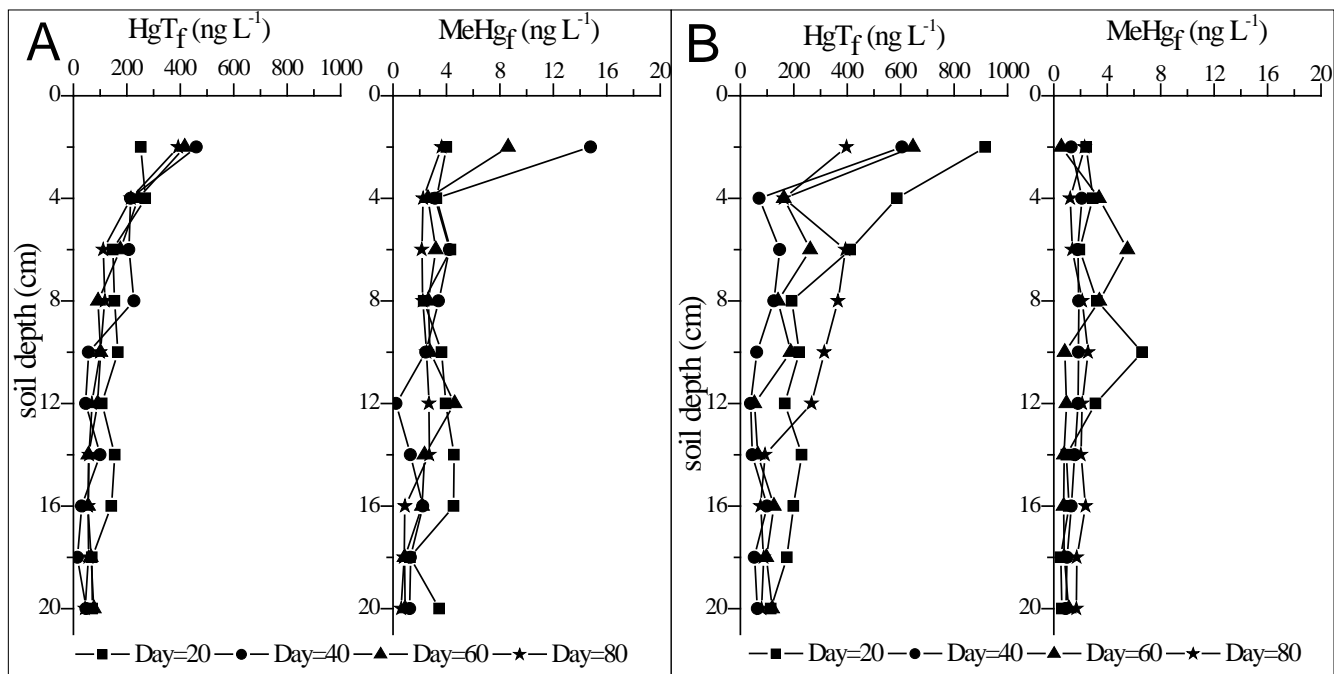
3



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).

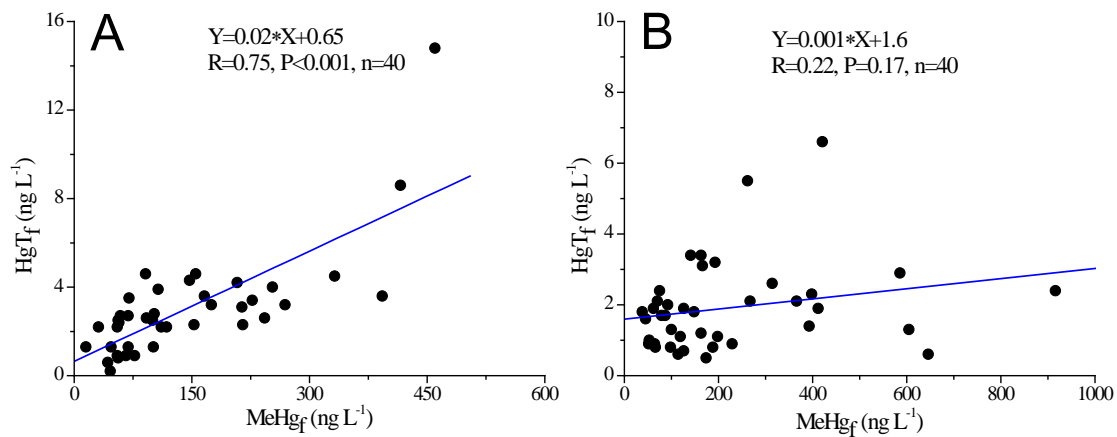
4



1

2 Figure 2. Concentration of HgT_f and MeHg_f (ng L^{-1}) in pore water during the rice growing season on
 3 Days 20, 40, 60, and 80 (A: artisanal Hg mining site of Gouxi; B: abandoned Hg mining site of Wukeng).

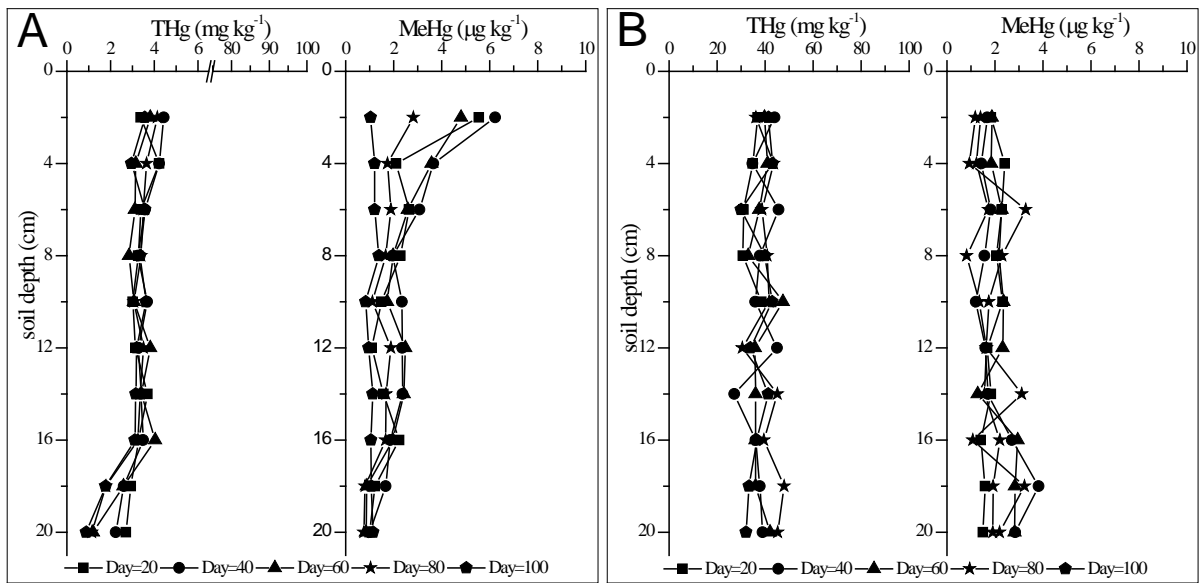
4



1

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