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Mercury methylation in rice paddies and its possible controlling factors in the Hg mining area, Guizhou province, Southwest China☆

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ABSTRACT

Understanding mercury (Hg) methylation/demethylation processes and the factors controlling methylmercury (MeHg) production within the rice paddy ecosystem of Hg mining areas is critical to assess the risk of MeHg contamination in rice grain. Two typical Hg-contaminated mining sites, a current-day artisanal site (Gouxi) and an abandoned site (Wukeng), were chosen in this study. We qualified the *in situ* specific methylation/demethylation rate constants in rice paddy soil during a complete rice-growing season. Our results demonstrate that MeHg levels in rice paddy soil were a function of both methylation and demethylation processes and the net methylation potential in the rice paddy soil reflected the measured MeHg production at any time point. Sulfate stimulating the activity of sulfate-reducing bacteria was a potentially important metabolic pathway for Hg methylation in rice paddies. We suggest that bioavailable Hg derived from new atmospheric deposition appears to be the primary factor regulating net MeHg production in rice paddies.

1. Introduction

Mercury (Hg) is one of the most hazardous heavy metals, posing a potential risk to humans and the environment. The mobility, bioavailability, and toxicological effects of Hg are strongly dependent on its chemical speciation (Ullrich et al., 2001). Methylation of inorganic Hg (IHg) is an important process that can fundamentally define its environmental behaviors (Boening, 2000). Of all the chemical forms of Hg, methylmercury (MeHg) poses the greatest toxicity concern to wildlife and human health due to its neurotoxicity and tendency to accumulate in food chains (Boening, 2000; King et al., 2002).

Concerning the exposure of MeHg, rice (*Oryza sativa*) has recently been of particular focus because rice grains are an intensive bio-accumulator of MeHg (Qiu et al., 2008; Meng et al., 2010, 2011; Zhang et al., 2010a). Rice paddy soil, as a typical ephemeral wetland, is known to be a significant setting for Hg methylation, which results in the accumulation of MeHg in rice grain (Meng et al., 2010). Recent studies elucidated that rice consumption, not fish, was the primary pathway of MeHg exposure for local residents in Hg mining areas in Southwest China (Feng et al., 2008; Zhang et al., 2010b). To maximize the benefits of rice-growing cultural activities, it is desirable to minimize the potential for MeHg formation in rice paddies. Thus, an understanding of the factors that control the MeHg production is crucial as a basis for reliable risk assessment of emission sources and as a guide to appropriate strategies for remediating contaminated soil.

Generally, Hg methylation occurs largely in anoxic soils and sediments (Olson and Cooper, 1976), with particularly high rates of methylation observed in wetland sediments (Driscoll et al., 1998). In particular, the mobility and methylation of Hg in ephemeral flooded soil is determined by a range of factors, such as redox potential, pH, dissolved organic carbon, sulfur, iron, and dissolved Hg concentration (e.g., Ullrich et al., 2001; Benoit et al., 2001). Hg methylation is largely facilitated by a subset of sulfate-reducing bacteria (SRB) (Gilmour et al., 1992) and/or iron-reducing bacteria (IRB) (Fleming et al., 2006) in anoxic conditions. However, Yu et al. (2013) and Gilmour et al. (2013) specified that methanogens can also methylate Hg under the physicochemical conditions typical of

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rice paddy soil.

Because numerous studies have reported high levels of MeHg in rice grain and in the corresponding soil in Hg mining areas (e.g., Feng et al., 2008; Zhang et al., 2010a; Qiu et al., 2008; Meng et al., 2010, 2014), concerns have been raised about the fate of Hg and MeHg production in the soil-rice system. The general consensus among Hg researchers is that the methylation of IHg in paddy soil primarily occurs under reducing conditions through a process mediated by sulfate-reducing bacteria (Peng et al., 2012; Rothenberg and Feng, 2012; Wang et al., 2014; Liu et al., 2009, 2014a; Somenahally et al., 2011). Therefore, intermittent flooding, as opposed to continuous flooding, can reduce soluble Hg concentrations and restrain Hg methylation in the rice rhizosphere, which would in turn decrease the accumulation of MeHg in rice grain (Peng et al., 2012). In particular, Rothenberg and Feng (2012) explored how the reduction of Fe^{3+}, which acts as an electron acceptor for sulfate-reducing bacteria, indirectly stimulates Hg methylation in paddy soil. Recently, Wang et al. (2014) indicated that water management activities can dramatically impact the community composition of sulfate-reducing bacteria. The authors further observed that Hg methylation was restrained as a result of decreased sulfate-reducing bacteria numbers and proportion of Hg methylators in the rhizosphere under aerobic conditions (Wang et al., 2014). Recently, Parks et al. (2013) reported a link between a two-gene cluster, hgcA and hgcB, and Hg methylation in two bacteria. A previous study conducted by Liu et al. (2014b) specified that hgcA microbes in rice paddy soil of the Wanshan Hg mining area were potentially related to Deltaproteobacteria, Firmicutes, Chloroflexi, Euryarchaeota, and two unclassified groups.

Earlier studies have suggested that newly deposited Hg is more readily transformed to MeHg in rice paddies (Meng et al., 2010, 2011). However, these conclusions are suggestive, and their characteristics are inferred from indirect evidence. Little attention has been paid to the process of Hg methylation after the deposition of atmospheric Hg. Current understanding is that transformations between IHg and MeHg occur predominantly in microbially mediated processes of oxidation, reduction, methylation, and demethylation; MeHg production is ultimately controlled by the presence and activity of those Hg-methylating bacteria, and is limited by the availability of electron acceptors (e.g. SO_4^{2-}, Fe^{3+}, and fumarate) and/or electron donors, and the bioavailability of Hg^{2+} to those bacteria (Marvin-DiPasquale et al., 2009).

The methylation of IHg in rice paddy soil constitutes a key step in the cycling of Hg in this special terrestrial ecosystem, especially within Hg mining areas. Despite advances in research to define the factors controlling MeHg production, mainly by relating MeHg concentrations in the soil to changes in environmental conditions (e.g., Peng et al., 2012; Wang et al., 2014), the compartmentalization of the Hg methylation process and the biogeochemical controls on Hg methylation in paddy soil are extremely complex and remain poorly understood (Peng et al., 2012; Wang et al., 2014). The balance between Hg methylation and MeHg demethylation determines the net production of MeHg in rice paddy soil; these processes and their dynamics have been rarely quantified in Hg-contaminated ecosystems. Therefore, the primary objectives of the current study were to 1) understand the Hg methylation/demethylation processes and 2) reveal possible controlling factors on MeHg production within the rice paddy ecosystem.

2. Materials and methods

2.1. Study area

Two typical sites, including an artisanal Hg mining site (Gouxi) and an abandoned Hg mining site (Wukeng), were selected for this study. These sampling sites are located in the Wanshan Hg mining district, eastern Guizhou province, China. Detailed information concerning sampling at the two sites is shown in the Supporting Information (SI).

Field sampling for the current research were performed by establishing a plot of 10×10 m^2 within a rice paddy and each site and implementing a sampling programme to approximately 20 cm of soil depth. The two experimental plots were cultivated during the period from 1st June to 10th September (~100 days). Once transplanted, standing water (2–8 cm) above the soil surface was required to maintain flooded soil conditions during the growing period from Day 0 to Day 80. The paddy fields were thereafter drained from Day 80, prior to the harvest between Days 90 and 100.

2.2. Sample collection and preparation

Five consecutive sampling campaigns were conducted during an entire rice-growing season. The first sampling was initiated 20 days after the plants were planted out (19thJune, 2012), and thereafter, at Days 40 (11th July), 60 (4th August), 80 (20th August), and 100 until the final harvest (10th September, 2012). At each sampling campaign, irrigation water and paddy water (overlying water) were collected for total (HgT_{unf}) and total MeHg ($MeHg_{unf}$) analysis.

Samples of soil pore water (to a depth of 20 cm) at both Gouxi and Wukeng were collected in the center of the rice paddy on days 20, 40, 60, and 80. The soil pore water was divided for dissolved total Hg (HgT_f), dissolved MeHg ($MeHg_f$), S^{2-}, SO_4^{2-}, Fe^{2+}, and Fe^{3+} analysis. At each sampling site and at each sampling time, a soil core (solid + liquid phases, with depth of 20 cm) was collected for total Hg (THg), MeHg, bioavailable Hg, organic matter, and pH analysis. Specific methylation rate (K_m) and demethylation rate (K_d) constants in soil cores from each of the two sampling sites were measured at Days 40, 60 and 80 during the rice-growing season. A detailed description of sample collection and preparation is given in the SI.

2.3. Sample analysis

Detailed information concerning analytical methods is described in the SI, including measurements of MeHg isotopes, calculation of specific methylation and demethylation rate constants, bioavailable Hg, organic matter, and pH analysis of the soil samples, Fe^{2+}, Fe^{3+}, S^{2-}, and SO_4^{2-} analysis of pore water samples.

2.4. QA/QC

Quality control for THg and MeHg determination in water samples and soil samples is available in a companion paper (Zhao et al., 2016). Standard quality assurance and control procedures for MeHg isotope determination in soil samples were conducted by method blanks, matrix spikes, certified reference materials. These data are available in the SI.

Statistical analysis was performed using SPSS 13.0 software (SPSS) The data in this study were normally distributed. Pearson correlation analyses among 7 biogeochemical parameters were subjected to regression analysis. The 7 parameters included Fe^{2+}, Fe^{3+}, S^{2-}, SO_4^{2-} concentrations in soil pore water and methylation rate constant (K_m), demethylation rate constant (K_d), net Hg methylation potential (K_m/K_d) in soil cores. Correlation coefficients (r) and significance probabilities (p) were computed for the linear regression fits. Differences are declared as significant in the case that $p < 0.05$. The Kolmogorov-Smirnov test (*K-S*) and Kruskal-Wallis test (*K-W*) were processed to compare significant differences between two or more independent datasets.

3. Results and discussion

3.1. General characteristics in soil pore water and soil cores

Sulfur (S^{2-} and SO_4^{2-}) concentrations and distribution in soil pore water were investigated during the rice growing season and are shown in Fig. 1. Our data showed that the sulfate (SO_4^{2-}) concentrations in soil pore water were in the range of 3.7–157 μM and 3.4–211 μM at Gouxi and Wukeng, respectively. Average sulfate concentrations in the pore water profiles of Gouxi and Wukeng were 48 ± 40 μM and 34 ± 48 μM, respectively. The highest sulfate concentrations were present in the surface soil layer across the two sampling sites during the rice growing season. Sulfate concentrations in pore water at Gouxi decreased gradually with depth. In comparison, pore water profiles at Wukeng showed a steep sulfate concentration gradient. Statistical analysis showed that the sulfate concentration in soil pore water was significantly higher at Wukeng than at Gouxi during the sampling season (*K-S* test, $p = 0.02$).

During the rice growing season, the mean concentrations of sulfide in soil pore water collected from Wukeng and Gouxi were 0.70 ± 0.36 μM (ranging from 0.07 μM to 1.2 μM) and 1.8 ± 0.79 μM (ranging from 0.69 μM to 3.8 μM), respectively. The concentration of sulfide in the soil pore water of Wukeng showed a narrow scale variation with depth. A more obvious vertical variation was present in the corresponding data at Gouxi. The highest value of sulfide in pore water at Gouxi was present in the surface soil layer, and the value decreased gradually with depth. Statistical analysis showed that sulfide concentrations in soil pore water at Gouxi were significantly higher than those at Wukeng throughout the three sampling campaigns (*K-S* test, $p < 0.001$). The relative temporal variation of sulfide concentrations among the three sampling campaigns at both Wukeng and Gouxi are less pronounced (*K-W* test, $p = 0.73$ and $p = 0.33$ for Wukeng and Gouxi, respectively).

The concentrations and distribution patterns of Fe^{2+} and Fe^{3+} in soil pore water collected from Gouxi and Wukeng are illustrated in Fig. 2. The average Fe^{3+} concentrations in soil pore water were 20 ± 12 μM (ranging from 0.45 μM to 48 μM) and 13 ± 13 μM (ranging from below the detection limit to 45 μM) at Gouxi and Wukeng, respectively. Furthermore, no discernible vertical trend in Fe^{3+} distribution was observed in the soil pore water across the two sampling sites during our sampling periods. Conversely, a clearly different vertical variation in Fe^{2+} distribution was observed between Gouxi and Wukeng. The Fe^{2+} concentrations in soil pore water at Gouxi exhibited a relative narrow range (41–417 μM), whereas they ranged widely from 2.3 μM to 843 μM at Wukeng during the sampling periods. In addition, the vertical distribution of Fe^{2+}in soil pore water at Gouxi showed little variation, with the exception of a slight peak at the depth of 12 cm–14 cm on Day 80. In contrast, the Fe^{2+} concentrations in soil pore water at Wukeng showed clear peaks in the depth from 8 cm to 12 cm throughout the 3 sampling periods, unlike the distribution patterns observed at Gouxi.

Organic matter in soil cores averaged 4.8 ± 0.75% (range: 3.7–6.2%) and 3.5 ± 0.59% (range: 2.4–4.6%) at Gouxi and Wukeng, respectively. The organic matter content in soil at Gouxi was slightly higher than that at Wukeng (*K-S* test, $p < 0.05$). The pH values in soil samples, which averaged 6.7 ± 0.10 (ranging from 6.6 to 6.9) at Gouxi and averaged 6.6 ± 0.14 (ranging from 6.3 to 6.9) at Wukeng, were nearly neutral during the rice growing season. Despite the pH values of the irrigation water and paddy water at Wukeng (irrigation water pH = 11 ± 0.45; paddy water pH = 8.6 ± 1.3) being significantly higher than those at Gouxi (irrigation water pH = 8.3 ± 0.24; paddy water pH = 7.2 ± 0.24) (Zhao et al., 2016), no significant difference of pH levels in soil cores was observed between these two sampling sites throughout the five sampling campaigns (*K-S* test, $p > 0.05$). These results indicated that the irrigation water and paddy water have little influence on the values and distributions of pH in soil cores. Furthermore, temporal and vertical distributions of organic matter content and pH in soil profiles of Gouxi and Wukeng showed little variation and remained nearly monotonic for each of the sampling campaigns.

3.2. Mercury in paddy water, irrigation water, and soil cores

Mercury in the ambient air, precipitation, irrigation water, and soil cores during the rice-growing season are documented in a companion paper (Zhao et al., 2016). Briefly, the mean concentrations of TGM in the ambient air at Gouxi (403 ± 399 ng m^{-3}) was much higher than that at Wukeng (28 ± 13 ng m^{-3}) during the rice growing season, which was attributed to the emission of TGM from nearby artisanal Hg smelters. Consequently, the HgT_{unf} concentration in precipitation at Gouxi was highly elevated (mean = 2599 ± 1874 ng L^{-1}) in comparison with the sampling site of Wukeng (mean = 445 ± 296 ng L^{-1}). However, no significant difference in $MeHg_{unf}$ concentration in precipitation between the two sampling sites was observed during the rice growing season (*K-S*, test, $p > 0.05$). During the rice growing season, the HgT_{unf} (mean = 513 ± 215 ng L^{-1}) and $MeHg_{unf}$ (1.7 ± 1.1 ng L^{-1}) concentrations in irrigation water (unfiltered) at Wukeng were significantly higher than those at Gouxi (mean

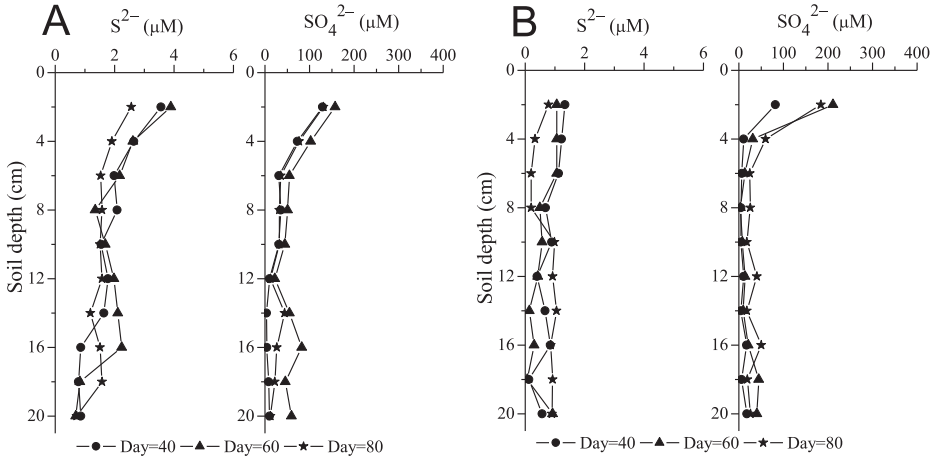

Fig. 1. Concentrations of S^{2-} and SO_4^{2-} in pore water collected from Gouxi (A) and Wukeng (B) during the rice growing seasons.

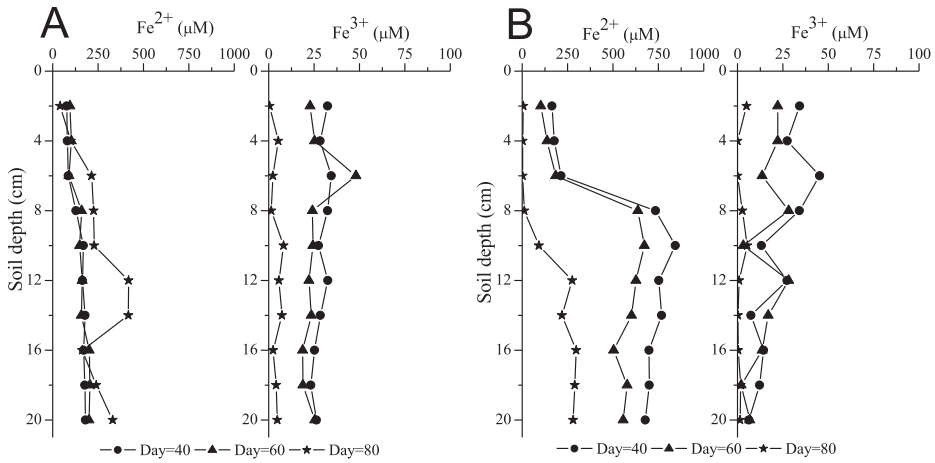

Fig. 2. Concentrations of Fe^{2+} and Fe^{3+} in pore water collected from Gouxi (A) and Wukeng (B) during the rice growing seasons.

$HgT_{unf} = 159 \pm 67$ ng L^{-1}; mean $MeHg_{unf} = 0.75 \pm 0.65$ ng L^{-1}) (*K-S*, test, $p < 0.001$ both for HgT_{unf} and $MeHg_{unf}$). Conversely, the highest values of $MeHg_{unf}$ in paddy water at each sampling time were all observed at Gouxi (mean = 13 ± 16 ng L^{-1}), whereas sampling at Wukeng (mean = 1.1 ± 0.52 ng L^{-1}) yielded a relatively low MeHg concentration in the paddy water throughout the rice growing season.

The mean concentration of HgT_f in soil pore water during the rice growing season was 142 ± 111 ng L^{-1} at Gouxi and 180 ± 160 ng L^{-1} at Wukeng, respectively. The corresponding THg concentration in soil cores (liquid phase plus solid phase) was 3.2 ± 0.75 mg 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. In contrast, the MeHg concentration in both soil pore water and soil cores collected from Gouxi was significantly higher than that for Wukeng throughout the rice growing season (*K-S* test, $p < 0.01$). Furthermore, the concentration of MeHg in pore water and soil cores was generally highest in the surface soil at Gouxi, and decreased with depth. However, the vertical distributions of MeHg in soil pore water and soil cores at Wukeng showed very little variation with depth.

3.3. Methylation/demethylation rate in paddy soil

The distributions of methylation/demethylation rate constants in soil cores collected from Gouxi and Wukeng are shown in Figs. 3 and 4. ^{202}Hg was added to the experimental plot exclusively in an inorganic form ($^{202}HgCl_2$). The only mechanism by which excess $Me^{202}Hg$ appears in paddy soil would be through *in situ* methylation. For soil samples collected from Gouxi and Wukeng rice paddies, the Hg methylation rate constant (K_m) ranged from 0.034×10^{-3} to 1.1×10^{-3} day^{-1} (mean = $(0.41 \pm 0.25) \times 10^{-3}$ day^{-1}) and from 0.054×10^{-3} to 0.81×10^{-3} day^{-1} (mean = $(0.20 \pm 0.15) \times 10^{-3}$ day^{-1}), respectively. Two-way ANOVA tests demonstrated that site variations of K_m were highly significant ($p < 0.001$). Statistical analysis indicated that Gouxi samples had significantly higher K_m than the Wukeng samples (*K-S* test, $p < 0.01$). To our knowledge, no data on K_m for paddy soil have been published previously. Methylation rates previously found from the Everglades (FL USA) ranged between 0.01 day^{-1} and 0.07 day^{-1} for wetland soil (Li et al., 2012), from Valdeazogue River (Spain) between 0.0038 day^{-1} and 0.13 day^{-1} for sediment (Gray et al., 2004), and from Oligotrophic Lake (WI USA) between 0.0051 day^{-1} and 0.028 day^{-1} for sediment (Korthals and Winfrey, 1987). The methylation rate we report in this paper for the Gouxi and Wukeng rice paddies are therefore relatively low.

As shown in Figs. 3 and 4, the vertical distribution patterns of the demethylation rate constant (K_d) in soil profiles were opposite to the corresponding K_m across the two sampling sites. Statistical analysis revealed that the K_d values in soil at Wukeng (0.55 ± 0.40 day^{-1}) were significantly higher than those at Gouxi (0.38 ± 0.23 day^{-1}) (*K-S* test, $p < 0.001$). Moreover, the lowest K_d in soil at Gouxi was present at the surface soil layer, and it showed a roughly increasing tread with soil depth. Generally, MeHg demethylation rates are pH value dependent (Ullrich et al., 2001). Gilmour and Henry (1991) showed that low pH not only increases methylation rate but also decreases demethylation rate in aquatic systems. As shown in a companion paper (Zhao et al., 2016), the sampling site for the Wukeng paddy was located next to a calcine pile and the proximity of this waste had a major impact on water chemistry. Both the irrigation water (pH = 11 ± 0.45) and paddy water (pH = 8.6 ± 1.3) were alkaline during the rice growing season (Zhao et al., 2016). Therefore, we proposed that the alkaline conditions of the irrigation at Wukeng could stimulate MeHg demethylation in paddy soil (Ullrich et al., 2001). Matilainen et al. (1991) reported a decrease in anaerobic demethylation in sediments with decreasing water pH. The findings of our study are in agreement with those of Matilainen et al. (1991). However, the data, which support the hypothesis, are limited. To better understand this observation, further work needs to be done. The K_d values in soil at Gouxi and Wukeng were comparable with reported data for soil from the Everglades in Florida, U.S.A. (0–0.25 day^{-1}) (Li et al., 2012), for Azogado creek sediment from Spain (0.0090–0.53 day^{-1}) (Gray et al., 2004), and for Gor Lake sediment from Ontario, Canada (0.39–0.53 day^{-1}) (Hintelmann et al., 2000).

Previous studies have demonstrated that methylation is not the only process regulating the MeHg concentrations in soil; demethylation of MeHg occurs simultaneously and is likely an important process limiting the increase in the proportion of MeHg in soil (Hintelmann et al., 2000). Net MeHg production is ultimately a function of the availability of Hg^{2+} to the community of Hg methylating bacteria and of the activity of those bacteria (Marvin-DiPasquale et al., 2009), as well as the rates of biotic (Marvin-DiPasquale et al., 2000) and abiotic (Sellers et al., 1996) processes that facilitate MeHg degradation. Indeed, it is important to note that because both methylation and demethylation processes occur, a state of equilibrium with a near-constant level of MeHg is typically a reflection of the combined effect of MeHg production and degradation that are occurring simultaneously and are mediated by a variety of microorganisms. Comparable relationships between *in*

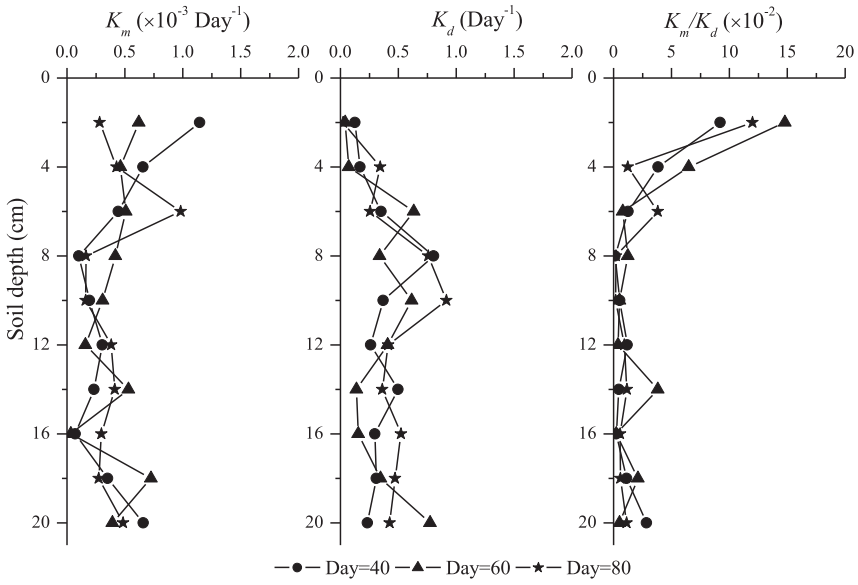

Fig. 3. Methylation rate constant (K_m), demethylation rate constant (K_d), and net Hg methylation potential (K_m/K_d) in the soil profile at Gouxi during the rice growing seasons.

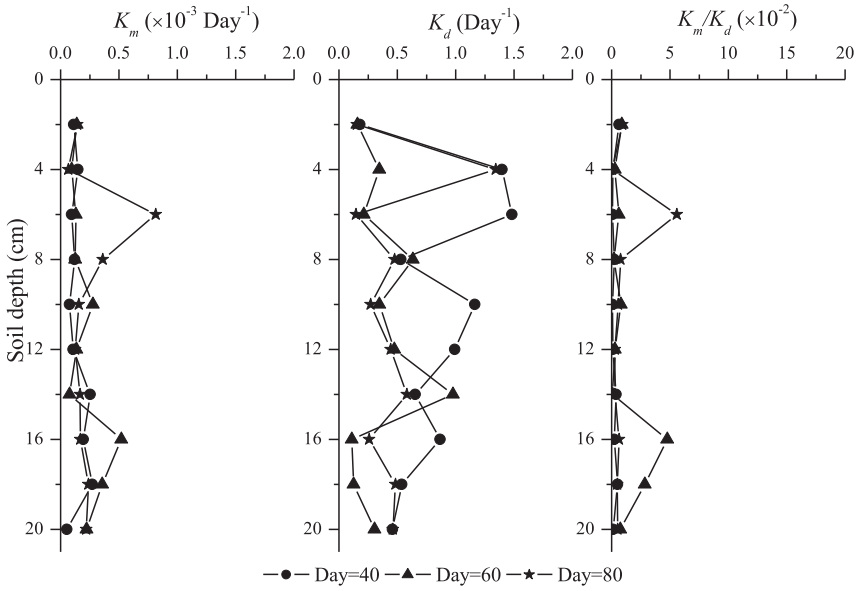

Fig. 4. Methylation rate constant (K_m), demethylation rate constant (K_d), and (K_m/K_d) in the soil profile at Wukeng during the rice growing seasons.

situ MeHg concentration and short-term K_m and K_d have been observed in this study. Our results showed that soil MeHg concentration was significantly positively correlated with K_m but significantly negatively correlated with K_d across the two sampling sites (Fig. 5). The strong correlation of soil MeHg with K_m and K_d suggests that MeHg levels in rice paddy soil were a function of both methylation and demethylation processes, and this is in agreement with previous observations (Drott et al., 2008).

The balance between MeHg production and degradation, namely, net Hg methylation potential, was calculated by using the ratio of Hg methylation relative to MeHg demethylation (K_m/K_d), to estimate the influence of methylation and demethylation processes on MeHg cycling and profile structure (Korthals and Winfrey, 1987). Results of this analysis showed that the maximum net Hg methylation potential was generally found at the surface layer at Gouxi, which varies during the different periods of the rice growing season and was similar to the natural MeHg concentration distributions, confirming the direct link between the extent of net Hg methylation and soil MeHg level. Both sites clearly showed a relatively strong positive relationship between the *in situ* MeHg concentration and the instantaneous net Hg methylation potential in soil (Gouxi: $r = 0.69$, $p < 0.001$, n = 30; Wukeng: $r = 0.52$, $p = 0.003$, n = 30), indicating that the net methylation potential in the rice paddy soil reflects the measured MeHg production at any time point. Moreover, results from the current study imply that the net Hg methylation process serves as a surrogate for MeHg production in ecosystems without significant exogenous sources of MeHg.

As shown above, Hg methylation rates in Gouxi were at the lower range of those reported from other wetland or riverine systems. However, the Hg concentration in both the solid and liquid phases of soil was high and representative of a mercury contaminated environment (Zhao et al., 2016). Furthermore, high K_m was

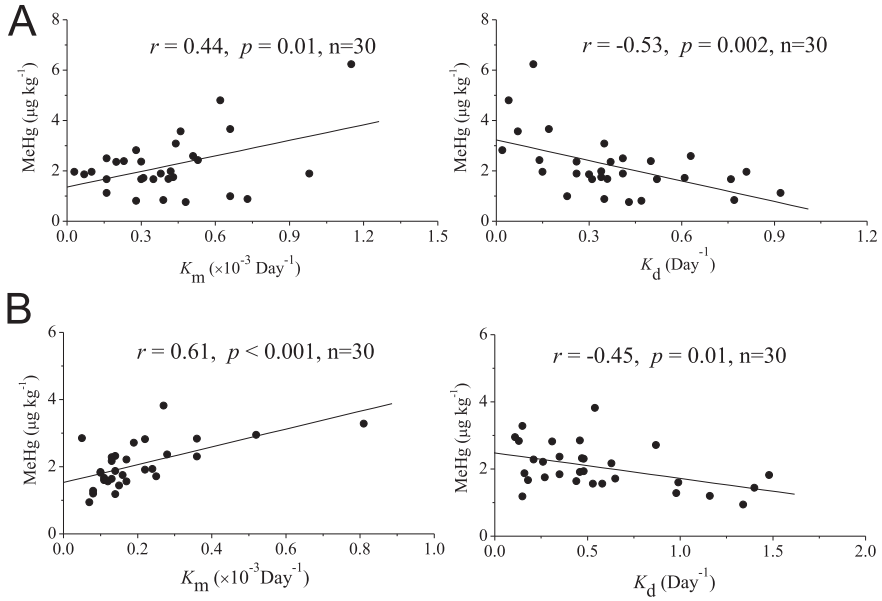

Fig. 5. Correlation between the MeHg concentration and methylation/demethylation rates in soil cores at Gouxi (A) and Wukeng (B) during the rice growing seasons.

accompanied by low K_d in paddy soil at Gouxi (Fig. 3). Consequently, the potential for net MeHg production, as indicated by the K_m/K_d ratios, were high, particularly so for the surface soil at Gouxi as samples from this site had a higher MeHg concentration than those at Wukeng. In comparison, low K_m was accompanied by high K_d in paddy soil at Wukeng, which resulted in the lower net Hg methylation potential when compared with the Gouxi soil profiles (Fig. 3). We propose that the lack of a surficial peak net Hg methylation potential in the Wukeng soil was not due to increased demethylation activity but to an increase in methylation relative to demethylation (Fig. 4). This implies that actual MeHg production in the surface soil of Wukeng may be restrained and that net methylation was reduced due to active MeHg degradation.

3.4. Factors controlling mercury methylation in rice paddies

A previous study suggested that SRB mediate the formation of sulfide as a result of respiration processes (King et al., 2002). Therefore, the presence of sulfide in pore water implies that sulfate-reducing bacteria were active in rice paddy soil during the rice growing season. Furthermore, the sulfide concentrations in soil pore water at Gouxi were much higher in the surface layer relative to deeper in the soil profile, indicating that maximum microbial sulfate reduction was stimulated in the uppermost layer of the soil.

Hydrogen sulfide plays an important role in the biogeochemical cycling of Hg in aquatic systems. In the presence of sulfide, Hg^{2+} can form insoluble mercuric sulfide (HgS) under anoxic conditions (Ullrich et al., 2001). Therefore, it is widely speculated that the inhibitory influence of sulfide on Hg methylation is attributed to the decreasing solubility and bioavailability of Hg^{2+} due to HgS precipitation (e.g., Craig and Bartlett, 1978; Winfrey and Rudd, 1990; Gilmour and Henry, 1991). However, HgS can be transformed into soluble Hg–S complexes (e.g., HgS_2^{2-}) in the presence of excess sulfide ions (Ullrich et al., 2001). A study conducted by Benoit et al. (1999) suggested that dissolved HgS could potentially be methylated. Consequently, the concentration of MeHg in sediment can increase as a function of an increasing sulfide concentration (Hintelmann and Wilken, 1995).

In this study, rice paddy soil from both Gouxi and Wukeng were serious contaminated due to historical large-scale Hg smelting combined with current-day artisanal Hg smelting activities. Consequently, HgT_f concentrations in soil pore water both from Gouxi and Wukeng were highly elevated during the rice growing season (Zhao et al., 2016). Regression analysis revealed a significant positive correlation between sulfide and sulfate concentrations in pore water at Gouxi ($r = 0.78$, $p < 0.001$, n = 30) (Table 1), implying that sulfate could enhance microbial sulfur reduction in rice paddies of Gouxi (production of sulfide) and then stimulate MeHg production. Statistical analysis showed that the net Hg methylation potential correlated positively with the concentrations of dissolved sulfate/sulfide in soil pore water at Gouxi (Table 1). Therefore, we speculate that, 1) methylation of Hg was likely stimulated in the soil cores at Gouxi due to the dependence of this process on soluble electron acceptors (e.g., sulfate); and 2) high concentrations of dissolved Hg^{2+} in soil pore water at Gouxi indicate that the solubility of HgS can be stimulated in the presence of excess sulfide, probably due to the formation of soluble Hg–S complexes. The findings of our study are in agreement with those of previous studies (e.g. Gagnon et al., 1997; Benoit et al., 1998). As summarized above (see detail in Introduction), Hg methylation is primary facilitated by a subset of bacteria including SRB (Gilmour et al., 1992), IRB (Fleming et al., 2006) and methanogens (Gilmour et al., 2013) in anoxic conditions. In this study, we cannot eliminate that other processes such as iron reduction and methane production may also influence Hg methylation by anaerobic microbes in the rice paddy soil. Thus, to better understand the processes of microbial Hg methylation in rice paddy, further work

Table 1
Pearson's Correlation Matrix, giving the Linear Correlation Coefficients (r) among the variables at the artisanal Hg mining site (n = 30).

	Fe^{2+}	Fe^{3+}	S^{2-}	SO_4^{2-}	K_m	K_d	K_m/K_d
Fe^{2+}	1	−0.47**	−0.53**	−0.50**	−0.10	0.26	−0.43**
Fe^{3+}		1	0.25	0.04	0.10	−0.05	−0.02
S^{2-}			1	0.78***	0.26	−0.39*	0.69***
SO_4^{2-}				1	0.37*	−0.49**	0.84***
K_m					1	−0.42*	0.49**
K_d						1	−0.65***
K_m/K_d							1

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2
Pearson's Correlation Matrix, giving the Linear Correlation Coefficients (r) among the variables at the abandoned Hg mining site (n = 30).

	Fe^{2+}	Fe^{3+}	S^{2-}	SO_4^{2-}	K_m	K_d	K_m/K_d
Fe^{2+}	1	0.18	−0.32	−0.47***	−0.15	0.17	−0.17
Fe^{3+}		1	0.26	−0.07	−0.37*	0.36	−0.26
S^{2-}			1	−0.22	−0.46*	0.05	−0.41*
SO_4^{2-}				1	0.10	−0.36*	0.07
K_m					1	−0.41*	0.91***
K_d						1	−0.49**
K_m/K_d							1

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

needs to be done.

Although the mean concentration of sulfate in pore water at Wukeng was significantly higher than that at Gouxi, the concentration of sulfide in pore water from Gouxi was significantly higher than that found from Wukeng. No relationship between sulfide and sulfate concentrations in pore water at Wukeng was observed during the rice growing season ($r = -0.20$, $p > 0.05$, n = 30) (Table 2). In addition, the relationship between sulfate/sulfide concentrations and net Hg methylation potential at Wukeng was less pronounced during the rice growing season (Table 2). These results suggest that Hg methylation and MeHg demethylation occurred throughout the rice paddy ecosystem; however, the specific microbiology of methylation and demethylation appears to vary within a rice paddy.

Multiple iron pools were tracked during the rice growing season, which provided a dynamic picture of temporal and vertical iron cycling. Significantly negative correlations between Fe^{2+} and sulfur (S^{2-} and/or SO_4^{2-}) in pore water across the two sampling sites were observed during the rice growing season ($p < 0.001$, n = 30 for Gouxi and Wukeng) (Tables 1 and 2). This implies that Fe^{2+} may restrain sulfur activity through the formation of solid FeS, apparently supported by a decrease in the concentrations of sulfide with the increase of Fe^{2+} (Coles et al., 2000). A previous study indicated that Hg^{2+} can be scavenged by solid FeS, which is known to retain trace metals by both adsorption and co-precipitation (Coles et al., 2000). Therefore, we speculate that iron cycling in rice paddies could impact the availability of Hg for methylation in pore water (Mehrotra and Sedlak, 2005).

Previous studies have reported that pH should have an impact on the methylation of Hg because acidic conditions might favor the diffusion of Hg from solid phase to liquid phase, which in turn may stimulate Hg methylation because of increased bioavailability (Ullrich et al., 2001). The results presented in the current study show a less clear effect of pH on the MeHg concentration in soil cores. Although the relationship between MeHg and pH was modest at Gouxi ($r = -0.41$, $p = 0.003$, n = 50), a mutual development between MeHg and pH was not obvious at Wukeng ($r = 0.04$, $p = 0.78$, n = 50), suggesting that additional factors are needed to explain MeHg variations. Alternately, the availability of organic matter could be an important factor controlling microbial Hg methylation in aquatic systems (Ullrich et al., 2001). Positive correlation between MeHg concentration and organic matter content in sediments and soil were widely observed (e.g., Lucotte et al., 1999; Meng et al., 2016), and this is generally attributed to a stimulating effect of organic nutrients (the decomposition of flooded vegetation and organic matter) on microbial methylation of IHg to MeHg. However, in the current study, a direct impact of organic matter content on MeHg concentrations cannot be detected across the two sampling sites (Gouxi: $r = 0.20$, $p = 0.17$, n = 50; Wukeng: $r = 0.04$, p = 0.78, n = 50). This suggests that the influence of organic matter on Hg methylation activity in rice paddy soil might be indirect.

MeHg levels do not necessarily correlate to the total amount of Hg in soil, indicating that the determination of THg is insufficient for understanding its biogeochemical cycle and potential effects (Zhao et al., 2016; Lin et al., 2012; Tessier et al., 1979). This is predominantly due to the lack of information concerning its reactivity, bioavailability, and toxicity. Instead, the most hazardous form of Hg in soil is that associated with the bioavailable fractions (soluble and exchangeable Hg fraction) due to the potential of this phase of Hg to be readily methylated (Bishop et al., 1998). Therefore, knowledge of the levels of bioavailable Hg in paddy soil is essential for understanding its transport behavior, potential bioavailability, and impact on the environment.

The concentration and distribution of bioavailable Hg in soil profiles collected from Gouxi and Wukeng are shown in Fig. S1. Briefly, the distribution of MeHg in soil cores paralleled the bioavailable Hg concentration throughout the rice growing season in Gouxi. Moreover, regression analyses yielded positive correlations when plotting MeHg vs. bioavailable Hg in Gouxi soil with a Pearson Correlation Coefficient of 0.80 ($p < 0.001$, n = 50) (Fig. 6). These results suggest that bioavailable Hg, which may serve as the substrate for the Hg methylation process, plays an important role in MeHg production in the paddy soil of Gouxi. Throughout the rice growing season, after initially increasing, bioavailable Hg values at Gouxi decreased when the rice paddy was drained and reached the lowest value during the rice-harvesting period (Fig. S1). Peng et al. (2012) observed that the HgT_f concentration in soil pore water declined dramatically in an aerobic treatment. Our results, together with the previous studies, confirm that periods of paddy field drying are associated with a decrease in the bioavailable Hg level, which could inhibit the Hg methylation during periods of desiccation and soil oxidation (Zhao et al., 2016).

On the contrary, no relationship between the MeHg and bioavailable Hg concentration in soil was observed at Wukeng ($r = -0.14$, $p = 0.34$, n = 50) (Fig. 6). This indicates that the bioavailability of Hg was not the key factor controlling MeHg soil distribution and was therefore not a useful indicator for predicting MeHg concentration in Wukeng soil. The influence of Hg chemistry on methylation was therefore site-specific. Differences in net MeHg production between the two sites may be due to differences in

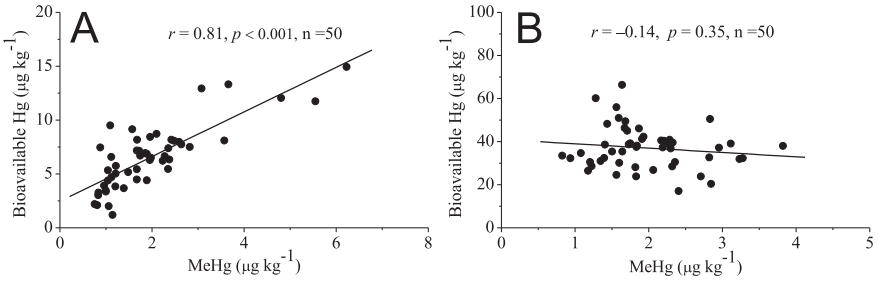

Fig. 6. Correlations between bioavailable Hg and MeHg concentrations in soil cores at Gouxi (A) and Wukeng (B) during the rice growing seasons.

atmospheric Hg flux between Wukeng and Gouxi (Zhao et al., 2016).

Since 2002, Hg levels in the air and the corresponding rate of Hg deposition to soil have declined at Wukeng, and this decline is attributed to a decrease in local Hg emissions. However, atmospheric deposition of Hg at Gouxi has stayed relatively constant over the past decade due to the ongoing artisanal Hg retorting activities using artisanal techniques. Subsequently, Hg levels in ambient air and deposition during the sampling campaigns were relatively low at Wukeng compared to Gouxi, as summarized in Section 3.2. We propose that bioavailable Hg in Wukeng soil originated from historical large-scale Hg retorting via wet and dry deposition; the limited range of this Hg form in Wukeng soil cores could be a result of low levels of primary Hg deposition together with continuous soil disturbance through cultivation. Therefore, we suggest that the relatively low MeHg concentration in soil at Wukeng is indicative of old Hg which has become tightly bound to soil complexes over time, and is unavailable for methylation (Zhao et al., 2016). In contrast, the elevated concentration of bioavailable Hg in the surface soil of Gouxi was primary attributed to the direct atmospheric deposition from ambient air. The elevated concentration of MeHg in the surface soil at Gouxi provides further evidence for the previously reported claim that newly deposited mercury can be expected to rapidly methylate after deposition (Zhao et al., 2016). However, that the importance of environmental conditions associated with sulfur (e.g., sulfide and sulfate) that may enhance MeHg production by stimulating Hg methylation at Gouxi cannot be excluded. Because MeHg can be demethylated to IHg biotically and abiotically in soil or paddy water, rapid cycling occurs between the IHg and MeHg pools. Current data were limited to only the rice growing season, not the entire year or a long period of time, and therefore our results represent an initial rather than a long-term assessment of the influence of bioavailable Hg on the MeHg production. Such a longer-term study for the rice paddy ecosystem is warranted based on the findings of the current work.

4. Conclusion

Active net Hg methylation and an elevated MeHg concentration in paddy soil at Gouxi were consistent with significantly higher levels of current-day Hg deposition to soil than at the historically contaminated Wukeng mining site. The elevated MeHg concentration in soil and net Hg methylation potential at Gouxi indicate not only that the newly deposited Hg is readily transformed to MeHg in paddy soil but also that the older Hg in soil can be tightly bound to organic material and is not available for methylation.

The peak in MeHg concentrations in the surface soil layer at Gouxi resulted from the combination of elevated net Hg methylation potential and the increasing availability of Hg. These results confirmed the importance of both bacterial activity and Hg speciation in controlling MeHg concentration in rice paddy soil. The balance between sulfate availability, which controls SRB activity, and sulfide production and accumulation, which control Hg bioavailability, are critical in controlling Hg methylation rates. Sulfate stimulation of SRB activity is a potentially important metabolic pathway for Hg methylation; bioavailable Hg derived from atmospheric deposition is the primary limiting factor in permanently flooded rice paddies. However, ongoing work is urgent to further ascertain the relative importance of microbial Hg methylation in rice paddy ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.05.001.

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