Effects of soil properties on production and bioaccumulation of methylmercury in rice paddies at a mercury mining area, China

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ABSTRACT

Rice paddy soil is recognized as the hotspot of mercury (Hg) methylation, which is mainly a biotic process mediated by many abiotic factors. In this study, effects of key soil properties on the production and bioaccumulation of Hg and methylmercury (MeHg) in Hg-contaminated rice paddies were investigated. Rice and soil samples were collected from the active Hg smelting site and abandoned Hg mining sites (a total of 124 paddy fields) in the Wanshan Mercury Mine, China. Total Hg (THg) and MeHg in soils and rice grains, together with sulfur (S), selenium (Se), organic matter (OM), nitrogen (N), phosphorus (P), mineral compositions (e.g., SiO₂, Al₂O₃ and Fe₂O₃) and pH in soils were quantified. The results showed that long-term Hg mining activities had resulted in THg and MeHg contaminations in soil-rice system. The newly-deposited atmospheric Hg was more readily methylated relative to the native Hg already in soils, which could be responsible for the elevated MeHg levels in soils and rice grains around the active artificial Hg smelting site. The MeHg concentrations in soils and rice grains showed a significantly negative relationship with soil N/Hg, S/Hg and OM/Hg ratio possibly due to the formation of low-bioavailability Hg-S(N)-OM complexes in rhizosphere. The Hg-Se antagonism undoubtedly occurred in soil-rice system, while its role in bioaccumulation of MeHg in the MeHg-contaminated rice paddies was minor. However, other soil properties showed less influence on the production and bioaccumulation of MeHg in rice paddies located at the Wanshan Mercury Mine zone.

Introduction

Mercury (Hg), especially methylmercury (MeHg), is highly toxic and has a large capability to be bioaccumulated and biomagnified in food webs. Inorganic Hg (IHg), undergoing biotic and abiotic processes, can be transformed to MeHg, which poses a potential threat to human and wildlife health (Ullrich et al., 2001). It is commonly believed that the consumption of fish is the main MeHg exposure to humans (Mergler et al., 2007). Recently, higher MeHg levels were found in rice (Oryza sativa L.) than other crops (e.g., corn and wheat), and studies indicated that rice paddies were hotspots for Hg methylation (Meng et al., 2014; Zhang et al., 2015).
As rice is a staple food for most populations in the world, MeHg bioaccumulation in rice is becoming a new concern, especially to inland inhabitants who seldom eat fish (Meng et al., 2010, 2014; Zhang et al., 2010a).

Previous reports showed that rice is a bioaccumulator of MeHg, which is mainly derived from paddy soils (Meng et al., 2010; Zhang et al., 2010a). The distribution of MeHg in rice tissues is different from Hg, and is usually greater in grains than other tissues (Meng et al., 2010; Zhang et al., 2010a). The MeHg content in paddy soil can be readily adsorbed into roots, where MeHg is combined with organic matter, polyaccharide, and nucleic acid, and then is transferred to grains during the ripening period (Meng et al., 2011). However, phytochelatin present in the roots can more effectively chelate Hg(II) than MeHg, which prevents the divalent Hg(II) from entering into rice grains (Krupp et al., 2009), whereas atmospheric Hg is one of the important sources of Hg in aboveground tissues (e.g., leaves) in Hg-contaminated area (Meng et al., 2010). Although many studies have been conducted, the mechanism of MeHg bioaccumulation in rice is still not well understood, and warrants further investigation (Meng et al., 2010, 2011, 2012, 2014; Qiu et al., 2008; Rothenberg et al., 2013; Zhang et al., 2010a).

Hg methylation in rice paddy soils is largely produced by sulfate-reducing bacteria (SRB), a principal methylator under anoxic conditions (Liu et al., 2014b; Wang et al., 2014b). This biotic process is usually mediated by a range of factors, such as bioavailability of Hg (Meng et al., 2014), source of Hg species (Zhao et al., 2016a), newly-deposited atmospheric Hg (Zhao et al., 2016b), water management (Wang et al., 2014b), selenite (Se) (Wang et al., 2016b; Zhang et al., 2012), sulfate (Liu et al., 2014b; Wang et al., 2016c), organic matter (OM) (Liu et al., 2014a), and pH (Zhao et al., 2016a). However, the potential effects of some key soil properties on Hg biogeochemistry in rice paddies, such as nutrients (e.g., nitrogen (N), phosphorus (P)) and mineral compositions (e.g., SiO2, Al2O3, Fe2O3), are less understood. As described above, the MeHg present in paddy soils greatly affects the contaminated levels of rice. Therefore, factors mediating soil Hg methylation will ultimately affect the MeHg bioaccumulation in rice. However, the effects of soil properties are usually ambiguous. For example, OM, containing various O-, N- and S-bearing ligands, can complex Hg(II) (Skyllberg et al., 2006; Skyllberg and Drott, 2010), while OM is an important electron donor for SRB (Graham et al., 2012). In many studies, Hg/dissolved OM (DOM) concentration ratio is used to estimate the Hg-ligands interaction or Hg mobility in soil/sediment (Aiken et al., 2003; ¨Akerblom et al., 2008; Frohne et al., 2012; Haitzer et al., 2002; Hesterberg et al., 2001). As a matter of fact, both sulfides (Han et al., 2008; Skyllberg and Drott, 2010) and Se (Zhang et al., 2012) in soils have high affinity with Hg(II), while sulfate is the key electron acceptor for SRB (Shao et al., 2012). Therefore, the biogeochemical controls on Hg methylation in paddy soils are extremely complex and need further investigation, especially in the areas contaminated by long-term mining activities.

Wanshan mercury mine (WMM) is the largest Hg mine in China. Long-term Hg mining activities have resulted in serious Hg contamination to the local ecosystem such as soil, sediment, water, atmosphere, plants, and humans in the WMM (Li et al., 2009; Qiu et al., 2005, 2009; Wang et al., 2007). One of the greatest concerns in WMM is Hg contamination of rice paddies, which is attributed to the fact that flooding conditions can facilitate Hg methylation, and rice has a higher capability to uptake MeHg than Hg (Zhang et al., 2010a). The bioaccumulation factor (BAF) of MeHg in rice was about 2 to 3 magnitudes higher than Hg (Zhang et al., 2010a). Extremely high levels of MeHg (>100 ng/g) are reported in the edible portion of rice from the WMM (Qiu et al., 2008), and consumption of rice has been demonstrated to be the major exposure pathway of MeHg to the local population (Li et al., 2015). Therefore, alleviating the exposure risk of MeHg caused by the Hg-contaminated rice is urgent.

In this study, we report the spatial distribution of total Hg (THg) and MeHg concentrations in soils and rice grains from 124 rice paddies of the WMM. These rice paddies were located either at abandoned Hg mining sites, or at an active artificial Hg smelting site. Meanwhile, multiple soil properties, such as S, Se, OM, N, P, mineral compositions (e.g., SiO2, Al2O3, Fe2O3) and pH, were measured to find the key factors determining the production and bioaccumulation of MeHg in rice paddies of the WMM. Better understanding of these key factors controlling the biogeochemical cycling of Hg in rice paddy ecosystem will help mitigate the problem of Hg-contaminated rice grains.

1. Materials and methods

1.1. Sample collection and preparation

Although large-scale mining activities in the WMM were ceased in 2001, small-scale artisanal smelting was still active when the samples for the current study were collected. Therefore, two typical areas were emphasized (i.e., the abandoned Hg mining sites and the active artisanal Hg smelting site, Fig. 1). Historical Hg mining activities have produced roughly 125.8 million tons of mine waste materials, and several large tailings were formed at the head of major rivers of the WMM (Li et al., 2013). Along these rivers, there are rice paddies which use the river water for irrigation. The concentrations of total gaseous Hg (TGM) in ambient air and THg in precipitate at artisanal Hg smelting site were significantly higher than those around the abandoned Hg mining sites during the rice growing season (Zhao et al., 2016a). However, THg concentrations in irrigation water were found the higher levels in abandoned Hg mining areas (Zhao et al., 2016a).

In September 2012, rice had entered into full ripe stage, and paddy soils were in a moist state. Rice grain and corresponding soil samples adjacent to root surface (0–10 cm in depth) were collected using a wooden shovel from 124 rice paddies in the WMM (numbers of paddies in the abandoned Hg mining sites and the active artisanal Hg smelting site, Fig. 1). Historical Hg mining activities have produced roughly 125.8 million tons of mine waste materials, and several large tailings were formed at the head of major rivers of the WMM (Li et al., 2013). Along these rivers, there are rice paddies which use the river water for irrigation. The concentrations of total gaseous Hg (TGM) in ambient air and THg in precipitate at artisanal Hg smelting site were significantly higher than those around the abandoned Hg mining sites during the rice growing season (Zhao et al., 2016a). However, THg concentrations in irrigation water were found the higher levels in abandoned Hg mining areas (Zhao et al., 2016a).
was collected from 3 to 4 points, and was sealed and double-bagged. Soil samples were preserved in situ with liquid nitrogen (approximately \(-196^\circ C\)). After transportation to the laboratory within 24 hr, soil samples were freeze-dried \((-60^\circ C)\), ground to pass through a 150 mesh, and then mixed homogenously. Rice samples were also freeze-dried, shelled to brown rice using a rice huller (Model ILG-4.5, Taizhou Food Instrument Factory, China), and grounded to pass through a 120 mesh using a high-speed crusher (Model DFT-100, Lin Da Machinery Co., Ltd., China).

1.2. Sample analysis

Rice total Hg (THg_{rice}) concentrations analysis followed a method by (Zheng et al., 2006). About 0.1–0.2 g of rice sample was digested with 5 mL of HNO₃ in a water-bath \((95^\circ C)\) for 3 hr. Hg in digest solutions was measured by a cold vapor atomic fluorescence spectroscopy (CVAFS) (MODELIII, Brooks Rand LLC, USA) with a detection limit of 0.6 pg.

Soil total Hg (THg_{soil}) concentrations analysis followed a method by (Zhang et al., 2010a). About 0.2 g of soil sample was digested with 5 mL of aqua regia (HCl:HNO₃ = 3:1, V/V) in a water-bath \((95^\circ C)\) for >30 min, before being determined by a cold vapor atomic absorption spectrometry (CVAAS, F732-S, Shanghai Huaguang Instrument Co., Ltd., China) with a detection limit of 0.1 μg/L.

Soil MeHg (MeHg_{soil}) concentrations were measured using the previous method by (He et al., 2004). Briefly, about 0.2 g of soil was leached using HNO₃ \((H_2O:HNO₃ = 3:1, V/V)\) and CuSO₄ (2 mol/L). MeHg in sample was extracted with CH₂Cl₂ solution, back-extracted from solvent phase into water, and then the aqueous phase ethylated using 1% sodium tetraethylborate (NaBEt₄). The CH₃CH₂CH₂Hg, ethyl analog of MeHg, was enriched onto a Tenax trap (Tenax-TA, 20/35 meshes, Restek corporation, USA) by purging with N₂, and then thermally desorbed, separated from other Hg species using an isothermal gas chromatography column in a 85°C oven (GC, 15%OV-3, Chromosorb W-AW80/100, Brooks Rand LLC, USA), and then reduced to Hg⁰ in a pyrolytic decomposition column (700°C). Finally the Hg⁰ was examined by CVAFS.

Rice MeHg (MeHg_{rice}) concentrations were measured according to the report by (Liang et al., 1996). About 0.15 g of rice sample was digested using a KOH-methanol/solvent extraction technique at a water-bath \((80^\circ C)\) for 3 hr. Subsequent extraction, back-extraction and detection were same with the MeHg_{soil} analysis.

Soil OM and pH were measured by methods reported previously (Meng et al., 2011). Mineral compositions \((e.g., SiO₂, Al₂O₃, and Fe₂O₃)\), P, S, Se and N concentrations of the soil samples were measured at the Institute of Geological Mining Key Laboratory, Guizhou province. SiO₂, Al₂O₃, Fe₂O₃ and P concentrations were measured using a fluorescence spectrophotometer (ZSX Primus II, Rigaku Industrial Corporation, Japan) (Basak et al., 2011). S and N were measured by combustion iodometry and the Kjeldahl method, respectively (Bremner, 1996; Zhou and Zhu, 2012). Soil Se concentrations were measured using an hydride generation atomic fluorescence spectrophotometer (XGY-1011A,
Institute of Geophysical and Geochemical Exploration, CAGS Co., Ltd., China (Zhang et al., 2012).

1.3. Quality assurance and quality control (QA/QC)

Quality control for sample detection was conducted by matrix spikes, duplicates and standard reference materials (SRM). GSB-11 (citrus leaf, GBW10020, Institute of geophysical and geochemical exploration, CAGS Co., Ltd., China) and GSS-5 (soil, GBW07405, Institute of geophysical and geochemical exploration, CAGS Co., Ltd., China) were used as SRM during the THgsoil and THgrice analysis, respectively. Recoveries for THg analysis ranged from 84 to 106%, and relative standard deviation (RSD) for duplicate analysis was <10%.

The SRM was ERM-CC580 (estuarine sediment) and TORT-2 (lobster hepatopancreas) for MeHgsoil and MeHgrice analysis, respectively. Recoveries for MeHg analysis were between 89% and 117%, and RSD for duplicate analysis was <8%. Quality control of MeHgrice analysis was also carried out using matrix spikes, and recoveries for matrix spikes were from 85% to 106%.

For SiO2, Al2O3, Fe2O3, P, S, N and Se analysis, GSS-5 was used as the SRM, which showed recoveries from 89 to 116%, and RSD for duplicate analysis was <7%.

2. Results

2.1. General properties in rice paddy soils

The pH values ranged from 4.71 to 6.90 with an average of 6.14, indicating acidic to slightly acidic of the soils. The soil OM and N concentrations ranged from 1.22% to 7.29% and 0.03% to 0.36% with average of 3.79% and 0.21%, respectively. Compared to other soils in China, most samples had higher OM and N values according to the national soil survey standard (Office of national soil survey, 1979). The P concentrations (0.03% to 0.13% with an average of 0.06%) were generally higher than the value of soil P-deficiency (0.03%) (Tiwari and Tiwari, 1993). High N and P levels indicated that the nutrients to rice growing were sufficient. The S concentrations (0.02% to 0.11% with an average of 3.79%) were in general higher than the average S (0.03%) in paddy soils of South China (Liu et al., 1990). Both N (Pearson correlation coefficient, r = 0.67, p < 0.01, n = 124, Fig. 2a) and S (r = 0.58, p < 0.01, n = 124, Fig. 2b) showed significant positive correlation with OM, demonstrating that OM was an important carrier for N and S in the rice paddy soils. This result indicated that the reactive S-, N-bearing ligands in soils might be elevated with the increase of soil OM (e.g., organic fertilizers input). Soil Se concentrations (0.16 to 15.72 μg/g with average of 1.71 μg/g) were well consistent with previous results in the WMM (Horvat et al., 2003), but were much higher than the maximum concentration of 0.05 μg/g in paddy soils of the Yangtze River delta (non-mercury contaminated zone) (Cao et al., 2001). According to the soil Se classification standard in China (Tan, 1996), approximately 60% of the rice paddies were in a Se-rich state (0.45–2.0 μg/g), and 10% of the sites exceeded the baseline of 3.0 μg/g for Se-excessive soil. Other properties, such as SiO2, Al2O3 and Fe2O3, were within the range of Chinese soil (Yan et al., 1997).

2.2. THg in soils and rice grains

The THgsoil concentrations ranged from 0.5 to 187.8 μg/g with an average of 31.0 μg/g, which unexceedingly exceeded the maximum Hg level (0.3 μg/g) restricted for Chinese agricultural soils (State Environmental Protection Administration of China, 1995). Like S and Se, the THgsoil concentrations were higher further upstream of several WMM rivers (Fig. 4a), indicating that Hg mining activities have resulted in the changes of not only the soil properties, but also the soil Hg levels. In comparison, the rice paddies around active artisanal Hg smelters had the extremely significantly lower soil THg concentrations than those at abandoned Hg mining sites (T-test, p < 0.01, Fig. 5a), indicating that the atmospheric Hg deposition rarely determined the distribution of THg in surface soils.

THgrice concentrations ranged from 7.9 to 316.9 ng/g, and > 60% of samples had THgrice higher than the maximum Hg level (20 ng/g) restricted for food in China (Ministry of Health Standardization Administration of China, 2005). The THgrice concentrations were also higher at the upstream of the rivers (Fig. 4b), however, we did not observe clear correlation between THgrice and THgsoil (p > 0.05). Although MeHg in rice was mainly derived from the soils (Meng et al., 2010, 2011), previous studies...
Fig. 3 – Spatial distribution of S and selenium (Se) concentrations in paddy soils of Wanshan mercury mine (WMM).

Fig. 4 – Spatial distribution of soil total Hg (TH_{soil}) (a), rice total Hg (TH_{rice}) (b), soil MeHg (MeHg_{soil}) (c) and rice MeHg (MeHg_{rice}) (d) in rice paddy soils of WMM.
had shown that, as well as soil Hg (Rothenberg et al., 2011), atmospheric Hg is an important source of Hg in rice tissues (Meng et al., 2010, 2012; Yin et al., 2013a). The elevated THgrice levels in the paddies upstream of the WMM rivers was consistent with the higher atmospheric Hg concentrations in these areas (Meng et al., 2012). This might be used to explain the absence of correlation between THgsoil and THgrice. In addition, the rice paddies near the active artisanal Hg smelters associated with high atmospheric Hg concentrations had extremely significant higher THg concentrations in rice grains compared to those in the areas with abandoned Hg tailings and wastes (T-test, \( p < 0.01 \), Fig. 5b), indicating that atmospheric Hg concentration was one of good indicators of the THg contamination level in rice grains. Hg0 derived from active artisanal Hg smelters could be converted to high bioavailable Hg species (e.g., Hg(II), undergoing ozone-mediated processes (Wang et al., 2011)), which was subsequently deposited onto rice paddy soil via wet and dry deposition. These Hg species could be readily adsorbed by roots, and be transported to aboveground parts, and this resulted in a high enrichment of THg in rice grains cultivated near the active Hg smelters.

2.3. MeHg in soils and rice grains

The MeHgsoil concentrations ranged from 0.7 to 6.7 ng/g with an average of 2.8 ng/g, which were much higher than the background levels in the WMM (0.1 to 0.3 ng/g) (Qiu et al., 2005). Previous studies reported that MeHg concentrations in paddy soils of WMM were higher than those in corn soils, and this was usually attributed to the formation of anoxic conditions caused by seasonal flooding, which could facilitate Hg methylation by sulfate- and iron-reducing bacteria (Qiu et al., 2005; Rothenberg et al., 2012; Zhao et al., 2016a, 2016b). As depicted in Fig. 4c, the MeHgsoil concentrations were higher further upstream of WMM rivers, and were positively correlated with THgsoil \( (r = 0.45, p < 0.01, n = 124) \). This could be attributed to the fact that tailings were usually formed further upstream of rivers, where rice paddies received more Hg through irrigation. Although active Hg smelting activities had less contribution to the THg levels in paddy soils, significant high MeHg concentrations were found (T-test, \( p < 0.05 \), Fig. 5d), indicating that MeHg production was increased in surface soils with high atmospheric deposition. The newly-deposited Hg (e.g., Hg(II), conversion of emitted Hg0 through ozone-mediated processes (Wang et al., 2011)) entering a low-pH anoxic paddy soils was believed to favor the in situ MeHg production (Zhao et al., 2016a), and this may explain the high MeHg concentrations in soils near the active artisanal Hg smelters. Contrary, old Hg already present in abandoned Hg mining area was less bioavailable due to its tightly bound to soil complexes over time (Hintelmann et al., 2002; Zhao et al., 2016a).

The MeHgrice concentrations ranged from 2.3 to 43.2 ng/g with an average of 13.2 ng/g, which were higher than previous results in pristine regions (Li et al., 2012). The MeHgrice was positively correlated with both MeHgsoil \( (r = 0.54, p < 0.01, n = 124) \) and THgsoil \( (r = 0.64, p < 0.01, n = 122) \), but not with THgsoil \( (p > 0.05) \), indicating that MeHg accumulation in rice was largely affected by Hg methylation efficiency in soils. This observation is consistent with previous results that rice paddies were hotspots for Hg methylation, and rice mainly received MeHg from soils (Meng et al., 2011). The MeHgrice exhibiting similar distribution patterns to THgsoil and MeHgsoil, but not to THgsoil, showed significant negative relationships with distance away from all abandoned Hg tailings located at the upstream of rivers (Pearson correlation, \( p < 0.01 \) for MeHgsoil, THgsoil and MeHgsoil) (Fig. 4b–d). However, no similar result was achieved in active artisanal Hg smelting site.
The proportion of MeHg to THg in soils (FMeHgsoil), an indicator of methylation efficiency of soil Hg (Frohne et al., 2012), ranged from 0.0 to 0.5% with an average of 0.05%, suggesting that soil Hg was mainly in the IHg form (Qiu et al., 2005). However, the percentage of MeHg to THg in rice grains (FMeHgrice) was much higher, which ranged from 7.1 to 68.4% with an average of 34.0%. A significant negative correlation between FMeHgrice and THgrice (\( r = -0.59, p < 0.01, n = 124 \)) showed less of a relationship with both MeHgsoil (\( p > 0.05 \)) and MeHgsoil (\( p > 0.05 \)). This indicated that FMeHgrice was largely determined by THg concentrations present in rice grains, which could be increased by elevated atmospheric Hg levels.

2.5. Bioaccumulation factors of THg and MeHg in rice grains

The bioaccumulation factors of THg (BAFTHg) and MeHg (BAFMeHg) in rice grains were calculated. The BAFTHg (0.0–8.3%) was much lower than the BAFMeHg (107.1%–1227.3%), suggesting that there was a stronger capability to accumulate soil MeHg than THg. A small portion of soil IHg was incorporated by plants due to the barrier effects of roots (Krupp et al., 2009). In addition, rice cultivated in the areas affected by active artisanal Hg smelting activities showed an obviously elevated BAFTHg compared to that around the abandoned Hg mining sites (T-test, \( p < 0.01, \) Fig. 5c), and this could be contributed to the high phytoavailability of deposited atmospheric Hg in surface soil. The BAFMeHg (T-test, \( p < 0.05, \) Fig 5f) followed the similar rule like BAFTHg. However, considering that the MeHg present in rice was mainly derived from soils rather than atmosphere (Zhao et al., 2016a), it was reasonable to believe that the in situ MeHg production in surface soils was greatly dependent on the elevated atmospheric Hg levels. The wet/dry deposition, occurring in active artisanal smelting areas, contributed to more new Hg (e.g., Hg(II)) in rice paddy soils (Wang et al., 2011), where these bioavailable Hg was more readily methylated. Then, more deposited Hg(II) and synthetic MeHg in rhizosphere were adsorbed and transported into rice bodies through roots, which resulted in the high bioaccumulation capacity of THg and MeHg near the active smelters. In other words, the biogeochemical cycling of atmospheric Hg was one of important factors determining the soil Hg methylation, especially in the active artisanal smelting areas, which warranted further investigation.

2.6. Influencing factors of production and bioaccumulation of MeHg in rice paddies

The THgsoil showed a significant relationship with soil Se (\( r = 0.50, p < 0.01, n = 124, \) Fig. 6a), indicating that Hg and Se might have the similar source. Previous studies demonstrated that Hg tailings were important source of Hg in the WMM soils, and these tailings had high Se concentrations (Qiu et al., 2005; Zhang, 2011; Zhang et al., 2014). Nevertheless, no clear correlation was observed between THgsoil and other soil properties (e.g., pH, OM, S, N, P, SiO2, Al2O3, and Fe2O3).

As described above, the organic ligands in soil OM were important factors affecting the bioavailability of Hg. In this study, the N/Hg, S/Hg and OM/Hg concentration ratio were calculated to estimate the Hg-ligands interaction in soils. Soil N/Hg ratio (\( r = -0.53, p < 0.01, n = 124, \) Fig. 7a), OM/Hg ratio (\( r = -0.52, p < 0.01, n = 124, \) Fig. 7c), and S/Hg ratio (\( r = -0.52, p < 0.01, n = 124, \) Fig. 7e) showed the significant negative relationships with MeHgsoil. This result indicated that the elevated N/Hg ratio, S/Hg ratio and OM/Hg ratio in soils inhibited the production of MeHg possibly due to the formation of Hg-OM, Hg-S-OM or Hg-N-OM complexes, which prevented Hg(II) from methylation in soils (Haitzer et al., 2002; Hesterberg et al., 2001; Skyllberg et al., 2006). Like MeHgsoil, the significant negative relationships of soil N/Hg ratio (\( r = -0.36, p < 0.01, n = 124, \) Fig. 7b), OM/Hg ratio (\( r = -0.34, p < 0.01, n = 124, \) Fig. 7d), and S/Hg ratio (\( r = -0.42, p < 0.01, n = 124, \) Fig. 7f) with MeHgsoil, were observed, indicating the distribution of MeHg in rice grains was largely controlled by the OM (N, S)-Hg interactions in soils.

In this study, there were no clear relationships between FMeHgsoil and soil properties such as pH, OM, P, N, SiO2, Al2O3, and Fe2O3 (\( p > 0.05 \)), whereas FMeHgsoil was negatively correlated with Se (\( r = -0.33, p < 0.01, n = 123, \) Fig. 6b). This implied that Se showed the potential inhibitory effects to Hg methylation in soils. Previous studies showed that bioavailable Hg(II) in soils could be immobilized by Se through the formation of Hg-Se complexes (Hg(II) + Se(II) → HgSe), which could reduce bioavailable Hg(II) from methylation (McNear et al., 2012; Wang et al., 2016c; Zhang et al., 2012).

The BAFTHg showed a significant negative correlation with soil Se concentrations (\( r = -0.26, p < 0.01, n = 122, \) Fig. 6c), but less relationship with other soil factors (\( p > 0.05 \)). A previous study demonstrated that the activity of membrane transporters of Hg was lowered by the addition of Se, which restricted the uptake of Hg into root cells (Wang et al., 2014a). Apart from that, Hg-Se antagonism mainly occurred in soils and roots rather than aboveground tissues (e.g., grains), which reduced Hg transportation from soils/roots to grains (Tang et al., 2017). This may explain the weaker relationship between THgsoil (MeHgsoil) and soil Se (both \( p > 0.05 \)). Moreover, no clear relationship between BAFMeHg and soil Se was found in this
study (Fig. 6d), indicating that soil Se did not suppress the bioaccumulation of MeHg in rice grains.

3. Discussions

3.1. Se–Hg antagonism in Hg-contaminated soils

It was commonly believed that Se–Hg antagonism occurred in mammals, aquatic organisms and humans, which alleviated the toxicity of Hg (Bjerregaard et al., 2011; Pelletier, 1986; Pinheiro et al., 2009). Recent studies had confirmed that the Se-Hg interactions also occurred in soil-root system, which could largely limit the bioavailability, adsorption, and bioaccumulation of Hg in rice paddies (Wang et al., 2014a, 2016c; Zhang et al., 2012). Our results indicated that Se in Hg-contaminated paddy soils possibly had the same source as Hg, such as irrigation water input. In addition, soil Se exhibited positive inhibitory effects on the methylation potential of Hg in soils and the bioaccumulation of THg in rice grains. The less bioavailable Hg-Se complexes were mostly responsible for the reduced MeHg production (Wang et al., 2016c; Zhang et al., 2012). Moreover, the development of apoplastic barriers in the endodermis might restrict the adsorption of Hg in rice tissues (Wang et al., 2014a). Hg-Se antagonism occurring in soil-root system may greatly alleviate the Hg exposure risk to humans. However, only 10% of the soil samples had a Hg/Se molar ratio < 1 (Fig. 6a), indicating that the Se present in Hg-contaminated soils may not effectively protect against the biotic toxicity of Hg. Previous studies revealed that Se-inhibited MeHg production was largely dependent on not only the formation of Hg-Se nanoparticles, but also Se dose alteration in soils (Wang et al., 2014a, 2016a, 2016c). It was speculated that a considerable portion of bioavailable Hg was still methylated in paddy soils, which resulted in little evidence of Se-inhibited bioaccumulation of MeHg in the MeHg-contaminated soils. This might explain the weaker relationship between soil Se and BAFMeHg (Fig. 6d). Although Se application as a remediation agent in the rice cultivation was proposed (Tang et al., 2017; Zhang et al., 2012), the Se-rich levels in paddy soils of the WMM may pose a Se exposure risk to local people. Therefore, dual role of Se acting as either an essential element at appropriate levels or a toxic substance at higher levels is one of most important concerns if Se–Hg antagonistic effects are utilized.

3.2. OM-Hg interaction in Hg-contaminated soils

The roles of OM in the biogeochemical cycling of Hg in soil, sediment and water are widely documented (Boszke et al., 2003; Frohne et al., 2012; Ullrich et al., 2001). Several studies confirmed that DOM was a positive contributor to convert Hg(II) to MeHg due to its key roles in inhibiting aggregation of β-HgS(s) (Graham et al., 2013), increasing bioavailability of Hg (Chiasson Gould et al., 2014), and enhancing activity of bacteria (Moreau et al., 2007). However, DOM represents only small proportion of total OM in soils (Bolan et al., 2011). Previous reports revealed preferential bonding of Hg(II) to reduced organic S sites in OM, meanwhile, the N-bearing ligands also play positive roles in competing for Hg(II) (Haitzer et al., 2002; Skyllberg et al., 2006), especially when the high-affinity organic S sites are saturated (Lin et al., 2010; Skyllberg et al., 2006).
In fact, S-bearing compounds (e.g., sulfate and sulfide) present in soils play key roles in Hg methylation. Previous studies demonstrated that sulfate could facilitate microbial production of MeHg via enhancing the SRB activities (Gilmour et al., 1992), whereas the organic S ligands in OM (e.g., thiol groups) have high affinity with Hg(II) to restrict its bioavailability (Skyllberg et al., 2006; Skyllberg and Drott, 2010). In comparison with soil Se, S exhibited conflicting effects on the biogeochemical cycling of Hg in soils, however, the mechanistic understanding is still unclear. In this study, S/Hg ratio instead of S alone in soils played a positive role in regulating the MeHg production in soils and its enrichment in rice grains. This could be mostly attributed to: (1) increasing the proportion of S-bearing ligands in soil OM that could bind more bioavailable Hg(II) (Haitzer et al., 2004; Skyllberg et al., 2006), which restricted the production of MeHg in rhizosphere, together with the transportation of which into rice tissues; (2) sulfides, the products of sulfate reduction in flooding paddy soils, had high affinity with Hg(II) in soil solution (Han et al., 2008; Skyllberg and Drott, 2010), which could also inhibit the conversion of Hg(II) to MeHg.

Although N could be an important factor controlling microbial Hg methylation in aquatic system (Liem-Nguyen et al., 2016), its effect on MeHg production in paddy soils was less apparent. As described above, N/Hg ratio, rather than N alone, showed significant negative relationship with MeHg_soil. The increasing N-bearing ligands in OM (e.g., amino groups) associated with the increase of N/Hg ratio could bind more Hg(II) to form low bioavailable Hg-N-OM complexes (Hesterberg et al., 2001; Skyllberg et al., 2006). The resulting inhibited MeHg production could alleviate the MeHg-contaminated levels in rice tissues.

OM, a complex mixture of molecules, is comprised of various reactive S- and N-bearing ligands, which can form stable complexes with Hg(II) (Graham et al., 2012; Hesterberg et al., 2001). The inhibitory effects of increasing the OM/Hg ratio on MeHg production in soils may largely depend on the S- and/or N-bearing ligands in OM. This explains the significant negative relationship between MeHg_soil and soil OM/Hg ratio rather than OM alone. To increase S- and N-bearing ligands in OM could protect against more Hg(II) from methylation in rhizosphere soils, and then ultimately alleviate the MeHg bioaccumulation in rice grains.

In our previous study input of organic fertilizers such as swine manures was found to pose a potential inhibitory effect on the MeHg production in soils and its subsequent bioaccumulation in rice (Yin et al., 2016). However, some recent studies revealed that incorporation of rice straw could facilitate MeHg production in soils via increasing DOM content and microbial activity (Liu et al., 2016; Zhu et al., 2015, 2016). This implied that OM derived from

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**Fig. 7** – The correlations of MeHg_soil and MeHg_rice with soil N/Hg ratio (a, b), OM/Hg ratio (c, d) and S/Hg ratio (e, f).
different sources has a positive or negative effect on inorganic Hg methylation possibly due to its heterogeneous structure, composition, and bioavailability (Bravo et al., 2017). In this study, those organic matters containing more S- and/or N-bearing ligands were expected to inhibit soil Hg methylation, and to be utilized in rice cultivation of the WMM area.

4. Conclusions

Long-term mining and smelting activities have resulted in the release of large volume of gaseous Hg and massive Hg wastes (e.g., gangues and calcines), which are two important sources causing Hg contamination in the rice paddy ecosystem of the WMM. In this study, we reported high THg (0.5 to 187.8 μg/g) and MeHg concentrations (0.7 to 6.7 ng/g) in paddy soils, and rice grains were characterized by high MeHg concentrations (2.3 to 43.2 ng/g) accounting for about 7.1%-68.4% of the THg.

The Hg mining activities definitely resulted in the high S and Se levels in paddy soils, especially further upstream of the rivers, where several Hg tailings were formed, which were comparable to the distribution of soil THg and MeHg. The newly-deposited Hg associated with high reactivity due to ozone-mediated conversion of Hg²⁺ derived from active Hg smelters, was readily methylated in a low-pH anoxic paddy soils, and this increased MeHg production and its subsequent bioaccumulation in rice paddies around the active Hg smelting site. The soil Se significantly restricted the bioaccumulation of THg in rice grains, however, showed less influence on MeHg bioaccumulation. Additionally, the soil OM, containing great amounts of S- and N-bearing ligands, could chelate the inorganic Hg(II) to form low bioavailable Hg-N(S)-OM complexes, which are speculated to be responsible for the reduced MeHg concentrations in soils and rice grains with the increase of soil S/Hg, N/Hg or OM/Hg ratio. Nevertheless, other soil factors had less influence on the production and bioaccumulation of Hg in paddy fields.

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Competing financial interests

The authors declare that they have no competing financial interests.

Data availability

For accessing to data, please contact the corresponding author (hetianrong@139.com).

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