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

Deliang Yin[1,2], Tianrong He[1,*], Runsheng Yin[3,4], Lingxia Zeng[1]

1. The Key Laboratory of Karst Environment and Geohazard Prevention, Guizhou University, Guiyang 550003, China
2. College of Resources and Environments, Southwest University, Chongqing 400715, China
3. State Key Laboratory of Ore Deposit Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China
4. Department of Civil and Environmental Engineering, Environmental Chemistry and Technology Program, University of Wisconsin-Madison, Madison, WI 53706, USA

ARTICLE INFO

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., SiO_2, Al_2O_3 and Fe_2O_3) 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.

© 2018 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

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.,

2010a). 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 IHg, and is usually greater in grains than other tissues (Meng et al., 2010; Zhang et al., 2010a). The formed MeHg in rhizosphere can be readily adsorbed into roots, where MeHg is combined with protein, polysaccharide and nucleic acid, and then is transferred to grains during the ripening period (Meng et al., 2011). However, phytochelatins 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 IHg 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., SiO_2, Al_2O_3, Fe_2O_3), 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; Åkerblom 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 IHg (Zhang et al., 2010a). The bioaccumulation factor (BAF) of MeHg in rice was about 2 to 3 magnitudes higher than IHg (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., SiO_2, Al_2O_3, Fe_2O_3) 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 areas and the active Hg smelting site were 113 and 11, respectively, Fig. 1). These paddies with a 500 m interval from each other were mainly along major rivers of the WMM (i.e., Gaolouping River (27°30′50.16″–27°30′39.85″N, 109°11′55.22″–109°10′26.17E), Aozhai River (27°32′53.381′–27°35′40.084″N, 109°12′17.002″–109°17′04.914″E), Huangdao River (27°30′46.20″–27°26′49.03″N, 109°14′00.11″–109°16′11.87″E), Dashuixi River (27°32′11.29′–27°32′05.38″N, 109°14′06.94″–109°18′26.53″E), Wawuping River (27°37′18.024″–27°37′13.388″N, 109°16′51.884″–109°21′28.511″E), Gouxi River (27°33′50.038″–27°33′45.849″N, 109°11′28.407″–109°09′59.242″E)) subjecting to obvious Hg contamination. At each rice paddy field, one composite sample of rice grain or soil
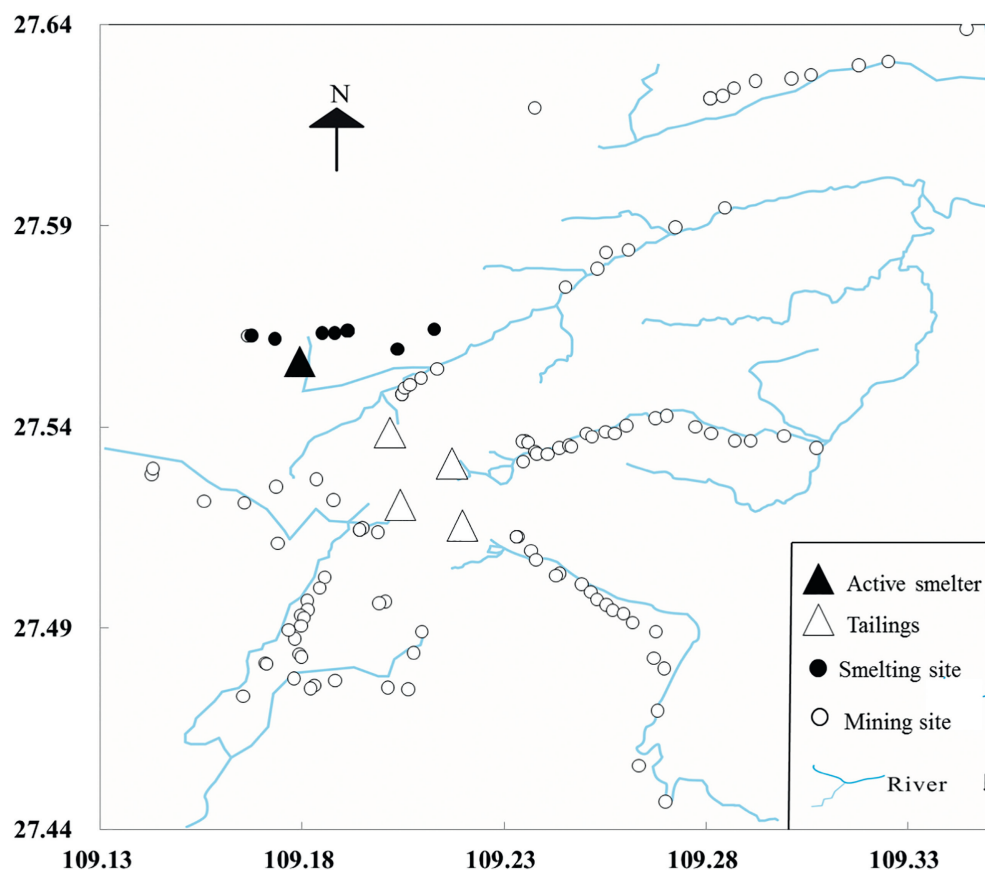
Fig. 1 – Study area and sampling sites including the abandoned Hg mining sites and active artisanal Hg smelting site.

was collected from 3 to 4 points, and was sealed and double-bagged. Soil samples were preserved *in situ* with liquid nitrogen (approximately −196°C). After transportation to the laboratory within 24 hr, soil samples were freeze-dried (−60°C), grounded 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 JLGJ4.5, 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 HNO_3 in a water-bath (95°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 ($HCl:HNO_3 = 3:1$, V/V) in a water-bath (95°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 HNO_3 ($H_2O:HNO_3 = 3:1$, V/V) and $CuSO_4$

(2 mol/L). MeHg in sample was extracted with CH_2Cl_2 solution, back-extracted from solvent phase into water, and then the aqueous phase ethylated using 1% sodium tetraethylborate ($NaBEt_4$). The $CH_3CH_3CH_2Hg$, ethyl analog of MeHg, was enriched onto a Tenax trap (Tenax-TA, 20/35 meshes, Restek corporation, USA) by purging with N_2, 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 Hg^0 in a pyrolytic decomposition column (700°C). Finally the Hg^0 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°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.*, SiO_2, Al_2O_3, and Fe_2O_3), P, S, Se and N concentrations of the soil samples were measured at the Institute of Geological Mining Key Laboratory, Guizhou province. SiO_2, Al_2O_3, Fe_2O_3 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 THg_{rice} and THg_{soil} 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 $MeHg_{soil}$ and $MeHg_{rice}$ analysis, respectively. Recoveries for MeHg analysis were between 89% and 117%, and RSD for duplicate analysis was <8%. Quality control of $MeHg_{rice}$ analysis was also carried out using matrix spikes, and recoveries for matrix spikes were from 85% to 106%.

For SiO_2, Al_2O_3, Fe_2O_3, 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 0.05%) 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 SiO_2, Al_2O_3 and Fe_2O_3, were within the range of Chinese soil (Yan et al., 1997).
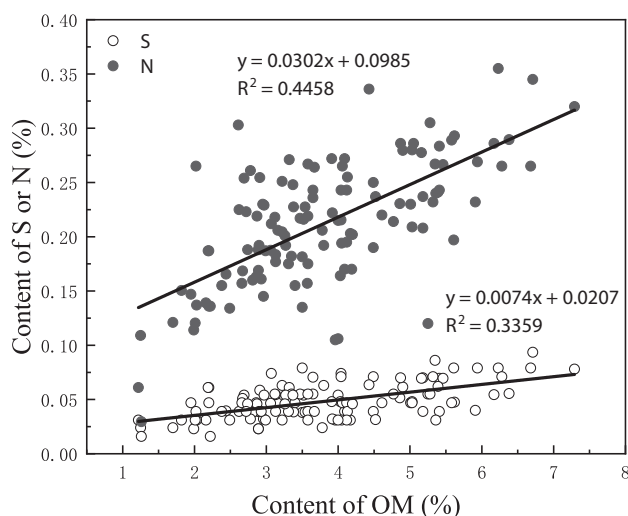

Fig. 2 – Relationships of nitrogen (N) and sulfur (S) with organic matter (OM) in paddy soils.

No clear spatial patterns of pH, OM, N, P, SiO_2, Al_2O_3, and Fe_2O_3 were observed, whereas the S and Se concentrations were higher at the upstream of several WMM rivers (Fig. 3). This can be explained by the fact that S was intimately associated with Se in Hg ores of the WMM (Qiu et al., 2005; Zhang, 2011; Zhang et al., 2014). Previous reports found that Se and S concentrations in the rivers around mine-waste calcines were elevated, which could enter paddy soils through irrigation (Zhang et al., 2014). Moreover, the deposited waste calcines from mining activities could carry large quantities of Se into rice paddies, causing Se-excessive soils. Se has a dual role as an essential element at appropriate levels and a toxic substance at higher levels (Zhang et al., 2014), and so the Se-rich status in paddies of WMM deserves more attention, especially near the abandoned Hg tailings.

2.2. THg in soils and rice grains

The THg_{soil} concentrations ranged from 0.5 to 187.8 μg/g with an average of 31.0 μg/g, which unexceptionally 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 THg_{soil} 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 significant 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.

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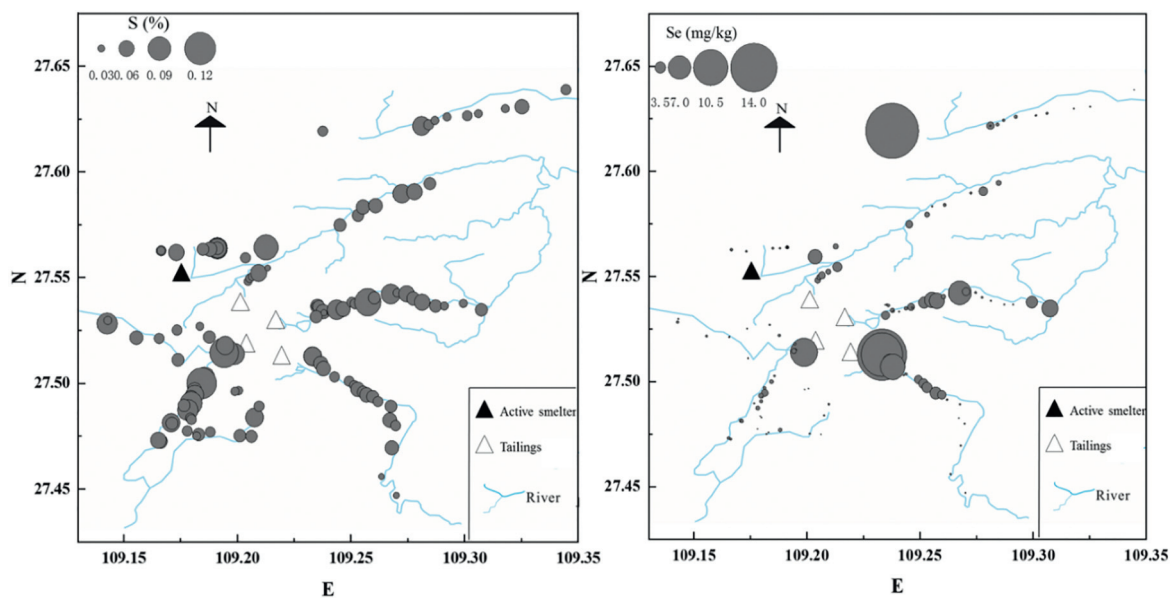
Fig. 3 – Spatial distribution of S and selenium (Se) concentrations in paddy soils of Wanshan mercury mine(WMM).
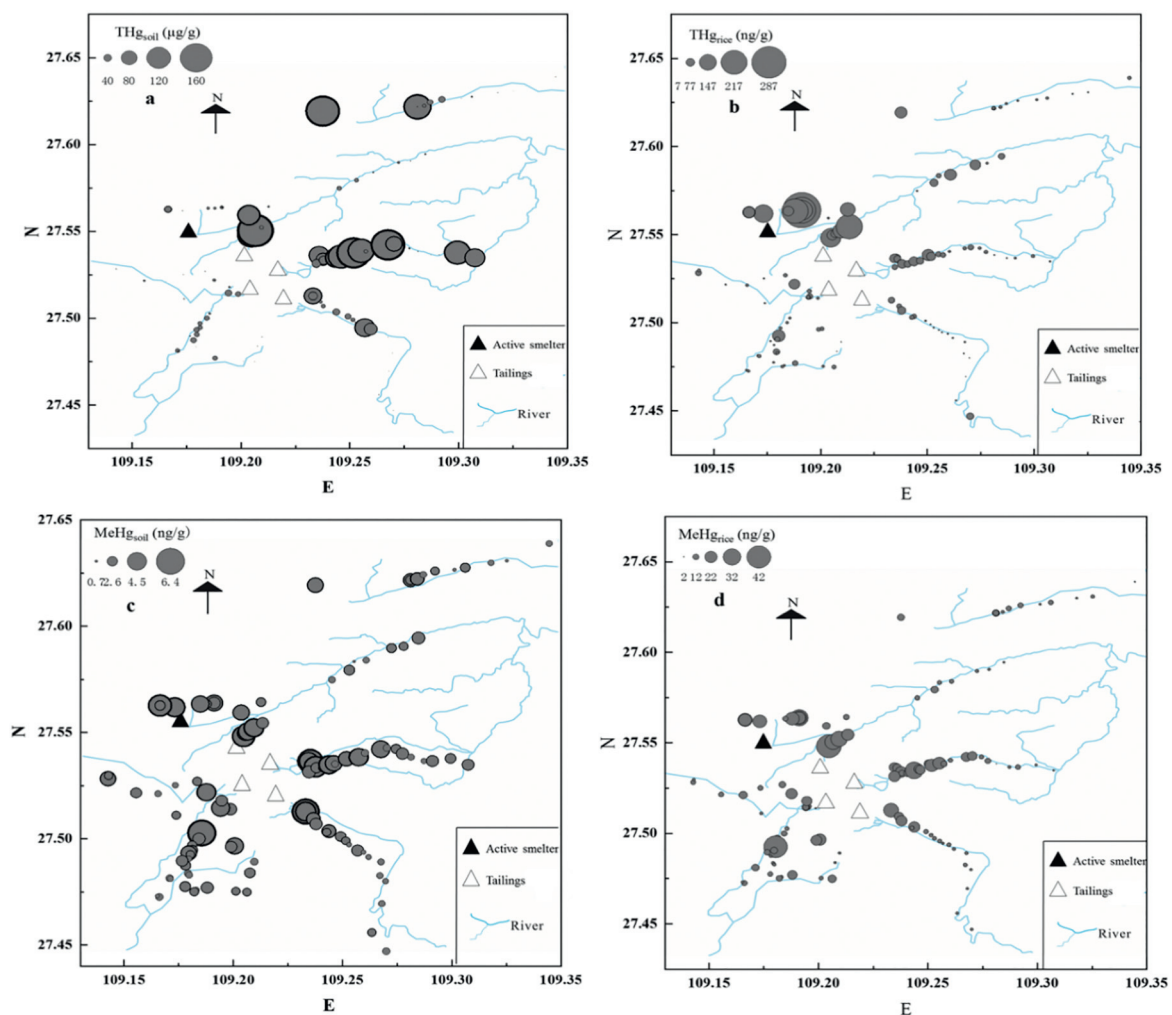

Fig. 4 – Spatial distribution of soil total Hg (THg$_{soil}$) (a), rice total Hg (THg$_{rice}$) (b), soil MeHg (MeHg$_{soil}$) (c) and rice MeHg (MeHg$_{rice}$) (d) in rice paddy soils of WMM.
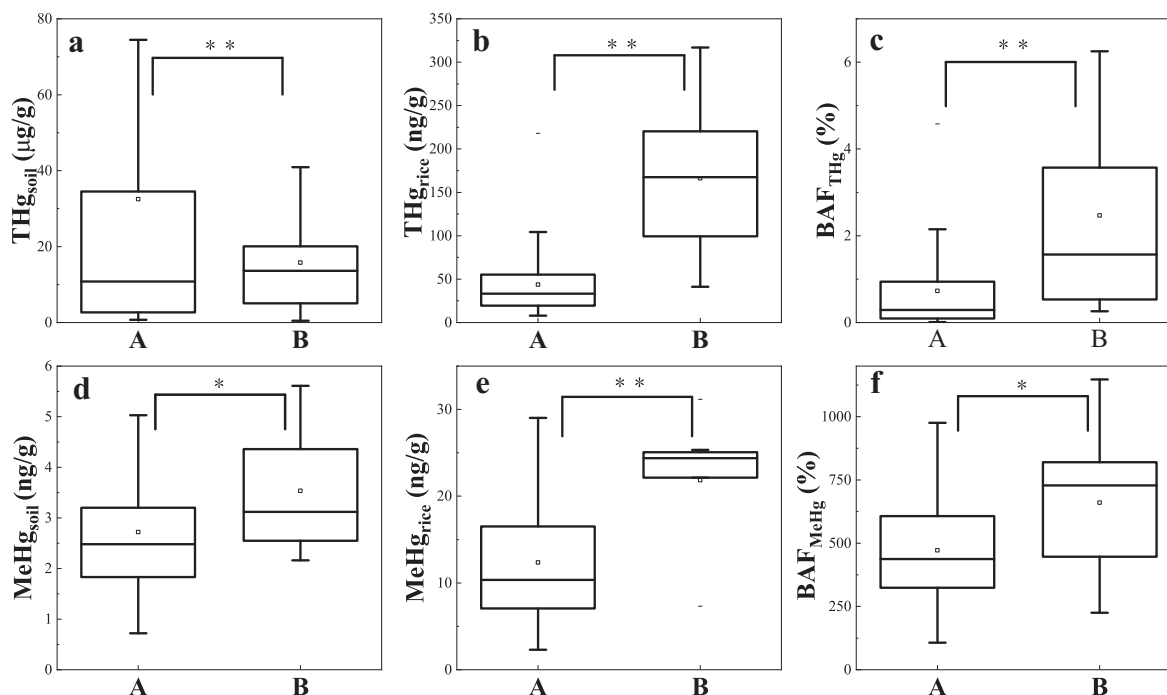
Fig. 5 – Concentrations and bioaccumulation factors of THg and methylmercury (MeHg) in paddy fields at the abandoned Hg mining sites (A) and the active artisanal Hg smelting site (B). A difference analysis is conducted by independent-samples T test. The * and ** represent significant difference ($p < 0.05$) and extremely significant difference ($p < 0.01$), respectively.

had shown that, as well as soil Hg (Rothenberg et al., 2011), atmospheric Hg is an important source of IHg in rice tissues (Meng et al., 2010, 2012; Yin et al., 2013a). The elevated THg_{rice} 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 THg_{rice} and THg_{soil}. 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. Hg^0 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 $MeHg_{soil}$ 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

$MeHg_{soil}$ concentrations were higher further upstream of WMM rivers, and were positively correlated with THg_{soil} ($r = 0.45$, $p < 0.01$, $n = 124$). 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 Hg^0 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 $MeHg_{rice}$ 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 $MeHg_{rice}$ was positively correlated with both $MeHg_{soil}$ ($r = 0.54$, $p < 0.01$, $n = 124$) and THg_{rice} ($r = 0.64$, $p < 0.01$, $n = 122$), but not with THg_{soil} ($p > 0.05$), 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 $MeHg_{rice}$ exhibiting similar distribution patterns to THg_{rice} and $MeHg_{soil}$, but not to THg_{soil}, showed significant negative relationships with distance away from all abandoned Hg tailings located at the upstream of rivers (Pearson correlation, $p < 0.01$ for $MeHg_{rice}$, THg_{rice} and $MeHg_{soil}$) (Fig. 4b–d). However, no similar result was achieved in active artisanal Hg smelting site

($p > 0.05$ for $MeHg_{rice}$, THg_{rice}, THg_{rice}, and $MeHg_{soil}$), implying that rice paddies located at this area had a distribution of Hg differing from that observed in abandoned Hg mining area. Correlation analysis also revealed that, among all abandoned Hg mining sites, only 3 of which showed significant negative relationships between THg_{soil} and distance away from abandoned Hg tailings ($p < 0.05$). This implied that MeHg and THg concentrations in both rice grains and soils (except for THg in soils) generally reduced with distance away from the abandoned Hg mining area, where large amounts of tailings and mine-waste calcines were produced. The diffusion of gaseous Hg^0 and runoff of waste particles resulted in the reduced Hg levels in atmosphere, river water/sediment and soil with distance away from the mining sites (Dai et al., 2012; Qiu et al., 2005; Yin et al., 2013b; Zhang et al., 2010b). In addition, the rice grains had extremely significant high MeHg levels near the active artisanal Hg smelters (T-test, $p < 0.01$, Fig. 5e), and this was consistent with the distribution of both THg_{rice} and $MeHg_{soil}$, indicating that elevated TGM was one of important factors determining the MeHg-contaminated levels in rice grains. This could be greatly attributed to the enhanced MeHg production in soil due to the methylation of bioavailable Hg derived from newly-deposited atmospheric Hg as described above.

2.4. Proportion of MeHg to THg in soils and rice grains

The proportion of MeHg to THg in soils ($FMeHg_{soil}$), 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 ($FMeHg_{rice}$) was much higher, which ranged from 7.1 to 68.4% with an average of 34.0%. A significant negative correlation between $FMeHg_{rice}$ and THg_{rice} ($r = −0.59$, $p < 0.01$, $n = 122$) was found, whereas $FMeHg_{rice}$ showed less of a relationship with both $MeHg_{rice}$ ($p > 0.05$) and $MeHg_{soil}$ ($p > 0.05$). This indicated that $FMeHg_{rice}$ 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 (BAF_{THg}) and MeHg (BAF_{MeHg}) in rice grains were calculated. The BAF_{THg} (0.0–8.3%) was much lower than the BAF_{MeHg} (107.1%–1227.3%), suggesting that rice had a stronger capability to accumulate soil MeHg than IHg. 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 BAF_{THg} 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 BAF_{MeHg} (T-test, $p < 0.05$, Fig. 5f) followed the similar rule like BAF_{THg}. 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 THg_{soil} 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 THg_{soil} and other soil properties (e.g., pH, OM, S, N, P, SiO_2, Al_2O_3, and Fe_2O_3).

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 $MeHg_{soil}$. 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 $MeHg_{soil}$, 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 $MeHg_{rice}$ 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 $FMeHg_{soil}$ and soil properties such as pH, OM, P, N, SiO_2, Al_2O_3, and Fe_2O_3 ($p > 0.05$), whereas $FMeHg_{soil}$ 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 BAF_{THg} 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 THg_{rice} ($MeHg_{rice}$) and soil Se (both $p > 0.05$). Moreover, no clear relationship between BAF_{MeHg} and soil Se was found in this
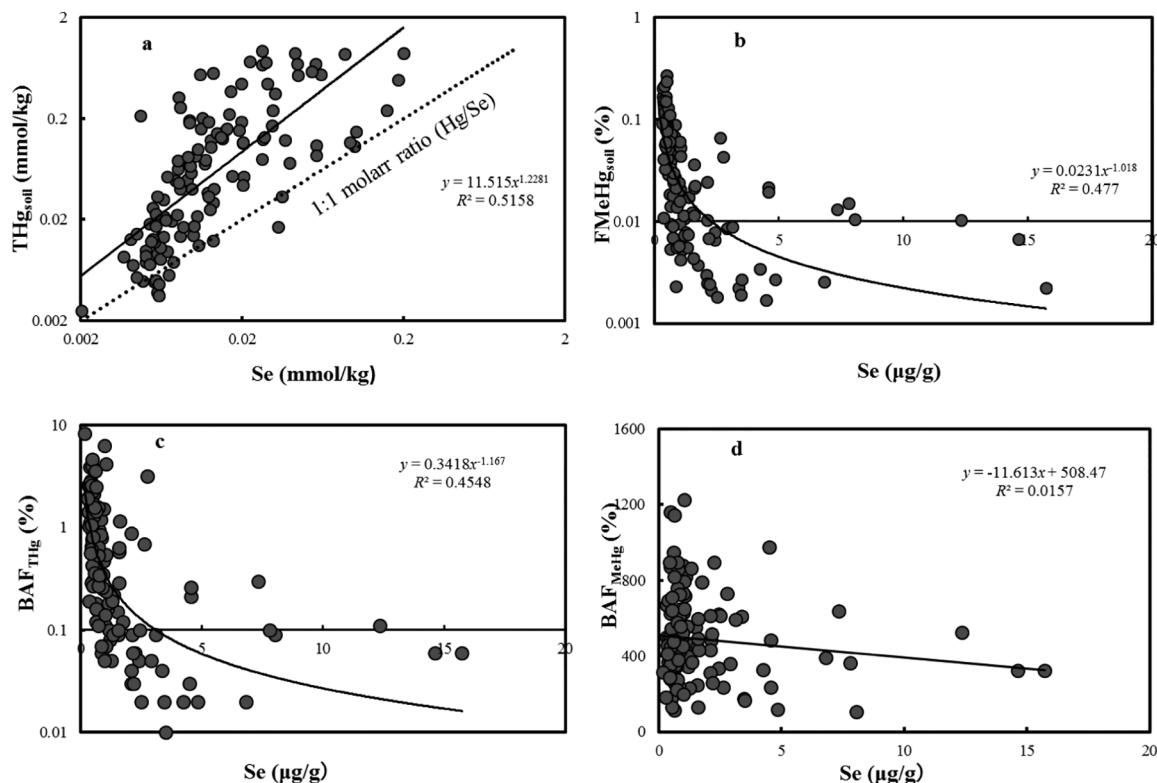
Fig. 6 – Relationships of THg_{soil} (a), proportion of MeHg to THg in soils ($FMeHg_{soil}$) (b), bioaccumulation factors of THg (BAF_{THg}) (c), and bioaccumulation factors of MeHg (BAF_{MeHg}) (d) with Se concentrations in paddy fields.

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 BAF_{MeHg} (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 (Chiassongould 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).
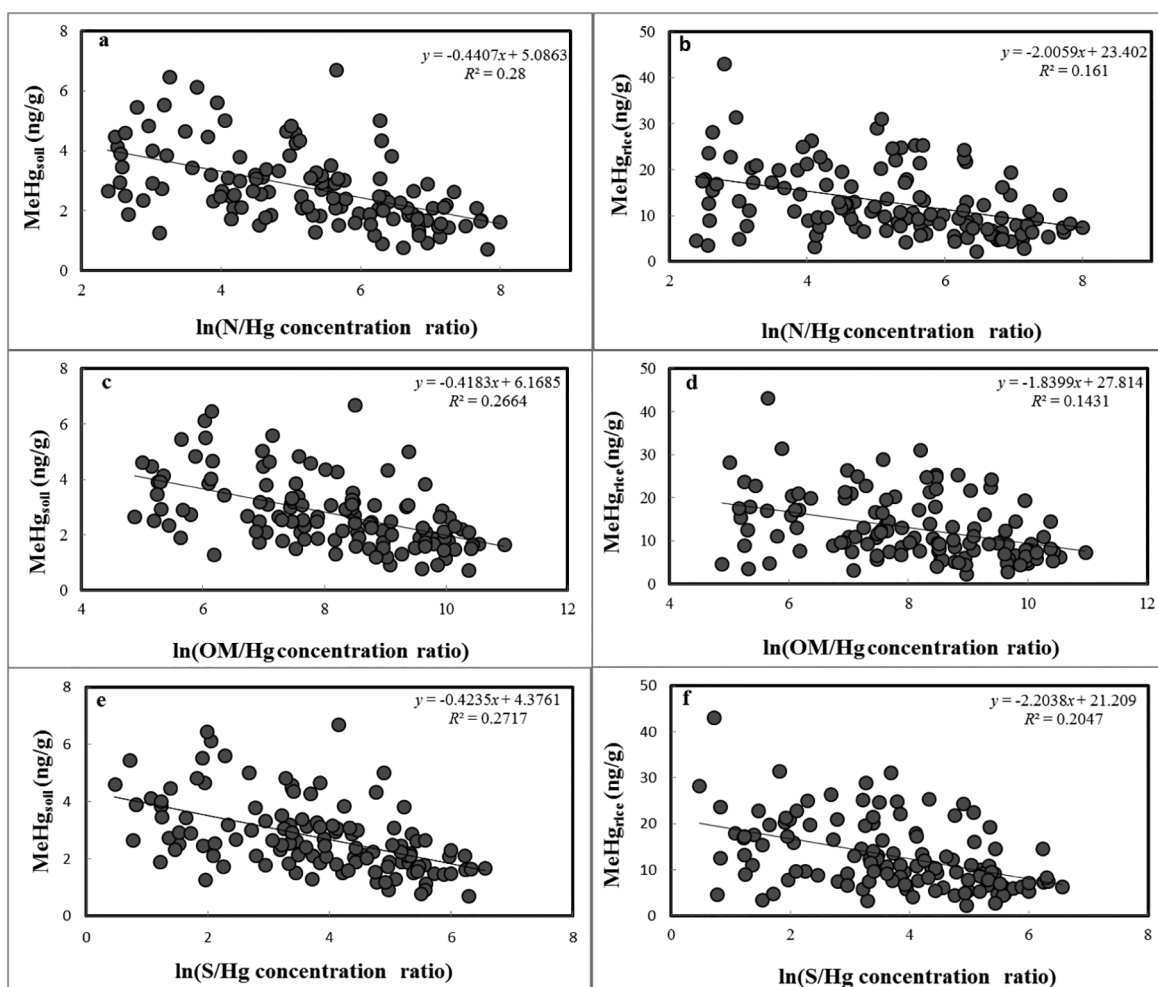
Fig. 7 – The correlations of MeHg$_{soil}$ and MeHg$_{rice}$ with soil N/Hg ratio (a, b), OM/Hg ratio (c, d) and S/Hg ratio (e, f).

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; Wiederhold et al., 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., 2002; 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

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 Hg^0 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.

Acknowledgment

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).

REFERENCES

Aiken, G., Haitzer, M., Ryan, J., Nagy, K., 2003. Interactions between dissolved organic matter and mercury in the Florida Everglades. J. Phys. IV 107, 29–32.

Åkerblom, S., Meili, M., Bringmark, L., Johansson, K., Dan, B.K., Bo, B., 2008. Partitioning of Hg between solid and dissolved organic matter in the humus layer of boreal forests. Water Air Soil Pollut. 189, 239–252.

Basak, M., Semra, C., Osman, N.E., 2011. Use of selected industrial waste materials in concrete mixture. Environ. Prog. Sustain. Energy 30, 368–376.

Bjerregaard, P., Fjordside, S., Hansen, M.G., Petrova, M.B., 2011. Dietary selenium reduces retention of methyl mercury in freshwater fish. Environ. Sci. Technol. 45, 9793–9798.

Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., Mcdowell, R., Senesi, N., 2011. Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. Adv. Agron. 110, 1–75.

Boszke, L., Kowalski, A., G³Osiñska, G., Szarek, R., Siepak, J., 2003. Environmental factors affecting speciation of mercury in the bottom sediments; an overview. Pol. J. Environ. Stud. 12, 5–13.

Bravo, A.G., Bouchet, S., Tolu, J., Bjorn, E., Mateos-Rivera, A., Bertilsson, S., 2017. Molecular composition of organic matter controls methylmercury formation in boreal lakes. Nat. Commun. 8.

Bremner, J.M., 1996. Nitrogen-total. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series No. 5. Soil Science Society of America& American Society of Agronomy, Madison, USA, pp. 1085–1121.

Cao, Z.H., Wang, X.C., Yao, D.H., Zhang, X.L., Wong, M.H., 2001. Selenium geochemistry of paddy soils in Yangtze River Delta. Environ. Int. 26, 335–339.

Chiassongould, S.A., Blais, J.M., Poulain, A.J., 2014. Dissolved organic matter kinetically controls mercury bioavailability to bacteria. Environ. Sci. Technol. 48, 3153–3161.

Dai, Z.H., Feng, X.B., Fu, X.W., Li, P., 2012. Spatial distribution of mercury deposition fluxes in Wanshan Hg mining area, Guizhou, China. Atmos. Chem. Phys. Discuss. 12, 5739–5769.

Frohne, T., Rinklebe, J., Langer, U., Laing, G.D., Mothes, S., Wennrich, R., 2012. Biogeochemical factors affecting mercury methylation rate in two contaminated floodplain soils. Biogeosciences 9, 493–507.

Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26, 2281–2287.

Graham, A.M., Aiken, G.R., Gilmour, C.C., 2012. Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions. Environ. Sci. Technol. 46, 2715–2723.

Graham, A.M., Aiken, G.R., Gilmour, C.C., 2013. Effect of dissolved organic matter source and character on microbial Hg methylation in Hg-S-DOM solutions. Environ. Sci. Technol. 47, 5746–5754.

Haitzer, M., Aiken, G.R., Ryan, J.N., 2002. Binding of mercury(II) to dissolved organic matter: the role of the mercury-to-DOM concentration ratio. Environ. Sci. Technol. 36, 3564–3570.

Han, S., Obraztsova, A., Pretto, P., Deheyn, D.D., Gieskes, J., Tebo, B.M., 2008. Sulfide and iron control on mercury speciation in anoxic estuarine sediment slurries. Mar. Chem. 111, 214–220.

He, T., Feng, X.B., Dai, Q.J., Qiu, G.L., Shang, L.H., Jiang, H.M., 2004. Determination of methyl mercury in sediments and soils by gc-cvafs after aqueous phase ethylation. Geol. Geochem. 32, 83–86.

Hesterberg, D., Chou, J.W., Hutchison, K.J., Sayers, D.E., 2001. Bonding of Hg(II) to reduced organic sulfur in humic acid as affected by S/Hg ratio. Environ. Sci. Technol. 35, 2741–2745.

Hintelmann, H., Harris, R., Heyes, A., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., et al., 2002. Reactivity and mobility of new and old mercury deposition in a Boreal forest ecosystem during the first year of the METAALICUS study. Environ. Sci. Technol. 36, 5034–5040.

Horvat, M., Nolde, N., Fajon, V., Jereb, V., Logar, M., Lojen, S., et al., 2003. Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. Sci. Total Environ. 304, 231–256.

Krupp, E.M., Mestrot, A., Wielgus, J., Meharg, A.A., Feldmann, J., 2009. The molecular form of mercury in biota: identification of novel mercury peptide complexes in plants. Chem. Commun. (28), 4257–4259.

Li, P., Feng, X.B., Qiu, G.L., Shang, L.H., Li, G.H., 2009. Human hair mercury levels in the Wanshan mercury mining area, Guizhou Province, China. Environ. Geochem. Health 31, 683–691.

Li, P., Feng, X.B., Yuan, X.B., Chan, H.M., Qiu, G.L., Sun, G.X., Zhu, Y.G., 2012. Rice consumption contributes to low level methylmercury exposure in southern China. Environ. Int. 49, 18–23.

Li, P., Feng, X.B., Qiu, G.L., Zhang, J.F., Meng, B., Wang, J.X., 2013. Mercury speciation and mobility in mine wastes from mercury mines in China. Environ. Sci. Pollut. Res. 20, 8374–8381.

Li, P., Feng, X.B., Chan, H.M., Zhang, X.F., Du, B.Y., 2015. Human body burden and dietary methylmercury intake: the relationship in a rice-consuming population. Environ. Sci. Technol. 49, 9682–9689.

Liang, L., Horvat, M., Cernichiari, E., Gelein, B., Balogh, S., 1996. Simple solvent extraction technique for elimination of matrix interferences in the determination of methylmercury in environmental and biological samples by ethylation gas chromatography cold vapor atomic fluorescence spectrometry. Talanta 43, 1883–1888.

Liem-Nguyen, V., Jonsson, S., Skyllberg, U., Nilsson, M.B., Andersson, A., Lundberg, E., et al., 2016. Effects of nutrient loading and mercury chemical speciation on the formation and degradation of methylmercury in estuarine sediment. Environ. Sci. Technol. 50, 6983–6990.

Lin, Y., Larssen, T., Vogt, R.D., Feng, X., 2010. Identification of fractions of mercury in water, soil and sediment from a typical Hg mining area in Wanshan, Guizhou province, China. Appl. Geochem. 25, 60–68.

Liu, C.Q., Cao, S.Q., Chen, G.A., Wu, X.J., 1990. Sulphur in the agriculture of China. Acta Pedol. Sin. 27, 398–404.

Liu, Y.R., Yu, R.Q., Zheng, Y.M., He, J.Z., 2014a. Analysis of the microbial community structure by monitoring an Hg methylation gene (hgcA) in paddy soils along an Hg gradient. Appl. Environ. Microbiol. 80, 2874–2879.

Liu, Y.R., Zheng, Y.M., Zhang, L.M., He, J.Z., 2014b. Linkage between community diversity of sulfate-reducing microorganisms and methylmercury concentration in paddy soil. Environ. Sci. Pollut. Res. 21, 1339–1348.

Liu, Y.R., Dong, J.X., Han, L.L., Zheng, Y.M., He, J.Z., 2016. Influence of rice straw amendment on mercury methylation and nitrification in paddy soils. Environ. Pollut. 209, 53–59.

McNear, D.H., Afton, S.E., Caruso, J.A., 2012. Exploring the structural basis for selenium/mercury antagonism in Allium fistulosum. Metallomics Integr. Biometal Sci. 4, 267–276.

Meng, B., Feng, X.B., Qiu, G.L., Cai, Y., Wang, D.Y., Li, P., et al., 2010. Distribution patterns of inorganic mercury and methylmercury in tissues of rice (Oryza sativa L.) plants and possible bioaccumulation pathways. J. Agric. Food Chem. 58, 4951–4958.

Meng, B., Feng, X.B., Qiu, G.L., Liang, P., Li, P., Chen, C.X., Shang, L. H., 2011. The process of methylmercury accumulation in rice (Oryza sativa L.). Environ. Sci. Technol. 45, 2711–2717.

Meng, B., Feng, X.B., Qiu, G.L., Wang, D.Y., Liang, P., Li, P., Shang, L. H., 2012. Inorganic mercury accumulation in rice (Oryza sativa L.). Environ. Toxicol. Chem. 31, 2093–2098.

Meng, M., Li, B., Shao, J.J., Wang, T., He, B., Shi, J.B., et al., 2014. Accumulation of total mercury and methylmercury in rice plants collected from different mining areas in China. Environ. Pollut. 184, 179–186.

Mergler, D., Anderson, H.A., Chan, L.H., Mahaffey, K.R., Murray, M., Sakamoto, M., et al., 2007. Methylmercury exposure and health effects in humans: a worldwide concern. Ambio 36, 3–11.

Ministry of Health Standardization Administration of China, 2005. Maximum Levels of Contaminants in Food (GB2762-2005).

Moreau, J.W., Roden, E.E., Gerbig, C., Kim, C.S., Aiken, G.R., Dewild, J.F., et al., 2007. The Role of Dissolved Organic Matter in Environmental Mercury Methylation by Sulfate-Reducing Bacteria, AGU Fall Meeting.

Office of national soil survey, 1979. Provisional Technical Regulations for the Second Soil Census in China. China Agricultural Press, Beijing.

Pelletier, E., 1986. Mercury-selenium interactions in aquatic organisms: a review. Mar. Environ. Res. 18, 111–132.

Pinheiro, M.D.C.N., Nascimento, J.L.M.D., Silveira, L.C.D.L., Rocha, J.B.T.D., Aschner, M., 2009. Mercury and selenium—a review on aspects related to the health of human populations in the Amazon. Environ. Bioindic. 4, 222–245.

Qiu, G.L., Feng, X.B., Wang, S.F., Shang, L.H., 2005. Mercury and methylmercury in riparian soil, sediments, mine-waste calcines, and moss from abandoned Hg mines in east Guizhou province, southwestern China. Appl. Geochem. 20, 627–638.

Qiu, G.L., Feng, X.B., Li, P., Wang, S.F., Li, G.H., Shang, L.H., Fu, X.W., 2008. Methylmercury accumulation in rice (Oryza sativa L.) grown at abandoned mercury mines in Guizhou, China. J. Agric. Food Chem. 56, 2465–2468.

Qiu, G.L., Feng, X.B., Wang, S.F., Fu, X.W., Shang, L.H., 2009. Mercury distribution and speciation in water and fish from abandoned Hg mines in Wanshan, Guizhou province, China. Sci. Total Environ. 407, 5162–5168.

Rothenberg, S.E., Feng, X., Dong, B., Shang, L., Yin, R., Yuan, X., 2011. Characterization of mercury species in brown and white rice (Oryza sativa L.) grown in water-saving paddies. Environ. Pollut. 159, 1283–1289.

Rothenberg, S.E., Feng, X.B., Zhou, W.J., Tu, M., Jin, B.W., You, J.M., 2012. Environment and genotype controls on mercury accumulation in rice (Oryza sativa L.) cultivated along a contamination gradient in Guizhou, China. Sci. Total Environ. 426, 272–280.

Rothenberg, S.E., Yu, X.D., Zhang, Y.M., 2013. Prenatal methylmercury exposure through maternal rice ingestion: insights from a feasibility pilot in Guizhou Province, China. Environ. Pollut. 180, 291–298.

Shao, D.D., Kang, Y., Wu, S.C., Wong, M.H., 2012. Effects of sulfate reducing bacteria and sulfate concentrations on mercury methylation in freshwater sediments. Sci. Total Environ. 424, 331–336.

Skyllberg, U., Drott, A., 2010. Competition between disordered iron sulfide and natural organic matter associated thiols for mercury (II) — an EXAFS study. Environ. Sci. Technol. 44, 1254–1259.

Skyllberg, U., Bloom, P.R., Qian, J., Lin, C., Bleam, W.F., 2006. Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. Environ. Sci. Technol. 40, 4174–4180.

State Environmental Protection Administration of China, 1995. Environmental Quality Standard for Soils GB 15618-1995, Beijing.

Tan, J.A., 1996. Chronic Keshan Disease and Environmental Elements of Life. Beijing.

Tang, W.L., Dang, F., Evans, D., Zhong, H., Xiao, L., 2017. Understanding reduced inorganic mercury accumulation in rice following selenium application: selenium application routes, speciation and doses. Chemosphere 169, 369–376.

Tiwari, R.B., Tiwari, J.P., 1993. Studies on the problem of leaf bronzing in guava induction of phosphorus deficiency symptoms under sand culture. Indian J. Hortic. 50, 53–56.

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

Wang, S.F., Feng, X.B., Qiu, G.L., Fu, X.W., Wei, Z.Q., 2007. Characteristics of mercury exchange flux between soil and air in the heavily air-polluted area, eastern Guizhou, China. Atmos. Environ. 41, 5584–5594.

Wang, J., Feng, X., Anderson, C.W., Zhu, W., Yin, R., Wang, H., 2011. Mercury distribution in the soil-plant-air system at the Wanshan mercury mining district in Guizhou, Southwest China. Environ. Toxicol. Chem. 30, 2725–2731.

Wang, X., Tam, N.F., Fu, S., Ametkhan, A., Ouyang, Y., Ye, Z., 2014a. Selenium addition alters mercury uptake, bioavailability in the rhizosphere and root anatomy of rice (*Oryza sativa*). Ann. Bot. 114, 271–278.

Wang, X., Ye, Z.H., Li, B., Huang, L.N., Meng, M., Shi, J.B., Jiang, G.B., 2014b. Growing Rice aerobically markedly decreases mercury accumulation by reducing both hg bioavailability and the production of MeHg. Environ. Sci. Technol. 48, 1878–1885.

Wang, Y.J., Dang, F., Evans, R.D., Zhong, H., Zhao, J.T., Zhou, D.M., 2016a. Mechanistic understanding of MeHg-se antagonism in soil-rice systems: the key role of antagonism in soil. Sci. Rep. UK 6.

Wang, Y.J., Dang, F., Zhao, J.T., Zhong, H., 2016b. Selenium inhibits sulfate-mediated methylmercury production in rice paddy soil. Environ. Pollut. 213, 232–239.

Wang, Y.J., Dang, F., Zhong, H., Wei, Z.B., Li, P., 2016c. Effects of sulfate and selenite on mercury methylation in a mercury-contaminated rice paddy soil under anoxic conditions. Environ. Sci. Pollut. Res. 23, 4602–4608.

Wiederhold, J.G., Cramer, C.J., Daniel, K., Infante, I., Bourdon, B., Kretzschmar, R., 2010. Equilibrium mercury isotope fractionation between dissolved Hg(II) species and thiol-bound Hg. Environ. Sci. Technol. 44, 4191–4197.

Yan, M.C., Gu, T.X., Chi, Q.H., Wang, C.S., 1997. Abundance of chemical elements of soils in China and supergenesis geochemistry characteristics. Geophys. Geochem. Explor. 21, 161–167.

Yin, R.S., Feng, X.B., Meng, B., 2013a. Stable Hg isotope variation in Rice plants (*Oryza sativa* L.) from the Wanshan Hg Mining District, SW China. Environ. Sci. Technol. 47, 2238–2245.

Yin, R.S., Feng, X.B., Wang, J.X., Li, P., Liu, J.L., Zhang, Y., Chen, J.B., Zheng, L.R., Hu, T.D., 2013b. Mercury speciation and mercury isotope fractionation during ore roasting process and their implication to source identification of downstream sediment in the Wanshan mercury mining area, SW China. Chem. Geol. 336, 72–79.

Yin, D.L., He, T.R., Zeng, L.X., Chen, J., 2016. Exploration of amendments and agronomic measures on the remediation of methylmercury-polluted Rice in a mercury mining area. Water Air Soil Pollut. 227.

Zhang, H., 2011. Impacts of Selenium on the Biogeochemical Cycles of Mercury in Terrestrial Ecosystems in Mercury Mining Areas. Springer Berlin Heidelberg.

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

Zhang, H., Feng, X.B., Larssen, T., Shang, L.H., Vogt, R.D., Lin, Y., et al., 2010b. Fractionation, distribution and transport of mercury in rivers and tributaries around Wanshan Hg mining district, Guizhou Province, Southwestern China: part 2 — methylmercury. Appl. Geochem. 25, 642–649.

Zhang, H., Feng, X.B., Zhu, J.M., Sapkota, A., Meng, B., Yao, H., et al., 2012. Selenium in soil inhibits mercury uptake and translocation in rice (*Oryza sativa* L.). Environ. Sci. Technol. 46, 10040–10046.

Zhang, H., Feng, X.B., Larssen, T., 2014. Selenium speciation, distribution, and transport in a river catchment affected by mercury mining and smelting in Wanshan, China. Appl. Geochem. 40, 1–10.

Zhao, L., Anderson, C.W.N., Qiu, G.L., Meng, B., Wang, D.Y., Feng, X.B., 2016a. Mercury methylation in paddy soil: source and distribution of mercury species at a Hg mining area, Guizhou Province, China. Biogeosciences 13, 2429–2440.

Zhao, L., Qiu, G.L., Anderson, C.W.N., Meng, B., Wang, D.Y., Shang, L.H., et al., 2016b. Mercury methylation in rice paddies and its possible controlling factors in the Hg mining area, Guizhou province, Southwest China. Environ. Pollut. 215, 1–9.

Zheng, W., Feng, X.B., Li, G.H., Li, Z.G., 2006. Determination of total mercury in plants by HNO_3 digestion in the water bath coupled with cold vapor atomic fluorescence spectrometry. Bull. Mineral. Petrol. Geochem. 25, 285–287.

Zhou, X.L., Zhu, Z.X., 2012. Determination of sulfur in the soil by cubic fitting equation. Guizhou Geol. 29, 68–70.

Zhu, H.K., Zhong, H., Evans, D., Hintelmann, H., 2015. Effects of rice residue incorporation on the speciation, potential bioavailability and risk of mercury in a contaminated paddy soil. J. Hazard. Mater. 293, 64–71.

Zhu, H.K., Zhong, H., Wu, J.L., 2016. Incorporating rice residues into paddy soils affects methylmercury accumulation in rice. Chemosphere 152, 259–264.