Mercury in soil, vegetable and human hair in a typical mining area in China: Implication for human exposure

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ABSTRACT

Concentrations of total mercury (T-Hg) and methylmercury (MeHg) in soil, vegetables, and human hair were measured in a mercury mining area in central China. T-Hg and MeHg concentrations in soil ranged from 1.53 to 1054.97 mg/kg and 0.88 to 46.52 μg/kg, respectively. T-Hg concentrations was correlated with total organic carbon (TOC) content (R^2 = 0.50, $p<0.01$) and pH values (R^2 = 0.21, $p<0.05$). A significant linear relationship was observed between MeHg concentrations and the abundance of sulfate-reducing bacteria (SRB) (R^2 = 0.39, $p<0.05$) in soil. Soil incubation experiments amended with specific microbial stimulants and inhibitors showed that Hg methylation was derived from SRB activity. T-Hg and MeHg concentrations in vegetables were 24.79 – 781.02 μg/kg and 0.01 – 0.18 μg/kg, respectively; levels in the edible parts were significantly higher than in the roots (T-Hg: $p<0.05$; MeHg: $p<0.01$). Hg species concentrations in rhizosphere soil were positively correlated to those in vegetables ($p<0.01$), indicating that soil was an important source of Hg in vegetables. Risk assessment indicated that the consumption of vegetables could result in higher probable daily intake (PDI) of T-Hg than the provisional tolerable daily intake (PTDI) for both adults and children. In contrast, the PDI of MeHg was lower than the reference dose. T-Hg and MeHg concentrations in hair samples ranged from 1.57 to 12.61 mg/kg and 0.04 to 0.94 mg/kg, respectively, and MeHg concentration in hair positively related to PDI of MeHg via vegetable consumption (R^2 = 0.39, $p<0.05$), suggesting that vegetable may pose health risk to local residents.

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Introduction

Mercury (Hg) is a hazardous and persistent environmental pollutant released from both natural and anthropogenic sources. It exists as the inorganic form Hg^0, Hg(II), Hg(I) and organic mercury compounds, such as methylmercury (MeHg). Hg toxicity depends on its chemical form (Clarkson and Magos, 2008). MeHg is considered the most toxic form, because of its tendency to bioaccumulate and biomagnify. Inorganic Hg species transform into MeHg through a variety of processes, involving both abiotic and biotic pathways. MeHg is produced by abiotic methylation *via* transmethylation reactions between Hg and other compounds containing methyl groups (Ullrich et al., 2001). Biological methylation is dominated by microorganisms and considered the main process of MeHg production (Yu et al., 2012). Parks et al. identified two gene clusters, *hgcA* and *hgcB*, which correlate with the ability of bacteria to methylate Hg (Parks et al., 2013). Sulfate-reducing bacteria (SRB) and iron-reducing bacteria (FeRB) are methylators of inorganic Hg, and utilize sulfate and Fe(III), respectively, as the

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terminal electron acceptor (Compeau and Bartha, 1985; Fleming et al., 2006). SRB plays the dominant role in methylation of inorganic Hg in the environment.

Hg methylation is prevalent in soil, and this process poses environmental risk to humans because MeHg can bioaccumulate and bioamplify in the soil–plant system. Multiple studies have focused on microbial or abiotic methylation of Hg in wetlands and sediment (Lehnherr et al., 2012; Schartup et al., 2013), but little attention has been paid to the microbial methylator and its contribution to MeHg production in soil. Since some studies reported that crops such as rice and vegetables were important pathways of Hg exposure for people living in Hg-mining areas (Zhang et al., 2010), concerns have been raised regarding Hg methylation in soil and transfer to edible plants.

The study area was a mining region located in central China. Hg was found there around 770 BC, and mining operations were taken over by the government in the 1980s. Intense Hg pollution in nearly all environmental compartments occurred from the mining and retorting activities. This Hg environmental contamination can pose serious threats to soil ecosystems and food safety (Zhang et al., 2009; Qiu et al., 2012). Therefore, it is necessary to quantify Hg methylation through abiotic or microbial processes. The aim of this study was to investigate Hg contamination and identify the main soil methylator of Hg in the study area. We also measured the concentrations of total mercury (T-Hg) and MeHg in vegetables and the corresponding rhizosphere soil collected, and estimated the health risk posed by T-Hg and MeHg in vegetables. To evaluate human exposure, T-Hg and MeHg levels in human hair samples were quantified.

1. Materials and methods

1.1. Study area

The study area was an Hg-mining area in a valley in the southern part of Shaanxi Province in central China. The mining area is approximately 5 km^2; a map of the area is shown in Fig. 1. The study area has a typical sub-tropical humid climate with an annual average rainfall of 859.4 mm. The annual mean temperature is 14.8°C, with temperature typical high of 38°C in July and low of 1°C in January. The soil is yellow-brown earth and the primary ore minerals are cinnabar (HgS) and stibnite (Sb$_2$S$_3$). Mining and smelting of Hg ores still occurs in the study area, and the locations of the air outlets from the mine, smelting workshop, ore-concentration workshop, and drain outlet are marked in Fig. 1. The smelting workshop and ore-concentration workshop are at the north end of the valley. A river flows eastward at the southern end of the valley in proximity to the mining area. The wastewater generated during the mining, concentrating, and smelting processes is treated. Some of the treated water is reused in the Hg production processes, and the rest is discharged directly into the river.

Approximately 200 inhabitants, including 100 workers and 96 residents, live in the study area. The area residents seldom eat fish, and rice and vegetables are the staple foods. Since residents have some awareness of the health risks associated with Hg mining activities, they consumed commercial rice from an uncontaminated area. However, the vegetables they consumed are grown on their own farmland.

1.2. Sample collection

Surface soil samples (n = 45) were collected from the study area in August of 2014. Four of the samples were collected within the Hg mining and smelting area, and the rest were collected from arable land along the river. The sampling sites are marked in Fig. 1. Three soil samples were collected in control area 2.5 km from Hg mining and retorting area as control or background samples. Each sample was taken from the surface layer 0–20 cm deep and was collected using a stainless steel shovel. Each soil sample was made up of five subsamples within an area of 1 m^2. All samples were sealed in a polyethylene bag and sent to the laboratory, where they were stored frozen until they were prepared for analysis.
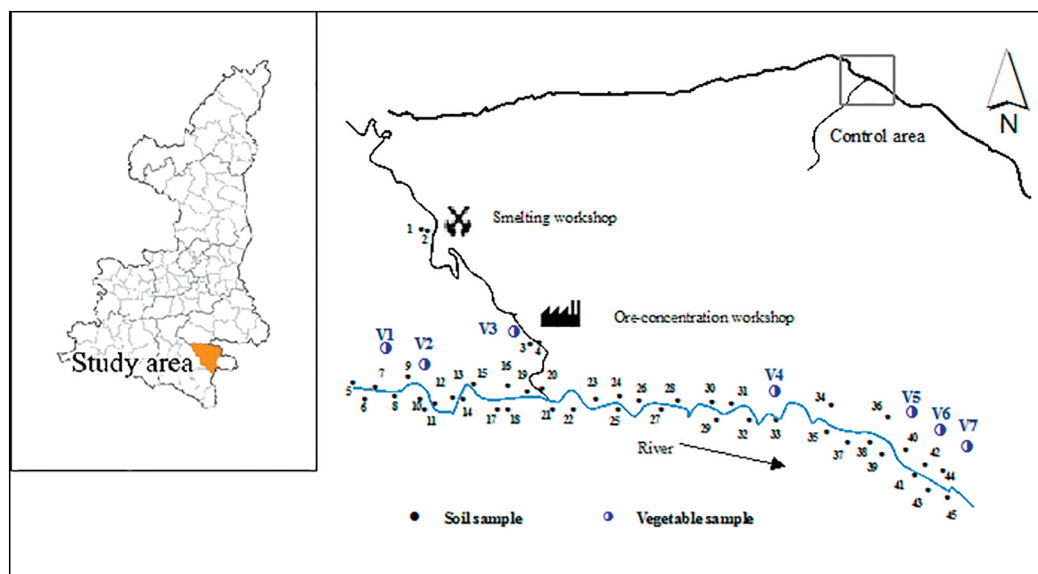

Fig. 1 – Soil and vegetable sampling sites in the study area. 1–45: soil samples; V1–V7: vegetable samples.

Vegetable samples (n = 38) were collected from seven domestic gardens; the sampling sites are marked in Fig. 1. To study the association between Hg concentrations in soils and vegetables, rhizosphere soil samples were also collected from the gardens. The vegetables sampled were bean (*Pisum sativum* Linn.), cabbage (*Brassica pekinensis* Rupr.), chili (*Capsicum annuum* L.), cucumber (*Cucumis sativus* Linn.), eggplant (*Solanum melongena* Linn.), scallion (*Allium fistulosum* L.), and tomato (*Lycopersicon esculentum* Mill.). Collected vegetable samples were rinsed with deionized water, cut and separated into edible parts and root parts. The weight of fresh vegetable was recorded to express the data on wet weight bases. Vegetable samples were wrapped well and stored under 4°C prior to freeze-drying.

Hair samples were collected from 18 local participants constituted 18.8% of total village population who had lived in their homes for at least 3 months prior to sampling. All participants were from local residents and no workers among them, for most workers are not locals and hired by factories for less than three months. The recruitment strategy was to include as many participants as possible. Most young adults in the study area worked in the surrounding cities, and some residents elected not to participate in the study, limiting the number of volunteers. All participants were asked to fill a questionnaire which included information on age, weight, profession, residential history, lifestyle (smoking and alcohol consumption), health status and food consumption such as average daily intakes of rice, vegetables, meat, and fish. Hair samples were cut with stainless steel scissors from the occipital region of the scalp, sealed in marked polyethylene bags, and sent to the laboratory for analysis.

1.3. Experimental setup

To study the relative contribution of the abiotic process, SRB, and FeRB to Hg methylation, a series of microcosm incubation experiments with potential stimulants or inhibitors of methylation was performed using contaminated farmland soil from the study area. Soil samples were collected from the surface (0–20 cm) of farmland in soil site 40. The soil sample had a neutral pH (7.1) and contained 3.3 mg/kg total organic carbon (TOC) and 7.57 mg/kg dissolved sulfate. The T-Hg and MeHg concentrations measured in the soil were (6.54 ± 1.21) mg/kg and (7.80 ± 0.03) μg/kg, respectively, with 33% moisture content. The samples were air-dried and sieved to an effective diameter of 2 mm and mixed homogenously. Approximately 50 g of soil were transferred to a glass jar; deionized water was added to obtain a final moisture content of 33%, and SRB and FeRB inhibitors and stimulants were added. There were totally five treatments, including abiotic control (Ab), SRB stimulant amendment (SS), SRB inhibitor amendment (SI), FeRB stimulant amendment (FS), and a blank treatment used as a control. Each treatment was performed in triplicate.

Abiotic control was prepared by autoclaving (SS-325i, TOMY Digital Biology, Japan) under high-pressure steam at 120°C for 2 hr. Sodium sulfate (Na_2SO_4) and sodium molybdate (Na_2MoO_4) were used as SRB stimulant and inhibitor, respectively (Pierre et al., 2014). Both compounds were added in the soil at 200 and 100 mg/kg, respectively. Amorphous iron(III) oxyhydroxide ($Fe(OH)_3$), which has been shown to be the most bioavailable form of iron for FeRB, was selected as FeRB stimulant (Lovley and Phillips, 1987). FeRB stimulant

amendment was prepared by amending 100 mg/kg of amorphous ferrihydrite ($Fe(OH)_3$) and 100 mg/kg of sodium molybdate. Stock solutions of Na_2MoO_4 (50 mmol/L) and Na_2SO_4 (50 mmol/L) were prepared with Milli-Q water (Millipore, Billerica, MA, USA). Amorphous ferrihydrite was synthesized according to a previously published protocol (Schwertmann and Cornell, 2000).

1.4. Sample preparation

1.4.1. Analyses of T-Hg and MeHg in soils
All soil samples were freeze–dried and passed through a 2-mm sieve. For T-Hg analyses, approximately 0.2 g of dry soil was digested in a 1:3 (V/V) mixture of HNO_3 and H_2SO_4 at 95°C in a water bath and shaken frequently. After cooling, an aliquot of each digest solution was prepared for analysis.

The method for MeHg extracted from soils was modified from a previous protocol (Obrist, 2012). Approximately 0.05 g of dry soil was added to a 50-mL centrifuge tube; H_2SO_4/KBr, $CuSO_4$ solution, and dichloromethane (CH_2Cl_2) were then added to the tube, which was shaken to mix the contents. After shaking for 2.5 hr, the organic phase was collected and the dichloromethane was back-extracted with Milli-Q water. Samples were buffered and ethylated for analysis.

1.4.2. Analyses of T-Hg and MeHg in hair and vegetables
All hair samples were washed with nonionic detergent, distilled water, and acetone, and dried in an oven at 60°C overnight. Vegetable samples were freeze–dried and ground into powder before analysis. For T-Hg digestion, hair and vegetable samples were digested in a 4:1 (V/V) mixture of HNO_3 and H_2SO_4 at 95°C in a water bath for 2.5 hr. After cooling, aliquots of each digest solution were prepared for analysis. For MeHg analysis, hair and vegetable samples were digested in a 25% solution of KOH in CH_3OH at 65°C in a water bath for 2.5 hr (Liang et al., 1996). Approximately 30 μL of solution was added to a vial filled with 30 mL of Milli-Q water and buffered and ethylated for analysis.

1.5. Mercury detection

T-Hg concentrations was determined by cold vapor atomic fluorescence spectrometry (CVAFS) following US Environmental Protection Agency (EPA) method 1631 (USEPA, 2002), with a detection limit of 0.005 μg/L. MeHg concentrations were measured using a MeHg analyzer (Tekran 2700, Knoxville, TN, USA), with a detection limit of 0.002 ng/L.

Quality control consisted of method blanks, matrix spikes, certified reference materials and duplicates. The measured concentration of T-Hg in certified reference materials (GSBZ50013-88, National Research Center for Certified Reference Materials, China) was (0.26 ± 0.03) mg/kg (n = 7), which was comparable to the certified value of 0.25 mg/kg. MeHg measured concentration was (72.52 ± 4.00) μg/kg (n = 7), in good agreement with the certified value of 75 μg/kg in certified reference materials (ERM-CC580, European Standards Agency, European). The measured T-Hg and MeHg concentration in hair certified reference material (NIES-13, National Institute for Environmental Studies, Japan) was (3.92 ± 0.51) μg/g (n = 7) and (4.00 ± 0.22) μg/g (n = 7), respectively, which were comparable to the certified value of 4.42

and 3.80 μg/kg. The recoveries from spiked samples ranged from 84% to 110% for T-Hg and from 88% to 117% for MeHg in vegetable samples. The relative standard deviation was lower than 9% for T-Hg and 8.2% for MeHg in soil, hair, and vegetable duplicate samples.

1.6. Analyses of environmental parameters in soils

The pH of each soil sample was measured using a pH electrode, with a soil-to-water ratio of 1:2.5 (m/m). The TOC contents of the soil samples were determined using the potassium dichromate oxidation spectrophotometric method, following Chinese National Standard method GB 7857-87. The dissolved SO_4^{2-} concentration in each soil sample was determined by ion chromatography using an IonPac AS14 column (Dionex, Sunnyvale, USA) (Mitchell et al., 2008).

Total DNA was extracted from 0.5 g soil using a FastDNA extraction kit (MP Biomedicals, Illkirch, France). Quantitative real-time Polymerase Chain Reaction (PCR) analysis of dsrAB gene was used to determine the abundance of SRB in the soil. The specific primers for the dsrAB gene β-subunit (350 bp) were DSRp2060F (5′-CAACATCGTYCAYACCCAGGG-3′) and DSR4R (5′-GTGTAGCAGTTACCGCA-3′), according to Geets et al. (2006). Amplification followed a three-step PCR procedure with 45 sec denaturation at 94°C, 45 sec annealing (52 and 72°C), and 30 sec elongation at 80°C. The amplification reactions were performed on an iCycler iQ PCR thermocycler (Bio-Rad, USA).

1.7. Calculation of probable daily intake (PDI)

To determine the daily intake of T-Hg and MeHg via consumption of vegetables, the PDI values were calculated by Eqs. (1) and (2):

$$PDI_{T-Hg} = C_{T-Hg} \times r/bw \qquad (1)$$

$$PDI_{MeHg} = C_{MeHg} \times r/bw \qquad (2)$$

where, PDI is given in micrograms per kilogram of body weight (bw) per day; C_{T-Hg} (μg/g) is the T-Hg concentration; C_{MeHg} (μg/g) is the MeHg concentration; and r (g/day) is the consumption rate. The consumption rate of vegetables for adults living in the study area was 304 g/day according to statistical data provided by the Chinese Environmental Protection Ministry (CNSA, 2015). For children, the consumption rate was estimated as 57% of that of adults. Average body weights of adults and children were 58 and 21.8 kg, respectively (CNSA, 2015).

1.8. Statistical analyses

All of the data were analyzed using the SPSS 17.0 statistical software package. The correlation coefficients were calculated between concentrations of T-Hg, MeHg and environmental factors (soil pH, contents of TOC, SO_4^{2-} and abundance of SRB). Pearson correlation tests were used to determine the relationship between Hg concentration in soil samples and vegetable samples. One way ANOVA test was used to determine differences between Hg concentrations in edible part and root part of vegetable samples. While, independent two-sample t-test was used to determine differences between Hg concentrations for

sampling and control sites. The p-values less than 0.05 were considered statistically significant.

2. Results and discussion

2.1. Hg in soil

2.1.1. Concentrations of T-Hg and MeHg
We quantified T-Hg and MeHg concentrations in soil collected from the mining activity areas, including the smelting workshop and ore concentration workshop, along with farmland along the river (Fig. 2). The mean concentration of T-Hg was (97.26 ± 195.79) mg/kg, and ranged from 1.53 to 1054.97 mg/kg. The highest T-Hg concentrations were found in the vicinity of the ore-concentrating workshop (sample site 4) and smelting workshop (sample site 1), indicating that ore smelting and concentration activities caused marked pollution in the surrounding soil. In samples collected from site 5 to site 45 located on farmland along the river, the highest T-Hg content was observed in sites 20 and 21, close to the drain outlet, which might be explained by the use of Hg-contaminated wastewater from the mine for irrigation. Elevated concentrations of MeHg were also observed in the samples and the mean value was (10.41 ± 8.77) μg/kg, ranging from 0.88 to 46.52 μg/kg. The peak concentration of MeHg was found in the soil (site 25) which was inundated by irrigation water. This is consistent with other studies that have demonstrated that inundated soils are favorable for Hg methylation (Drott et al., 2008; Qiu et al., 2006).The mean concentration of T-Hg in study area was significantly higher (p < 0.01) than that in control area (T-Hg: (1.34 ± 0.73) mg/kg). In addition, there was no MeHg concentration detected in soil samples collected in control area. Therefore, mining and retorting activities have posed significant Hg contaminant to soil in the study area.

These T-Hg levels are lower than those found in a Slovenian Hg mining area (0.18–2759 mg/kg) (Gosar et al., 2006) and in an Alaskan Hg mine area (0.03–5326 mg/kg) (Bailey et al., 2002), and similar to levels found in the soil of the Wanshan mining area in China (0.1–719 mg/kg) (Qiu et al., 2005). Similarly, MeHg concentrations were lower in our study than in soil collected in the Slovenian mining area (1.3–78 μg/kg), but was 2–4 times higher than those found in Wanshan (0.1–23 μg/kg) (Qiu et al., 2005), Lanmuchang (0.4–8.8 μg/kg) (Qiu et al., 2006), or Yanwuping (0.4–7.3 μg/kg) (Qiu et al., 2013) in Guizhou Province, China. The soil collected from our study area had slightly higher MeHg concentrations, suggesting that more attention should be paid to Hg methylation in soil.

2.1.2. Association of Hg and environmental factors
Mercury distribution and methylation in soil are complex processes, affected by many environmental factors. Table 1 shows the correlation analysis of T-Hg and MeHg and environmental factors, including SRB abundance, TOC and SO_4^{2-} contents, and pH level in soil. Soil collected from the study area contained 4.96 mg/kg (1.58–12.13 mg/kg) TOC and 53.04 mg/kg (7.57–159.51 mg/kg) SO_4^{2-}; the mean pH value was 7.57, ranging from 5.15 to 14.72. SRB abundance in the soil ranged from 0.53×10^8 to 1.41×10^8 copies/g (dry soil), a lower abundance than that found by Wang et al. (2014) in a paddy
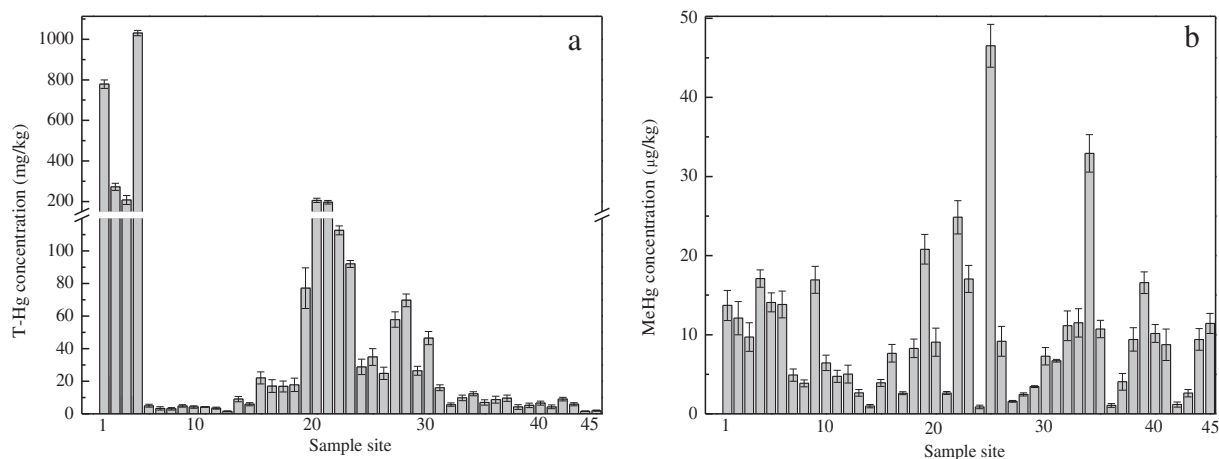
Fig. 2 – Hg concentrations in soil samples collected from study area. (a) T-Hg; (b) MeHg. T-Hg: total mercury; MeHg: methylmercury.

field (0.6×10^8 to 3.5×10^8 copies/g dry soil), due to the seasonal irrigation of paddy fields producing anaerobic conditions, which favored SRB survival.

There was a positive relationship between TOC and T-Hg concentrations, likely due to the absorption and fixation of Hg by organic carbon. In addition, positive correlation between T-Hg and pH was also observed in soils. This finding is similar to the findings from other Hg mining areas (Qiu et al., 2005; Li et al., 2012). The rocks in the study area are dolomite and carbonate, so high pH values in mining ore and calcines were predictable. During weathering and flooding events, large quantities of alkali compounds and Hg species in ore and calcines are released into the soil, explaining partially why the T-Hg concentration was correlated with pH in soil samples. No relationship was found between T-Hg levels and SRB abundance, indicating that Hg concentrations did not affect SRB abundance. This finding contrasted to previous study based on the shorting time cultivation that Hg concentration was negatively correlated to SRB numbers and activities of SRB was inhibited at high Hg levels (Liu et al., 2010; Zhou et al., 2012). Some soil SRB might exhibit resistance or adapt to high Hg concentrations over time.

No relationship was observed between T-Hg concentrations and MeHg concentrations, indicating that MeHg levels were not influenced by T-Hg levels. This contrasted with previous studies, in which soil containing high concentrations of T-Hg favored the production of MeHg (Rimondi et al., 2012;

Liu et al., 2014). Only the dissolved aqueous cation Hg^{2+} could be methylated to MeHg, rather than all forms of Hg (Hsu-Kim et al., 2013), and the dominant Hg form present was low-solubility cinnabar.

In the present study, no liner correlation was observed between MeHg and TOC concentrations, contrasting with studies reported that high levels of TOC favored Hg methylation (Schartup et al., 2013; Obrist, 2012).TOC may favor Hg methylation by stimulating the activities of Hg methylator microorganisms (Ullrich et al., 2001). However, TOC may also inhibit MeHg production by reducing the concentration of dissolved aqueous cation Hg^{2+} taken up by microorganisms (Frohne et al., 2012). We observed a positive correlation between MeHg and sulfate in the soil samples. Since sulfate is an electron acceptor, high concentrations in soil may stimulate SRB and yield greater MeHg production (Branfireun et al., 1999). Our findings likely indicated that SRB played an important role in Hg methylation in the soil. The correlation between MeHg and the number of SRB abundance in the present study may confirm this point. However, we could not infer causality from the linear relationship between MeHg and SRB abundance, so we further evaluated the main Hg methylator in Section 2.1.3.

2.1.3. Main Hg methylator in soil

To identify the main methylator in the soil of our study area, we conducted a series of incubation experiments using specific stimulants and inhibitors of microorganism which was considered to be the methylator of Hg. As shown in Fig. 3, during the 30-day incubation period, the abiotic control treatment (Ab) exerted no apparent effects on Hg methylation, indicating that MeHg could not be generated abiotically in our soil samples, and microbial methylation was the predominant process of MeHg production in the study area. The highest Hg methylation potential was observed in the SRB-stimulated (SS) treatment, and MeHg concentrations increased from (7.8 ± 0.03) to (8.18 ± 0.05) μg/kg. In contrast, MeHg production following SRB-inhibited (SI) treatment and FeRB-stimulated (FS) treatment was low, yielding only 0.02 μg/kg MeHg for SI and 0.05 μg/kg MeHg for FS. These results suggested that SRB rather

Table 1 – Correlations between abundance of sulfate-reducing bacteria (SRB), pH, and concentrations of total organic carbon (TOC), SO_4^{2-}, total mercury (T-Hg), and methylmercury (MeHg) in soil ($n = 45$).

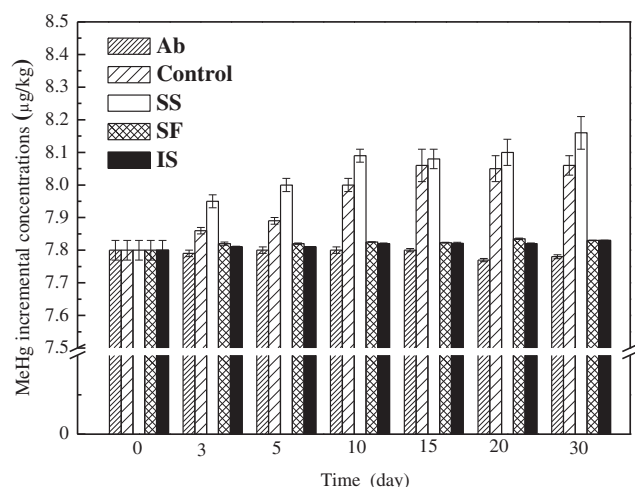|	Abundance of SRB	SO_4^{2-}	TOC	pH	T-Hg
SO_4^{2-}	0.23[*]				
TOC	0.14	0.076			
pH	0.011	0.049	0.033		
T-Hg	0.059	0.055	0.50[**]	0.21[*]	
MeHg	0.39[*]	0.36[*]	0.10	0.01	0.072

[*] $p < 0.05$; [**]$p < 0.01$.

Fig. 3 – MeHg concentrations in soils. Ab: abiotic control; SS: sulfate-reducing bacteria (SRB)-stimulated; FS: iron-reducing bacteria (FeRB)-stimulated and SRB-inhibited; SI: SRB-inhibited treatments.

Table 2 – T-Hg and MeHg concentrations and MeHg/T-Hg ratio in vegetables and soil rhizosphere ($n = 38$).				
Sample	Min	Max	Mean	SD
Edible part of vegetable T-Hg (μg/kg)	24.79	781.02	169.81	71.23
MeHg (μg/kg)	0.02	0.18	0.065	0.029
MeHg/T-Hg (%)	0.004	0.33	0.11	0.01
Root part of vegetable T-Hg (μg/kg)	25.50	277	112.88	38.25
MeHg (μg/kg)	0.01	0.06	0.03	0.01
MeHg/T-Hg (%)	0.01	0.12	0.05	0.01
Rhizosphere soil T-Hg (mg/kg)	15.37	224.28	94	12.28
MeHg (μg/kg)	1.48	18.05	7.71	0.95
MeHg/T-Hg (%)	0.007	0.024	0.013	0.006

than FeRB was the primary soil methylator of Hg in the study area.

SRB abundance in the soil was analyzed by real-time PCR (Fig. 4). SRB abundance followed the same trend of the increments of MeHg concentrations. The highest SRB numbers were observed following the SS treatment, and ranged from 0.96×10^8 to 1.28×10^8 copies/g (dry soil). In contrast, SRB numbers were lowest following the SI treatment, reaching a low of 0.64×10^8 copies/g (dry soil). SRB abundance has been reported to be related to the net MeHg production (Du et al., 2017). Therefore, higher SRB abundance may result in higher levels of MeHg produced in the soil. Our findings further confirmed that SRB played the critical role in Hg methylation in the soil of the study area.
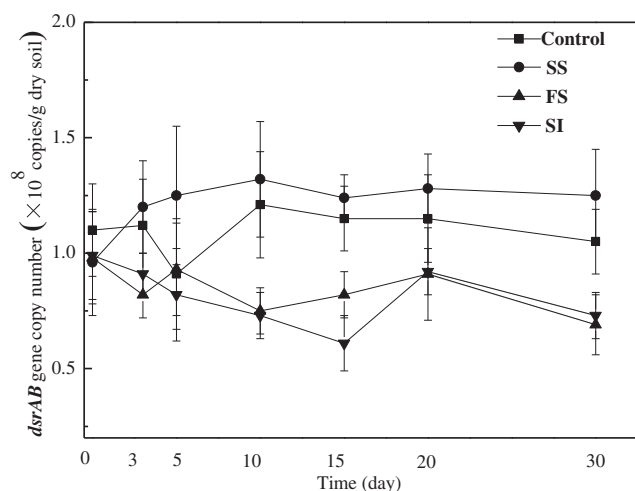
2.2. Hg levels in vegetables and human health risk assessment

The average concentration of T-Hg detected in vegetable samples from seven domestic gardens was (156.91 ± 65.31) μg/kg, ranging from 24.79 to 781.02 μg/kg. The mean concentration of MeHg was (0.052 ± 0.017) μg/kg, ranging from 0.01 to 0.18 μg/kg (Table 2). There was a significant linear correlation between T-Hg and MeHg levels in vegetable samples ($R^2 = 0.21$, $p < 0.05$). The mean ratio of MeHg to T-Hg was 0.07%, and ranged from 0.004% to 0.33%.

Fig. 5 displays concentrations of T-Hg and MeHg in the edible and root parts of vegetables sampled from the study area. T-Hg and MeHg concentrations in edible parts (T-Hg: (169.8 ± 27.5) μg/kg; MeHg: (0.065 ± 0.029) μg/kg, $n = 38$) were significantly higher (T-Hg: $p < 0.05$; MeHg: $p < 0.01$) than in root parts (T-Hg: (113 ± 8.5) μg/kg; MeHg: (0.029 ± 0.013) μg/kg). This finding might suggest that the edible parts of vegetables were more likely to accumulate Hg species than the root parts (Spada et al., 2012). The highest T-Hg concentrations were observed in the edible parts of vegetable samples collected from site V3, in the vicinity of the ore-concentration workshop, where ongoing activities likely released Hg species into the environment. In addition, T-Hg concentrations in all vegetables were higher than the limit of 10 μg/kg in food recommended by the Chinese National Standard Agency (GB 2762–2012). Mining activities evidently contaminate local vegetable crops.

Table 3 summarizes published reports on T-Hg and MeHg concentrations measured in vegetables. Compared with levels in non-Hg mining areas, T-Hg levels measured in vegetables in this study were higher than those reported by other studies. Compared to other Hg mining area, T-Hg concentration was similar to those obtained in Wanshan mining area, but lower than those collected from Idrijia, Slovenia. For MeHg, similar level was observed from Wanshan, Qingzhen, Weining and Pearl River Delta, but lower than those obtained in Lanmuchang and Gaohong in China. In addition, there was a significant positive association between T-Hg concentrations in rhizosphere soil and in vegetables ($R^2 = 0.72$, $p < 0.01$). A similar association was also noted between MeHg concentrations in rhizosphere soil and in vegetables ($R^2 = 0.57$, $p < 0.01$). These results suggest that vegetables accumulated Hg species, and Hg-contaminated soil may be a major source of Hg in vegetables.

Table 4 shows the PDI of T-Hg and MeHg from vegetable consumption for both adults and children in the study area.

Fig. 4 – *dsrAB* gene copy numbers in soil samples following treatment.
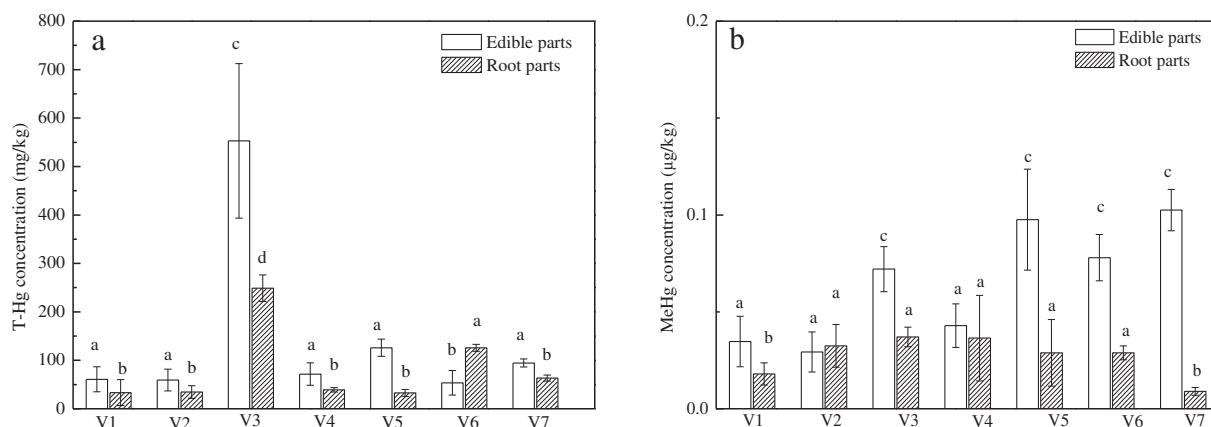
Fig. 5 – Hg concentrations in edible parts and root parts of vegetable samples. (a) T-Hg; (b) MeHg. Columns with the same letters indicate no significant differences among vegetable samples ($p < 0.05$).

Table 3 – Concentrations of Hg (µg/kg) in vegetable samples reported in the literature.

	T-Hg	MeHg	Reference
Wanshan Hg mining area, Guizhou Province, China	130	0.097	Zhang et al. (2010)
Lanmuchang Hg mining area, Guizhou Province, China	175.0 ± 40.8	2.59 ± 0.77	Qiu et al. (2006)
Idrija Hg mining area, Slovenia	215	25	Miklavčič et al. (2013)
Qingzhen coal-fired plant area, Guizhou Province, China	4.0	0.03	Zhang et al. (2010)
Weining Zn-smelting area, Guizhou Province, China	2.5	0.02	Zhang et al. (2010)
Tuscanychlor-alkali plant area, Italy	19.0 ± 20.5	0.27 ± 0.21	Gibicar et al. (2009)
Gaohong compact fluorescent lamp factories area, Zhejiang Province, China	18.6 ± 6.5	0.11 ± 0.03	Shao et al. (2012)
Pearl River Delta, South China	1.3 ± 0.6	0.03 ± 0.01	Shao et al. (2013)
Hg mining area, China	156.9 ± 65.3	0.05 ± 0.02	This study

The average PDI of T-Hg was 0.82 µg/(kg body weight·day) (range: 0.13–4.25 µg/(kg bw·day)) for adults and 1.21 µg/(kg bw·day) (range: 0.22–6.20 µg/(kg bw·day)) for children. For MeHg, the mean PDI was 0.34×10^{-3} µg/(kg bw·day) (range: 0.10–0.96×10^{-3} µg/(kg bw·day)) for adults and 0.49×10^{-3} µg/(kg bw·day) (range: 0.15–1.40×10^{-3} µg/(kg bw·day)) for children. The Joint WHO/FAO Expert Committee on Food Additives (JECFA) established a provisional tolerable daily intake (PTDI) value of 0.57 µg/(kg bw·day) for T-Hg in 2010 (JECFA, 2010). In the present study, 30% of vegetable samples exceeded the T-Hg PTDI value for adults, and 61% of vegetable samples exceeded the T-Hg PTDI value for children. The reference dose (RfD) for MeHg

recommended by the US EPA (2001) is 0.1 µg/(kg bw·day) (USEPA, 2001). The PDI of MeHg for all vegetable samples was well below the RfD for both adults and children.

2.3. Hg levels in hair samples

Table 5 summarizes the information of T-Hg and MeHg concentrations in human hair collected from different resident settlements. T-Hg concentrations in human hair ranged from 1.57 to 12.61 mg/kg, with a mean value of (4.29 ±3.25) mg/kg. The highest T-Hg concentrations were found in hair samples collected from participants living near the ore-concerning factory

Table 4 – Estimated daily intake of T-Hg and MeHg *via* vegetable consumption by adults and children in the study area (unit: µg/(kg bw·day)).

Sample site	T-Hg		MeHg	
	Adult	Children	Adult	Children
V1	0.33 (0.19–0.42) [a]	0.48 (0.27-0.7)	0.20×10^{-3} (0.15×10^{-3}-0.29×10^{-3})	0.29×10^{-3} (0.20×10^{-3}-0.43×10^{-3})
V2	0.23 (0.13–0.39)	0.34 (0.2–0.57)	0.16×10^{-3} (0.10×10^{-3}-0.23×10^{-3})	0.24×10^{-3} (0.15×10^{-3}-0.33×10^{-3})
V3	3.11 (2.38–4.15)	4.54 (3.47–6.21)	0.39×10^{-3} (0.33×10^{-3}-0.45×10^{-3})	0.49×10^{-3} (0.45×10^{-3}-0.66×10^{-3})
V4	0.42 (0.24–0.55)	0.61 (0.36–0.8)	0.25×10^{-3} (0.13×10^{-3}-0.43×10^{-3})	0.37×10^{-3} (0.19×10^{-3}-0.62×10^{-3})
V5	0.71 (0.17–1.35)	1.04 (0.24–1.98)	0.52×10^{-3} (0.37×10^{-3}-0.65×10^{-3})	0.76×10^{-3} (0.54×10^{-3}-0.95×10^{-3})
V6	0.33 (0.19–0.49)	0.48 (0.28–0.72)	0.43×10^{-3} (0.25×10^{-3}-0.61×10^{-3})	0.62×10^{-3} (0.37×10^{-3}-0.89×10^{-3})
V7	0.35 (0.21–0.48)	0.51 (0.31–0.70)	0.56×10^{-3} (0.16×10^{-3}-0.96×10^{-3})	0.81×10^{-3} (0.23×10^{-3}-1.4×10^{-3})

[a] The table shows mean values, while the Min and Max values are shown in parentheses.

Table 5 – Mercury concentrations (mg/kg) in hair of residents from seven sample sites.

Participant	Gender	Age	T-Hg	MeHg	MeHg/T-Hg (%)	Sample site
p1	Male	27	1.88	0.04	2.13	V1
p2	Male	27	3.01	0.15	4.98	V1
p3	Male	30	4.43	0.37	8.35	V2
p4	Female	43	6.17	0.14	2.27	V2
p5	Male	50	3.57	0.45	12.61	V2
p6	Male	33	11.37	0.07	0.62	V3
p7	Female	35	12.61	0.19	1.51	V3
p8	Female	40	2.11	0.17	7.96	V4
p9	Female	41	1.70	0.04	2.55	V4
p10	Male	44	2.11	0.06	2.65	V4
p11	Male	42	1.57	0.20	12.69	V5
p12	Male	47	1.91	0.30	15.77	V5
p13	Male	33	7.01	0.54	7.72	V6
p14	Female	46	2.10	0.73	34.90	V6
p15	Male	48	5.91	0.90	15.18	V7
p16	Female	37	4.33	0.90	20.86	V7
p17	Female	33	3.21	0.94	29.19	V7
p18	Female	50	2.34	0.88	37.45	V7

(sample site V3). It has been reported that T-Hg concentration in hair associated with both endogenous Hg contamination through consumption of food contaminated by Hg species and Hg concentrations in the air, since elemental Hg could adhere to hair (Li et al., 2008; Kobal et al., 2017). Although the concentrations of Hg in the air were not measured in the present study, our earlier study showed that the average Hg concentration in air around the ore-concerning factory was 642–923 ng/m^3, significantly higher than that along the river (27–60 ng/m^3), where other study participants lived (Zhang et al., 2015). The results in the present study indicated that elevated Hg concentration in hair might be affected by Hg level in air.

MeHg concentration ranged from 0.04 to 0.94 mg/kg, with a mean value of (0.35 ± 0.33) mg/kg. MeHg constituted approximately 11.4% of T-Hg in hair samples (0.6%–37.5%). An abnormally low ratio of MeHg to T-Hg was observed in hair sample collected in site V3. This might be contributed to that Hg vapor released from ore-concerning factory could deposit to hair surface and not be easily wash away during pre-treatment. Furthermore, high T-Hg concentrations that were also found in

vegetable samples collected from V3 and T-Hg could be accumulated in hair *via* consumption of vegetable contained Hg species. The similar lower ratio of MeHg to T-Hg was also found in previous study, such as workers recruited by compact fluorescent lamp (CFL) manufactory, for the high Hg contents in air (Liang et al., 2015). There was no relationship observed between T-Hg and MeHg concentrations in hair samples ($R^2 = 0.007$, $p > 0.05$). This may be explained by different exposure sources. T-Hg concentrations in human hair have been shown to be a good indicator of Hg exposure, either by ingestion of contaminated food or by deposition from the air (Li et al., 2011a). However, the main exposure route of MeHg in humans is diet, and exogenous contamination is rare (Liang et al., 2015).

Table 6 summarizes information of hair Hg concentrations in published studies, and compares the findings with those of the present study. T-Hg concentration of hair samples in our study was lower than those of workers, but similar with those of residents from other mining area in Guizhou Province. MeHg concentrations were mostly lower than those found in other studied populations, especially where fish consumption was considered the main exposure source of MeHg.

Previous studies reported that dietary exposure was the main route of MeHg exposure compared to through air and water (Feng et al., 2008; Zhang et al., 2010). Therefore, the pathway of MeHg exposure *via* food consumption was investigated in the present study. Residents in the studying area rarely eat fish due to their eating habits. In addition, rice consumed by local inhabitants was mostly from non-contaminated area. However, vegetables consumed by residents were from domestic gardens. A correlation analysis of MeHg in human hair and the PDI of MeHg from vegetable consumption was conducted. There was a positive correlation observed between hair MeHg concentrations and the PDI from vegetable consumption ($R^2 = 0.39$, $p < 0.05$), as shown Fig. 6. This suggests that vegetable consumption may be an important exposure way for local inhabitants.

3. Conclusions

We found high concentrations of T-Hg and MeHg in soil, vegetables, and human hair samples in the study area. T-Hg

Table 6 – Mercury concentrations (mg/kg) in hairs and exposure source reported in the literature.

Location	Population	Hg concentration		Exposure source	Reference
		T-Hg	MeHg		
Wanshan Hg mining area, China	Residents	7.3 (2.1–58.5)[a]	2.8 (0.8–5.6)	Rice	Feng et al. (2008)
Wuchuan Hg mining area, China	Workers	34 (7.6–93.1)	0.95 (0.5–1.7)	Rice	Li et al. (2011b)
	Residents	2.7 (1.1–4.3)	1.8 (0.9–3.1)		
Compact fluorescent lamp factory area, China	Workers	1.4 (0.1–22.8)	0.2 (0.003–1.2)	Rice	Liang et al. (2015)
	Residents	0.7 (0.2–1.7)	0.1 (0.004–0.9)		
Zhoushan island, China	Male	5.7 (1.3–29.9)	3.8 (0.9–9.5)	Fish	Cheng et al. (2009)
	Female	2.3 (0.8–6.4)	1.8 (0.3–4.1)		
Pearl River Delta, China	Residents	1.08 ± 0.94	0.58 ± 0.59	Fish	Shao et al. (2013)
Amazon	Residents	8.2 (0.7–20.1)	2.1 (0.1–5.7)	Fish	Passos and Mergler (2008)
Japan	Residents	17.7 ± 11.5	NA	Fish	Endo and Haraguchi (2010)
Hg mining area, China	Residents	4.3 (1.6–12.6)	0.35 (0.04–0.9)	vegetable	This study

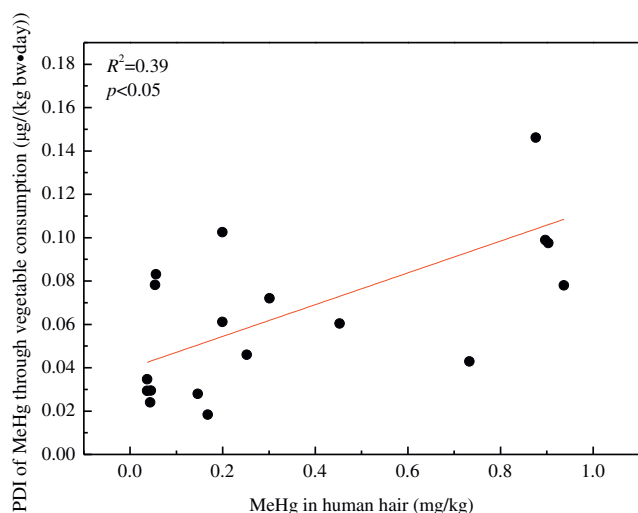[a] The table shows mean values, while the Min and Max values are shown in parentheses.

Fig. 6 – Correlation between MeHg in human hair and PDI of MeHg through vegetable consumption. PDI: probable daily intake.

concentrations in soil were affected by TOC concentrations and soil pH. MeHg concentrations correlated with SRB abundance. Soil incubation experiments suggested that the main Hg methylation route was microbial, and SRB was the dominant methylator in the soil.

T-Hg and MeHg levels were higher in the edible parts of vegetables than in the roots. Hg concentrations in vegetables correlated with those in the rhizosphere soil samples, indicating that T-Hg and MeHg contents in the vegetables were derived in part from the rhizosphere soil. The mean estimated daily T-Hg intake *via* vegetables for both adults and children was higher than the PTDI value recommended by the Food and Agriculture Organization. However, the mean probably daily MeHg intake was lower than the RfD. MeHg concentration in hair of local residents was positively related to PDI of MeHg *via* vegetable indicating that vegetable intake in the study mining area may pose a health risk of MeHg exposure to local habitants.

Acknowledgments

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