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Compound specific stable isotope determination of methylmercury in contaminated soil

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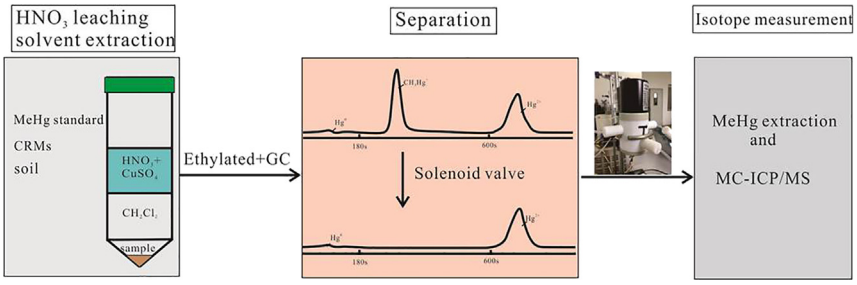
HIGHLIGHTS

- A new compound-specific method was developed for determining stable Hg isotopes in MeHg in soils and sediments.
- No significant MDF and MIF occurred during extraction processes.
- Significant differences of δ^{202}Hg values were observed between MeHg and THg in paddy soils.
- MeHg isotope analysis can be used to understand MeHg migration and transformation processes in soil–rice systems.

GRAPHICAL ABSTRACT



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ABSTRACT

Rice is one of the main sources of methylmercury (MeHg) to humans, and soil is the main source of MeHg to rice grains. Determining the Hg isotope composition in environmental samples is a good way of characterizing sources of Hg pollution and investigating environmental processes. We developed a new compound-specific method for determining stable Hg isotopes in MeHg in contaminated soil and sediment. The method involved HNO_3 leaching/solvent extraction, chemical ethylation, and separation by gas chromatography with a solenoid valve optimized to enrich MeHg. The method was optimized by using MeHg standard solution, certified reference materials and paddy soil samples. The δ^{202}Hg precision for replicate MeHg isotope analyses was 0.14‰ (2 × standard deviation, $n = 11$), and no fractionation of Hg stable isotopes was found during the separation processes. The δ^{202}Hg values for MeHg in paddy soils were −1.78‰ to −1.30‰, which were lower than the δ^{202}Hg values for total Hg (−1.32‰ to −0.44‰). The results indicated that methylation (rather than demethylation) was the dominant process in the paddy soils. The method developed in this study can help us to better understand MeHg migration and transformation processes in paddy soil-rice ecosystem.

1. Introduction

Mercury (Hg) is one of the most toxic pollutants and its ecological and toxicological effects are strongly dependent on its chemical forms (Ullrich et al., 2001). Methylmercury (MeHg), an organic Hg species, is one of the most poisonous Hg species and can bioaccumulate and biomagnify in food chains (Gilmour et al., 1992). Fish is the most important source of MeHg to humans in many regions of the world, but rice is the main source of MeHg in areas contaminated with Hg such as in southwest China where rice is the staple food supply (Feng et al., 2008; Zhang et al., 2010). Much attention has been paid to the sources of MeHg to rice grains (Qiu et al., 2008; Li et al., 2010; Li et al., 2017). MeHg concentrations and distributions in rice plants were evaluated

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throughout entire rice growth cycle and it suggested that paddy soil was the main source of MeHg to rice grains (Meng et al., 2011). MeHg in paddy soil was mainly produced by in situ methylation of Hg(II). The amount of Hg in paddy soils originated from atmospheric deposition and irrigation was negligible relative to the amount of Hg pool in the soil (Zhao et al., 2016a, 2016b). Total Hg (THg) stable isotope patterns suggested that soil and ambient air both contributed Hg to rice (Yin et al., 2013a). Results obtained from a greenhouse experiment using spiked Hg isotope approach found that MeHg was synthesized by Hg methylating microorganisms in the saturated soil and then transported to the rice grains (Strickman and Mitchell, 2016).

Paddy fields are special short-term wetlands in which both methylation and demethylation of Hg can occur (Mark et al., 2014; Zhao et al., 2016a, 2016b). The MeHg concentrations in paddy soils will be the net results of both methylation and demethylation, and MeHg production at different times will reflect the net methylation potential of the paddy soil (Zhao et al., 2016a, 2016b). Microorganisms such as sulfate-reducing bacteria, iron-reducing bacteria, and methanogens play important roles in the methylation and demethylation processes (Compeau and Bartha, 1984, 1985; Fleming et al., 2006; Gilmour et al., 1992, 2013; Oremland et al., 1991; Yu et al., 2013). The MeHg migration and transformation processes that occur in soil–rice ecosystems are still poorly understood.

Stable Hg isotope analysis techniques are useful for studying Hg sources and the processes that affect the environmental behavior of Hg (Blum et al., 2014; Yin et al., 2014). Mass-dependent fractionation (MDF, quantified as the δ^{202}Hg value) and mass-independent fractionation (MIF, quantified as the Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg values) commonly occur during these environmental processes, such as hydrothermal reaction, sorption/desorption, photochemical reduction, and photochemical degradation (Bergquist and Blum, 2007; Blum et al., 2014). The mechanisms of fractionation are considered as equilibrium fractionation and kinetic fractionation (Criss, 1999; Young et al., 2002). The MIF of Hg can be predicted based on the magnetic isotope effect (Buchachenko, 2001; Buchachenko et al., 2004) and the nuclear volume effect (Bigeleisen, 1996; Schauble, 2007). Laboratory experiments aimed at studying methylation and demethylation commonly use stable Hg isotopes as tracers to allow Hg isotope fractionation during the studied environmental processes. In Hg methylation experiments, sulfate-reducing bacteria in a pure culture caused MDF of stable Hg isotopes and generated MeHg with lighter Hg isotopes than Hg(II) isotope (Rodríguez-González et al., 2009; Perrot et al., 2015). Similar results have been found in abiotic methylation experiments using methylcobalamin and other methyl group donors (Jiménez-Moreno et al., 2013; Perrot et al., 2013). The MeHg remaining in the reactors became progressively heavier (increasing the δ^{202}Hg value) over time as MeHg was degraded to give volatile elemental Hg (Hg(0)), similar to what has been found during the photodecomposition of dissolved MeHg (Kritee et al., 2009; Malinovsky et al., 2010). Different Hg isotope fractionation patterns have been found during Hg methylation and demethylation of Hg, meaning that stable Hg isotope fractionation can be useful for distinguishing the effects of methylation and demethylation in paddy soils. Analyses of the stable Hg isotopes in MeHg in soil can provide data to improve our understanding of Hg methylation and demethylation of Hg in the environment.

Gas chromatography (GC) coupled with multicollector inductively coupled plasma mass spectrometry (MC-ICP/MS) was involved in measurement of compound specific Hg isotope ratios (Epov et al., 2008; Dzurko et al., 2009). But the external precision (2SD) was high as 0.56‰ for δ^{202}Hg measurement (Epov et al., 2008) and high Hg species concentration samples were needed to be digested and introduced into measurement system (Dzurko et al., 2009). An offline method was developed to measure the Hg isotope compositions of MeHg in estuarine sediments, involving distillation, ethylation, GC, and separation using a physical solenoid valve (Janssen et al., 2015). High precision and sensitivity of offline method can measure Hg isotopes in MeHg form in environmental samples such as sediments with extremely low MeHg ratio (<1%). However, Liang et al. (2004) found that unlike isolating Hg by HNO_3 leaching/solvent extraction, distillation decreases the MeHg concentration when the inorganic mercury (Hg(II)) concentration is >2 μg/g. It is therefore necessary to optimize the pretreatment method used to separate MeHg from soil samples with high THg concentrations.

In this study, we aimed to develop a new method to determine compound specific stable isotope of MeHg in contaminated soil. HNO_3 leaching/solvent extraction, chemical ethylation, and separation by gas chromatography were combined to enrich MeHg for Hg isotope analysis by MC-ICP/MS. We validated this method by experiments on MeHg standard solution and certified reference materials. The method developed in this study enables us to determine Hg isotope compositions in MeHg form in paddy soils, which can help us to better understand the mechanism of Hg methylation and demethylation and MeHg bioaccumulation in the rice grain in soil–rice systems.

2. Materials and methods

2.1. Sample collection

Samples were collected from two typical Hg-contaminated areas in Guizhou Province, southwest China. One area is in Qingzhen (N 26°34′–26°38′, E 106°28′–106°30′) polluted with Hg by emissions from a chemical plant and the other one is in Wanshan (N 27°33′, E 109°12′) polluted through Hg mining activities. Rice is an important crop in the studied areas. Surface soil samples (0–10 cm deep) were collected in each area. Each sample was double-bagged and stored at −20 °C. Later, each sample was freeze-dried, and was then grounded using a mortar and passed through a 200 mesh sieve before being analyzed for Hg contents.

2.2. THg measurements and preparation for THg isotope analysis

A 0.1 g aliquot of a soil sample was digested in a freshly prepared 3:1 (v/v) mixture of HCl and HNO_3 at 95 °C in a water bath for 45 min (Yin et al., 2013a). The THg concentration in the digest was determined by $SnCl_2$ reduction and cold-vapor atomic fluorescence spectrometry (CVAFS) (Method 1631, 2002). The effectiveness of the digestion process was verified by digesting an aliquot of European certified reference material CC580 (estuarine sediment). MeHg working standards were dissolved in 20% (v/v) HNO_3 and oxidized with 25% (w/w) BrCl. These solutions were analyzed to allow estimating the original Hg isotope composition.

The recoveries of THg concentrations in CC580 ranged from 90% to 98%, and the relative percentage differences for THg concentrations in duplicate samples were <5%. The method blank was 0.04 ng/mL. Each digest solution was diluted to give a THg concentration of 1 ng/mL before the Hg isotope composition in the solution was determined.

2.3. MeHg measurements and isotope analysis

The working MeHg standard solution (10 ng/mL, diluted from a MeHg stock solution; Brooks Rand, USA) and soil samples were dealt with HNO_3 leaching solvent extracted, and then ethylated. The solutions were then purged with the vapor passing through a trap. The trapped compounds were then released and analyzed by GC-CVAFS (Bloom, 1989; Liang et al., 2004). The MeHg separation and trapping system described by Janssen et al. (2015) was used. The GC column was designed as a helical U-tube (0.8 cm inner diameter, 90 cm length) to accumulate large amounts of MeHg. And it was filled with 15% OV-3 Chromosorb W-AW 60/80 and kept at 70 °C. The trap column was packed with 100 mg of Tenax to ensure that the derivatives were completely captured. A Teflon solenoid valve was placed between the pyrolysis system

and the atomic fluorescence spectrometer to divert MeHg-derived Hg (0) to a gold trap.

The recoveries of MeHg for the certified reference materials CC580 (sediment) and TORT-2 (lobster hepatopancreas) were 80%–102% ($n = 4$), and the relative percentage differences for MeHg concentrations in duplicate samples were <8%.

An overview of the protocol used to collect MeHg for Hg isotope analysis is shown in Fig. 1. First, MeHg working standards solution were dissolved in 20% (v/v) HNO_3 and oxidized with 25% (w/w) BrCl. These solutions were analyzed to estimate the original MeHg isotope composition. Second, working MeHg standard solution was ethylated and purged for 50 min with the Tenax trap capturing the volatile chemicals released. Organomercury species were desorbed from the Tenax trap at 200 °C and transferred using a carrier gas flow rate of 130 mL/min to the GC column. The Teflon solenoid valve opened at the end of the Hg(0) peak (at 180 s) and closed at the beginning of the Hg(II) peak (at 600 s). The Hg collected by the gold trap reflected the amount of MeHg trapped in the Tenax trap. The amount of Hg in the gold trap indicated the ethylation efficiency. Once exposed to Hg, the gold trap was heated to 500 °C for 3 min using a carrier gas flow rate of 25 mL/min, and the desorbed Hg was collected in an absorption solution (a 3:1 (v/v) mixture of 20% HNO_3 and HCl). The absorption solution was then added to 3% (v/v) BrCl and stored at 4 °C until Hg isotope analysis was performed. This MeHg isotope value reflects the effect of ethylation process. Third, MeHg standard solution, certified reference materials (CC580 and TORT-2), and soil samples were treated with HNO_3 leaching/solvent extraction to decrease the effects of high Hg(II) concentrations in the extracts. The extracts were also subjected to the subsequent processes described above. The MeHg concentrations in the soil samples were low, so a large amount of each digest was used to supply sufficient Hg to one gold trap. This MeHg isotope value reflects the effect of pretreatment process.

2.4. Stable Hg isotope analysis

The Hg isotopes in the prepared solutions were measured by MC-ICP/MS (Nu Instruments, UK) at the Chinese State Key Laboratory of Environmental Geochemistry, at the Institute of Geochemistry. The measurement procedures were described in detail by Yin et al. (2010).

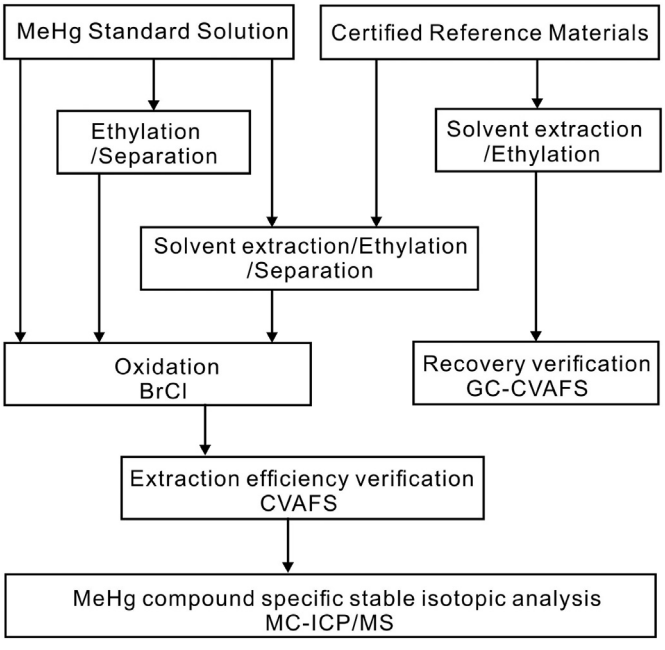

Fig. 1. Validation protocol for MeHg separation, MeHg quantification, and MeHg isotope measurement.

Thallium internal correction and an external standard and sample bracketing method were used to make precise and accurate measurements. Each sample was measured in triplicate and bracketed with US National Institute of Standards and Technology standard reference material 3133, and the results were expressed in delta notation (δ), calculated using Eq. (1),

$$\delta^{xxx}Hg_{sample}(‰) = \left[\left({}^{xxx/198}Hg_{sample}/{}^{xxx/198}Hg_{NIST3133}\right)-1\right] \times 1000, \quad (1)$$

where xxx is 199, 200, 201, 202, or 204. The MDF was defined using δ-notation, but MIF was defined using capital delta (Δ) notation (Blum and Bergquist, 2007), calculated using Eqs. (2) and (3).

$$\Delta^{199}Hg = \delta^{199}Hg - \delta^{202}Hg \times 0.252 \quad (2)$$

$$\Delta^{201}Hg = \delta^{201}Hg - \delta^{202}Hg \times 0.752 \quad (3)$$

The results of the analysis of the UM–Almadén standard solution (δ^{202}Hg −0.59‰ ± 0.11‰, Δ^{199}Hg 0.03‰ ± 0.08‰, Δ^{201}Hg 0.01‰ ± 0.05‰, 2σ, $n = 15$) were similar to those found in a previous study (Blum and Bergquist, 2007). The THg stable isotope results for the European reference material CC580 (δ^{202}Hg −0.51‰ ± 0.26‰, Δ^{199}Hg −0.01‰ ± 0.08‰, Δ^{201}Hg −0.04‰ ± 0.1‰, 2σ, $n = 2$) agreed with those of a previous study (Janssen et al., 2015). This was the first time the Hg isotopes in MeHg in the European reference material CC580 (δ^{202}Hg −2.62‰ ± 0.29‰, Δ^{199}Hg 0.01‰ ± 0.06‰, Δ^{201}Hg −0.02‰ ± 0.04‰, 2σ, $n = 3$) were measured. The stable Hg isotope results for the MeHg in the certified reference material TORT-2 (δ^{202}Hg 0.61‰ ± 0.04‰, Δ^{199}Hg 1.04‰ ± 0.06‰, Δ^{201}Hg 0.83‰ ± 0.01‰, 2σ, $n = 2$) were similar to those of a previous study (Masbou et al., 2013). The detail data of THg and MeHg isotope compositions were showed in Table 1.

3. Results and discussions

3.1. Relationship between the ethylation efficiency and the MeHg isotope results

In the aqueous phase, $NaBEt_4$ reacts with HgX_2 to form diethylmercury and with labile CH_3HgX species to form methylethylmercury (Rapsomanikis et al., 1986). These volatile derivatives were purged and then captured by the Tenax tube (Liang et al., 1994). The ethylation efficiencies achieved using the working MeHg standard solution were 55%–80%. This value decreased from 80% to 55% within two months because $NaBEt_4$ is a very sensitive reagent to oxygen (Jr and Riddle, 1961), and the conditions of $NaBEt_4$ preparation and storage may significantly affect its ethylation efficiency. In a previous study, labile CH_3HgX species were completely converted into methylethylmercury (Bloom, 1989; Janssen et al., 2015). We tested $NaBEt_4$ purchased from different companies and optimized the pretreatment method, but the ethylation efficiency remained relatively low. However, no MDF or MIF was found when the standard MeHg

Table 1
Hg isotopic compositions of THg and MeHg fractions in UM-Almadén standard solution and certified reference materials (mean ± 2SD).

	Fraction	n	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{201}Hg (‰)
UM-Almadén	THg	15	−0.59 ± 0.11	0.03 ± 0.08	0.01 ± 0.05
CC580	THg	2	−0.51 ± 0.26	−0.01 ± 0.08	−0.04 ± 0.1
CC580[a]	THg	5	−0.48 ± 0.06	−0.01	−0.03
TORT-2	MeHg	2	0.61 ± 0.04	1.04 ± 0.06	0.83 ± 0.01
TORT-2[b]	MeHg	9	0.54 ± 0.15	1.04 ± 0.12	0.83 ± 0.12
CC580	MeHg	3	−2.62 ± 0.29	0.01 ± 0.06	−0.02 ± 0.04

[a] Values were adopted from Janssen et al., 2015.
[b] Values were adopted from Masbou et al., 2013.

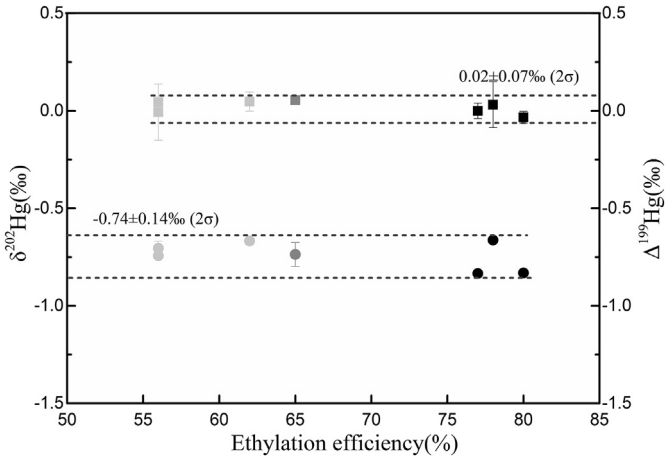

Fig. 2. Mercury isotope compositions of the standard MeHg solution ethylated using different batches of ethylation reagents.

solution was subjected to the pretreatments (Fig. 2). The δ^{202}Hg values for the standard MeHg solution were between −0.66‰ and −0.83‰ with a mean of −0.74‰ ± 0.14‰ (2σ, $n = 7$). The Δ^{199}Hg values were between −0.03‰ and 0.05‰ with a mean of 0.02‰ ± 0.07‰ (2σ, $n = 7$).

Measurable δ^{202}Hg MDFs of up to 3.56‰ ± 0.09‰ have been found when <90% of the Hg(II) was ethylated by $NaBEt_4$ (Yang and Sturgeon, 2009). We determined the Hg concentration in the solution after performing ethylation and found that <5% of the MeHg remained, indicating that >95% of the MeHg was ethylated by the $NaBEt_4$. In a previous study, the Hg isotope composition did not change when 66% of the MeHg was produced by the action of methylcobalamin, which methylated 98% of the Hg(II) (Jiménez-Moreno et al., 2013). In our study, the pyrolysis system and gold trap used during the purging process were connected in series after the Tenax tube. The Hg in the Tenax tube and the gold trap together gave recoveries of 95%–100%, indicating that some derivatives were produced that could not be trapped by the Tenax.

3.2. Pretreatment and MeHg isotope analysis

We tested three main parts of the separation system step by step and found that the sample pretreatment procedure and ethylation and GC procedures did not produce significant Hg fractionation. The initial Hg isotope composition of the standard MeHg solution was determined by oxidizing the MeHg with BrCl in an acidic solution. The mean δ^{202}Hg was −0.73‰ ± 0.13‰ (2σ, $n = 8$). The δ^{202}Hg value of the standard MeHg solution remained constant, at a mean of −0.74‰ ± 0.14‰ (2σ, $n = 7$), throughout the ethylation and separation processes. The mean δ^{202}Hg after HNO_3 leaching/solvent extraction, ethylation and GC separation, and solenoid valve separation was −0.78‰ ± 0.10‰ (2σ, $n = 4$), which was similar to the initial δ^{202}Hg value (Table 2). We concluded that Hg isotope fractionation during the pretreatment and ethylation procedures was not significant ($p > 0.05$). No MIF was found in any sample subjected to the three procedures (Δ^{199}Hg = −0.1‰ to 0.1‰). These results indicated that the separation method we developed did not induce MDF or MIF and could therefore be used to analyze natural samples.

The HNO_3 leaching/solvent extraction method for determining MeHg in sediment was developed by Bloom (1989) and improved by Liang et al. (1994). A high Hg(II) concentration in soil may interfere with the determination of MeHg using the distillation method because demethylation could occur during the pretreatment processes (Liang et al., 2004). However, HNO_3 leaching/solvent extraction gave high recoveries and good precisions for samples with high Hg(II) concentrations and for samples spiked with Hg(II) standards. This method has been widely used to analyze soil samples from Hg-contaminated areas, and has given good recoveries of 84%–101% (Liu et al., 2011; Meng et al., 2015; Yan et al., 2013). In the present study, the soils in Hg contaminated areas show high THg concentrations and low MeHg concentrations (Li et al., 2009; Mendes et al., 2016). The HNO_3 leaching/solvent extraction method can therefore be used in MeHg isotope analyses.

3.3. THg isotope composition

The Hg isotope compositions of THg and MeHg in the paddy soils are shown in Fig. 3. The mean THg δ^{202}Hg value for the Wanshan paddy soil was −0.44‰, similar to the mean of −0.43‰ ± 0.12‰ found in a previous study (Feng et al., 2013). The mean δ^{202}Hg values for cinnabar and calcine from Wanshan were −0.74‰ ± 0.11‰ (2σ, $n = 14$) and 0.08‰ ± 0.20‰ (2σ, $n = 11$), respectively (Yin et al., 2013a). The δ^{202}Hg values for the Wanshan paddy soils were therefore in the ranges for Hg ore and Hg in waste calcine, indicating that the Hg in the paddy soil was mainly derived from Hg ore and Hg in waste calcine (Yin et al., 2013a). The THg δ^{202}Hg values for the Qingzhen paddy soils were lower, ranging from −1.08‰ to −1.32‰. Hg pollution in Qingzhen is caused by a chemical plant that uses metallic Hg as a catalyst to produce acetic acid. The Hg isotope characteristics of sediment from Baihua Reservoir (δ^{202}Hg = −0.60‰ to −1.32‰) matched the isotope characteristic of the chemical plant waste and were similar to the Hg isotope characteristics of our samples from Qingzhen (Feng et al., 2010). The most important sources of Hg to soil are atmospheric deposition and supply from background sources (Qiu et al., 2005; Biswas et al., 2008; Blum et al., 2014). Many geological processes, such as hydrothermal reactions, sorption/desorption, and the burial of organic matter, can cause MDF, as can anthropogenic influences (Sonke et al., 2010; Blum et al., 2014). The THg Δ^{199}Hg values for the Wanshan and Qingzhen paddy soils were not significantly different, and ranged from −0.03‰ to 0.06‰. The photoreduction of aqueous Hg(II) and the photodegradation of MeHg could cause MIF, enriching the odd-numbered isotopes in the residual Hg(II) and MeHg (Bergquist and Blum, 2007; Malinovsky et al., 2010). Paddies are short-term wetlands in which photoreduction of aqueous Hg(II) and photodegradation of MeHg can occur. However, the samples from Wanshan and Qingzhen indicated that paddy soils in these areas have large Hg pools (THg ranged from 1.39 to 58.0 μg/g), so photochemical processes will probably affect only a tiny fraction of the Hg pool in paddy soil and we would not expect detecting MIF (Feng et al., 2013).

3.4. MeHg isotope compositions

The THg and MeHg concentrations in the soil samples are shown in Table 3. The Qingzhen soil samples had significantly higher MeHg concentrations (11.0 ± 3.47 ng/g, 2σ, $n = 3$) and higher MeHg/THg ratios (0.54%–0.91%) than did the Wanshan soil samples (MeHg concentration

Table 2
Chemical recovery and Hg isotope compositions of MeHg standards collected from various stages of the MeHg separation and trapping system (mean ± 2σ).

Process	*n*	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{200}Hg (‰)	Δ^{201}Hg (‰)	%MeHg recovery
MeHg standard	8	−0.73 ± 0.13	0.08 ± 0.05	0.04 ± 0.10	0.08 ± 0.15	–
Ethylation/separation	7	−0.74 ± 0.14	0.02 ± 0.07	0.00 ± 0.10	−0.01 ± 0.19	56–80
Solvent extraction/ethylation/separation	4	−0.78 ± 0.10	−0.01 ± 0.08	−0.02 ± 0.05	−0.01 ± 0.04	73–78

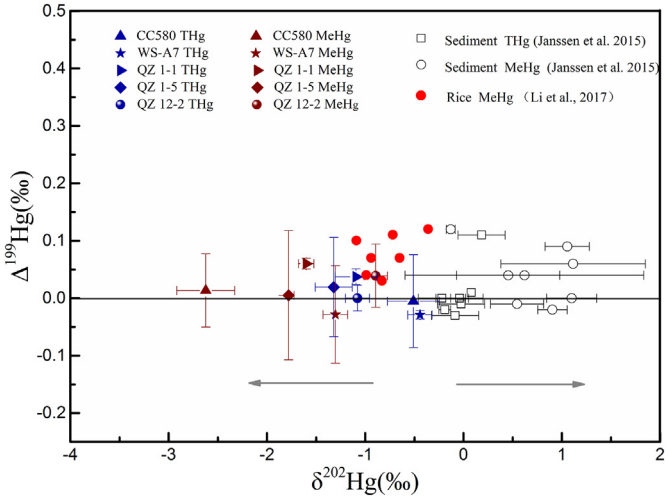

Fig. 3. Isotope compositions of the MeHg and total Hg in the samples and certified reference materials.

2.20 ng/g, MeHg/THg ratio 0.004%). Wastewater discharged from the chemical plant in Qingzhen has caused high THg and acetic acid concentrations in the soil in the nearby paddy soils, favoring Hg methylation (Yan et al., 2013).

Some sulfate-reducing and/or iron-reducing bacteria strongly accelerate Hg methylation under hypoxic conditions (Fleming et al., 2006; Gilmour et al., 1992). The methylation of inorganic Hg in paddy soils occurs mainly through processes mediated by sulfate-reducing bacteria (Peng et al., 2012; Rothenberg et al., 2012; Wang et al., 2014; Zhao et al., 2016a, 2016b). Methylation and demethylation both occur in paddy soils, and the dominant process controls the MeHg concentration. Hg isotope analysis is a good way of examining the balance between methylation and demethylation.

Microbial methylation (Barkay and Wagner-Döbler, 2005; Branfireun et al., 2005; Perrot et al., 2015), abiotic methylation (Jiménez-Moreno et al., 2013), MeHg photodegradation (Bergquist and Blum, 2007; Chandan et al., 2015; Malinovsky et al., 2010; Rose et al., 2015), and microbial demethylation (Bergquist and Blum, 2007) have been widely studied. The mean δ^{202}Hg values for the MeHg in the Wanshan soil sample A7, the Qingzhen soil sample 1-1, and the Qingzhen soil sample 1-5 were −1.30‰ ± 0.13‰, −1.60‰ ± 0.08‰, and −1.78‰ ± 0.06‰, respectively, and were lower than the corresponding δ^{202}Hg values for the THg in the soil samples, which were −0.44‰ ± 0.12‰, −1.09‰ ± 0.21‰, and −1.33‰ ± 0.19‰, respectively. The mean net difference between the δ^{202}Hg values for the MeHg and THg in the soil samples was 0.86‰ ± 0.12‰. Preferential methylation of light Hg isotopes has been found during the biotic and abiotic methylation of bioavailable Hg(II) (Jiménez-Moreno et al., 2013; Martín-Doimeadios et al., 2004; Perrot et al., 2015; Rodríguez-González et al., 2009), and the highest MDF found was 3.08‰ (Jiménez-Moreno et al., 2013). Yin et al. (2013b) found that the bioavailable Hg fraction in soil from Wanshan (with a mean δ^{202}Hg of 1.28‰ ± 0.25‰, $n = 8$) had heavier Hg isotopes than the THg, and the bioavailable Hg δ^{202}Hg value was 1.3‰ higher than the THg δ^{202}Hg value (−0.02‰ ± 0.16‰, $n = 8$). Isotope fractionation can occur during the methylation of bioavailable Hg in soil, resulting in the MeHg δ^{202}Hg value being lower than the THg δ^{202}Hg value. This probably drives isotope fractionation, the reaction products preferentially containing lighter and more mobile Hg isotopes. The MeHg concentration in paddy soil is a transient signal of methylation and demethylation processes, reflecting the net methylation potential of the paddy soil (Zhao et al., 2016a, 2016b). The MeHg δ^{202}Hg values were lower than δ^{202}Hg values of THg in soils, which indicated that methylation through microbial activities dominates MeHg isotope fractionation in paddy soil (Zhao et al., 2016a, 2016b). However, MeHg in rice grains from Wanshan has been found to have mean δ^{202}Hg values of −0.80‰ ± 0.25‰ (2σ, $n = 8$) (Li et al., 2017). The δ^{202}Hg values of MeHg form in rice grains were higher than the δ^{202}Hg values of MeHg in soil from Wanshan (Fig. 3). However, the MeHg isotope values for the roots, stems, and leaves of rice plants need to be determined to gain a full understanding of Hg isotope fractionation and MeHg accumulation in rice grains.

The Hg methylation rate is particularly high in wetland sediment (Driscoll et al., 1998). The δ^{202}Hg value was higher for MeHg (mean −0.89‰ ± 0.12‰) than THg (mean −1.08‰ ± 0.12‰) in the Qingzhen soil sample 12-2. Janssen et al. (2015) found that MeHg in estuarine sediment was enriched in heavier isotopes relative to THg. This was probably caused by demethylation because the MeHg isotopes became progressively heavier in a microbial demethylation experiment (Kritee et al., 2009). We found no significant differences between the MeHg and THg MIF values for the paddy soil samples. Photodegradation of MeHg mainly occurs in surface layer of water body, and therefore it plays a minor role in isotope fractionation of MeHg in the lower layer soil and sediment (Jackson et al., 2008).

Table 3
Total Hg and MeHg concentrations in the soil samples.

ID	Site	THg (μg/g)	MeHg (ng/g)	[MeHg]/[THg] (%)
A7	Wanshan	58.0	2.21	0.00381
1-1	Qingzhen	2.09	11.3	0.541
1-5	Qingzhen	1.39	12.6	0.906
12-2	Qingzhen	1.62	9.18	0.567
CC580	CRM	132	75.5	0.0572

4. Conclusions and environmental implications

We developed a method for extracting MeHg from soil and sediment to allow performing Hg isotope analysis. No significant MDF or MIF occurred throughout the separation process when standard MeHg solutions were analyzed. The δ^{202}Hg values were lower for MeHg than THg in the soil samples, indicating that methylation was dominant in the paddy soils. Methylation and demethylation are dynamic processes and both present in the ecosystem. While the fractionation of MeHg isotopes in soil maybe a good indicator to evaluate the dominated process. Long-term monitoring of the MeHg isotopes in the soils is needed to trace these dynamic processes.

The isotopes of MeHg in rice have been reported (Li et al., 2017) and soil is confirmed to be the major source of MeHg in rice (Meng et al., 2011, 2014, 2015; Strickman and Mitchell, 2016). However, the mechanism of MeHg bioaccumulation in rice grains is not well understood. Isotopic evidence of MeHg in the soils and rice plant are needed to understand transformation and migration of MeHg in the paddy field.

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