



Optimization of pretreatment procedure for MeHg determination in sediments and its applications

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Abstract

Methylmercury (MeHg) in sediment is difficult to be determined due to its low concentration and binding compounds like sulfide and organic matter. Moreover, wet sediment samples have been suggested to behave differently from certified reference materials in MeHg analysis. Optimal pretreatment procedure for MeHg determination in sediments has not been ascertained and whether the procedure could apply to sediment samples with complex matrix merits further research. This work firstly compared recovery results of five pretreatment procedures for MeHg determination using ERM-CC580. Using the optimal pretreatment procedure, recovery results were analyzed in different sediment samples after manipulation of moisture content, organic matter, and acid volatile sulfide. The procedure using CuSO₄/HNO₃ as leaching solutions and mechanical shaking as extraction method was proved to produce the most satisfactory recovery results (100.67 ± 6.75%, mean ± standard deviation). And when moisture content varied from 20 to 80%, average recovery results in sediment samples ranged from 100 to 125%. Furthermore, before and after the manipulation of organic matter or acid volatile sulfide, spiking recovery results varied little and were all within acceptable limit (85–105%). Therefore, the procedure of CuSO₄/HNO₃-mechanical is proposed as a universal pretreatment method for MeHg determination in sediment samples with various characteristics.

Keywords Methylmercury · Sediment · Pretreatment · Moisture content · Organic matter · Acid volatile sulfide

Introduction

Mercury (Hg), a toxic metal, is highly persistent and able to travel globally (Poullain and Barkay 2013). In surface waters, inorganic mercury can be methylated to methylmercury (MeHg) (Krabbenhoft and Sunderland 2013), whose content

corresponds with changes of mercury inputs (Harris et al. 2007). As a lipophilic and protein-binding neurotoxin, MeHg can pose an even severer threat to human beings after bioaccumulation and biomagnification through food chain (Clarkson and Magos 2006). Dietary intake, especially sea food, is the dominant pathway for general populations to be exposed to MeHg (Jiang et al. 2006). The element of Hg occurs naturally but can be anthropogenically introduced into the environment by industrial activities like mining and smelting. It is suggested that anthropogenic perturbations to global mercury cycle have tripled the mercury content of surface waters than that of pre-industrial times (Lamborg et al. 2014). In aquatic system, sediment is both an important sink and source of Hg and also a potential hotspot for Hg methylation (Ullrich et al. 2001). Maximum Hg methylation rate usually occurs at sediment-water interface, leading to the considerable MeHg content in surface sediment (Gilmour et al. 1992; Lambertsson and Nilsson 2006). As a result, accurate and feasible analysis of MeHg concentrations in sediment is of great necessity for environmental risk assessment.

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In sediment matrices, MeHg is difficult to be isolated due to binding compounds like sulfide and organic substances, especially humic substances (Horvat et al. 1993). Moreover, MeHg concentrations in sediment are rather low, usually as nanogram per gram (Caricchia et al. 1997). With gas chromatography and cold vapor atomic fluorescence spectrometry becoming the well-acknowledged analysis system for MeHg (Mao et al. 2008), there is still some dissent over the pretreatment procedures, including leaching solutions (Kodamatani et al. 2017a; Liang et al. 2004; Liu et al. 2015), extraction solutions (Liang et al. 1996; Maggi et al. 2009), and extraction methods (Bloom et al. 1997; Gu et al. 2013; He et al. 2015). Thus, it is necessary to specifically optimize the pretreatment procedure for MeHg determination in sediment samples.

Recently, researchers mostly choose dry sediment samples to determine MeHg, either after air-dried (Mikac et al. 1999; Qiu et al. 2005) or freeze-dried (Hoggarth et al. 2015; Meng et al. 2015), but there are still others using wet samples directly for analysis (Mikac et al. 1999; Yu et al. 2012). Also, owing to their different moisture content and chemical compositions, practical sediment samples have been suggested to behave differently from certified reference materials (CRMs) in MeHg determination (Liang et al. 2004). Whether the optimal pretreatment procedure could be applied to sediment samples with distinct characteristics merits further research. Typically, researchers would collect worldwide sediment samples to testify their analytical methods, which is quite demanding and lacks truth values (Kodamatani et al. 2017a; Liang et al. 2004; Maggi et al. 2009). In this work, instead of collecting a diversity of sediment samples, we manipulated certain chemical compositions of sediment physically to present different features. Then, we spiked methylmercury chloride (MeHgCl) standard solution to the manipulated sediment and analyzed the recovery results.

The objectives of this work are to evaluate the optimal pretreatment procedure for MeHg determination in sediment and then to study the applicability of this procedure to practical sediment samples with different characteristics relative to MeHg determination. For this aim, five pretreatment procedures were compared, including $\text{CuSO}_4/\text{HNO}_3$ as leaching solutions with mechanical shaking or manual shaking as extraction methods (short as $\text{CuSO}_4/\text{HNO}_3$ -mechanical and $\text{CuSO}_4/\text{HNO}_3$ -manual), $\text{KBr}/\text{H}_2\text{SO}_4/\text{CuSO}_4$ as leaching solutions with mechanical shaking or manual shaking as extraction methods (short as $\text{KBr}/\text{H}_2\text{SO}_4/\text{CuSO}_4$ -mechanical and $\text{KBr}/\text{H}_2\text{SO}_4/\text{CuSO}_4$ -manual), and $\text{KOH}/\text{CH}_3\text{OH}$ as leaching solutions with mechanical shaking as extraction method (short as $\text{KOH}/\text{CH}_3\text{OH}$). And practical sediment samples were manipulated physically to achieve different content of moisture, organic matter (OM), and acid volatile sulfide (AVS). Recovery results of MeHg analysis using the optimal pretreatment procedure were compared in sediment samples with and without manipulation.

Materials and methods

Reagents

The following reagents were used for the pretreatment procedures for MeHg determination in sediments: 65% nitric acid (Merck, Germany), 36% hydrochloric acid (Gaoheng, Beijing Institute of Chemical Reagents, China), copper sulfate pentahydrate (Sinopharm Chemical Reagent Co., Ltd., China), dichloromethane (J.T.Baker® Chemicals, USA), 98% sulfuric acid (Sinopharm Chemical Reagent Co., Ltd., China), potassium bromide (Sigma-Aldrich, USA), potassium hydroxide (Sigma-Aldrich, USA), methanol (LiChrosolv®, Merck, Germany), sodium tetraethylborate (Strem Chemicals Inc., USA), citric acid monohydrate (Sigma-Aldrich, USA), sodium citrate dihydrate (Sigma-Aldrich, USA).

Certified reference materials included ERM-CC580 (MeHg content, $75.5 \pm 3.7 \text{ ng g}^{-1} \text{ Hg}$, European Reference Materials, Institute for Reference Materials and Measurements, Belgium) and GSD-10 (THg content, $0.28 \pm 0.03 \text{ } \mu\text{g g}^{-1}$, GBW07310, IGGE, China). Standard solutions included methylmercury chloride standard solution ($65.5 \pm 2.5 \text{ } \mu\text{g g}^{-1} \text{ Hg}$, GBW08675, National Institute of Metrology, China). ERM-CC580 and GSD-10 were kept at $4 \text{ }^\circ\text{C}$ in dark. MeHgCl standard solution was diluted to $10.0 \text{ mg L}^{-1} \text{ Hg}$ by ultra-pure water ($18.2 \text{ M}\Omega$, Millipore, Darmstadt, Germany) and stored in dark. MeHgCl working solution was stepwise diluted by ultra-pure water when it would be used.

Sediment pretreatment procedures

Around 0.25 g of ERM-CC580 was weighed into a 50 mL polypropylene centrifuge tube (Corning, USA) for each treatment. The pretreatment procedures evaluated were as follows. All experiments were performed in triplicate.

$\text{CuSO}_4/\text{HNO}_3$ leaching, CH_2Cl_2 extraction, mechanical/manual shaking

1.5 mL of $2 \text{ mol L}^{-1} \text{ CuSO}_4$ and 7.5 mL of 25% (v/v) HNO_3 were added to the 50-mL centrifuge tubes with ERM-CC580 and waited 1 h for MeHg to be leached out thoroughly. To realize extraction, 10.0 mL of CH_2Cl_2 was added to each tube and the mixture was shaken mechanically (with a reciprocating shaker) at 350 r min^{-1} for 1.5 h (He et al. 2004) or manually for 0.5 h (Gu et al. 2013). Different lengths of the extraction time were applied according to the reported procedures. After leaching and extraction, these tubes were centrifuged at 3000 r min^{-1} for 15 min. Then, the mixture was filtered with phase separators (Whatman, GE Healthcare Life Sciences, UK) and the organic phase with MeHg was kept. 4.0 mL of the organic phase was added to tubes with around 20 mL distilled water and 2–3 pieces of boiling stones

(Saint-Gobain Performance Plastics, France) in them. These tubes were heated at 65 °C for 6 h to remove organic solvent. After heating, each sample was brought to 20.0 mL with ultra-pure water. 200.0 μL of the extract was pipetted into 40-mL amber glass vials (Agilent Technologies, USA) for MeHg analysis by the MERX-M Automatic Methylmercury System (Brooks Rand Laboratories, USA) following USEPA method 1630 (USEPA 2001).

KBr/H₂SO₄/CuSO₄ leaching, CH₂Cl₂ extraction, mechanical/manual shaking

5.0 mL of 18% (*m/v*) KBr dissolved in 5% (*v/v*) H₂SO₄ and 1.0 mL of 1 mol L⁻¹ CuSO₄ were added to centrifuge tubes with about 0.25 g ERM-CC580 and waited 1 h. Then, 10.0 mL of CH₂Cl₂ was added to each tube and the mixture was shaken mechanically at 350 r min⁻¹ for 1.5 h or manually for 0.5 h. The following procedure and analytical method were the same as CuSO₄/HNO₃ procedure.

KOH/CH₃OH leaching, heating

This pretreatment procedure was based on the published procedure with several improvements (Liang et al. 1996). 3.0 mL of 25% (*m/v*) KOH/CH₃OH was added to centrifuge tubes with around 0.25 g ERM-CC580. After heating at 75 °C for 3 h, 10.0 mL CH₂Cl₂ and 2.3 mL concentrated HCl were slowly added to each tube. These tubes were shaken at 300 r min⁻¹ for 10 min. The following procedure and analytical method were the same as CuSO₄/HNO₃ procedure.

Practical sample collection and analysis

Practical surface sediment samples were collected from Xingfu Reservoir, Qingnian Reservoir, and Taihu Lake in 2016. Spatial distribution of the sampling sites is shown in Fig. 1. The two reservoirs are located in Wanshan City of Guizhou Province, southwest of China. Even though they were constructed mainly for drinking and irrigation use, these reservoirs suffered severe mercury pollution from local mercury mining area (Du et al. 2016; Li et al. 2009; Qiu et al. 2009). In addition, sediment samples of Taihu Lake (Jiangsu Province) were collected from two lake regions, Meiliang Bay and Zhushan Bay. Taihu Lake is a eutrophic lake with mild mercury pollution and a high organic matter content (Guo 2007; Wang et al. 2012). After collection, all sediment samples were transferred to the lab instantly. Around 200 g sediment samples were separated and centrifuged at 3000 r min⁻¹ for 15 min to extract pore water. Then, the pore water samples were filtered through a 0.22- μm syringe filters (ANPEL Laboratory Technologies (Shanghai) Inc., China) for further analysis. The remaining sediment samples were lyophilized to achieve constant weight and then grounded and homogenized

to a size of 200 meshes per inch. All sediment samples prepared were stored in amber glass vials with Teflon lids at 4 °C.

Analysis of pore water samples

Sulfate (SO₄²⁻) concentrations of filtered pore water samples were determined by Ion Chromatography (IC6200, WAYEAL, China). The samples were separated using an anion column (IC SI-52 4E, 4 mm ID \times 250 mm) with the eluent (3.6 mmol L⁻¹ Na₂CO₃) flow rate of 0.8 mL min⁻¹ and column temperature of 45 °C (Liu et al. 2016). Concentrations of total iron and ferrous iron (Fe²⁺) were determined using 1,10-phenanthroline method with a UV-visible spectrophotometer (Shanghai Sunny Hengping, 756PC, China) (Tamura et al. 1974). Concentration of ferrous iron (Fe³⁺) could be obtained by subtracting the concentration of Fe²⁺ from total iron. Total mercury (THg) concentrations in pore water samples were determined with MERX-T Automatic Total Mercury System (Brooks Rand Laboratories, USA) following USEPA 1631, Revision E (USEPA 2002).

Analysis of sediment samples

The concentrations of total carbon (TC) and total nitrogen (TN) in sediment samples were determined with an elemental analyzer (Elementar, Vario EL III, Germany). The determination of TC and TN was performed in triplicate. Sediment moisture content was measured using a weight loss method with a lyophilizer. THg content of lyophilized sediment samples was determined by Leeman mercury analyzer (Leeman Labs Hydra II C, USA) according to the USEPA 7473 (USEPA 2007). MeHg concentrations of the samples were determined according to the predetermined optimal pretreatment procedure. Analysis of MeHg content was performed in triplicate.

Manipulation of sediment characteristics

The lyophilized surface sediment samples with different concentrations of moisture, organic matter, and acid volatile sulfide were achieved through physical methods. Then, we immediately determined the MeHg concentrations in the manipulated samples using the predetermined optimal pretreatment procedure. The MeHg concentration analysis experiments were performed in triplicate.

Moisture content

Considering the high background concentrations of MeHg in Xingfu Reservoir and Qingnian Reservoir, sediment samples there were suitable for investigating whether the optimal pretreatment procedure could apply to sediment with different moisture content. Lyophilized surface sediment samples from Xingfu Reservoir and Qingnian Reservoir were mixed with different

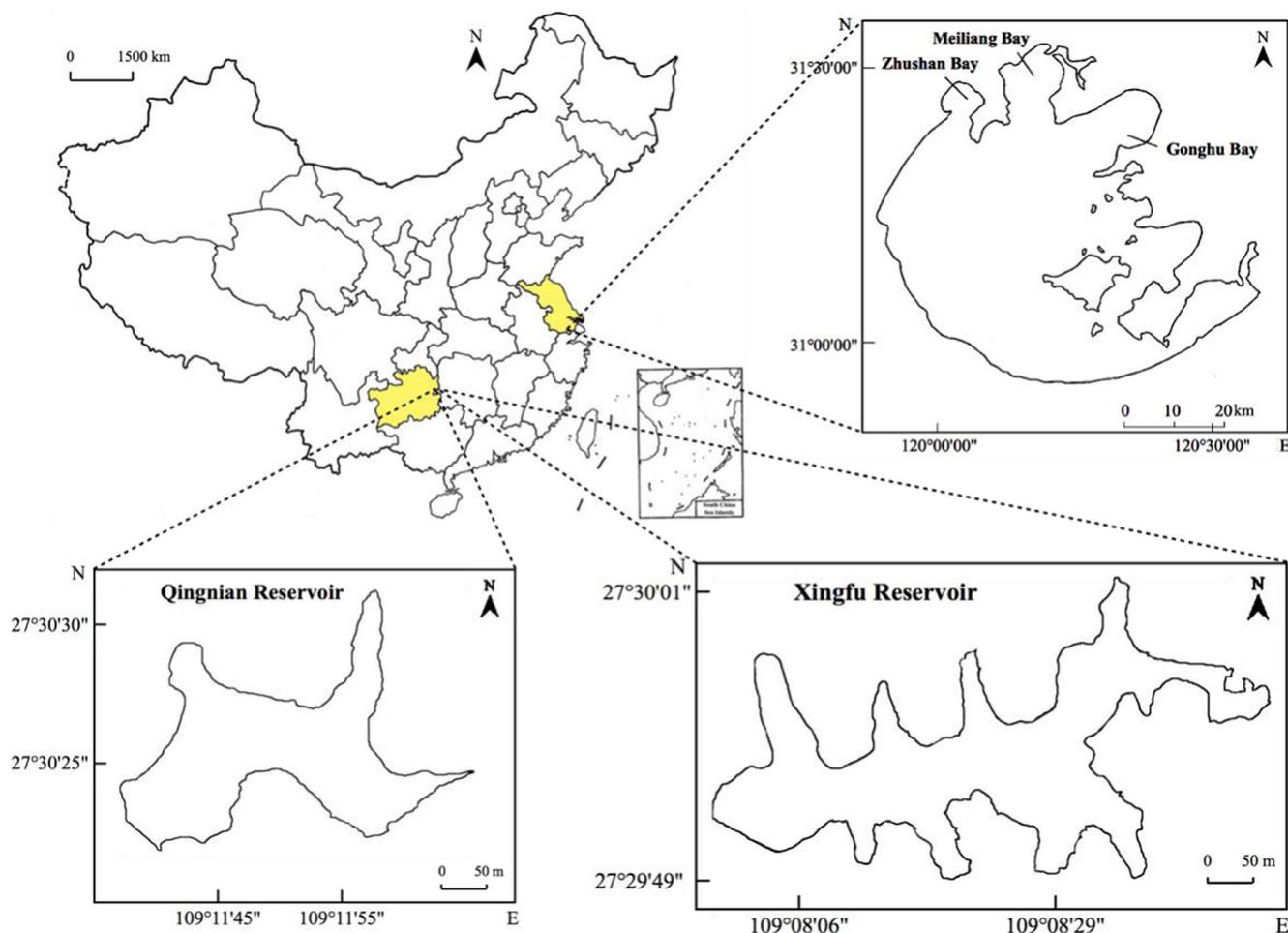


Fig. 1 Map of sampling sites in Meiliang Bay and Zhushan Bay of Taihu Lake, together with Qingnian Reservoir and Xingfu Reservoir. Taihu Lake is in Jiangsu Province, which is in the east of China. Qingnian Reservoir and Xingfu Reservoir are in Guizhou Province, south-west of China

aliquots of ultra-pure water to produce wet samples with different moisture content. After homogeneous mixing, we determined the MeHg content in these wet samples directly. In detail, 0.25 g wet sediment samples with 20, 40, 60, and 80% moisture content were spiked with MeHgCl working standard solution containing 2.0 ng Hg. During the pretreatment,

4.0 mL in 10.0 mL extraction solutions was pipetted in the filtration and 2.0 mL in 20.0 mL back-extraction solutions was brought for MeHg analysis after heating. For convenience, all the recovery results in wet sediment samples were converted to the ratio of MeHg content in lyophilized samples following Eq. (1):

$$\text{Corrected recovery (\%)} = \frac{\text{MeHg content in wet sediment (ng g}^{-1}, \text{ wet weight)}}{\text{MeHg original content (ng g}^{-1}, \text{ dry weight)} \times (1 - \text{moisture content})} \quad (1)$$

Organic matter

Organic matter in lyophilized surface sediment samples from Meiliang Bay and Zhushan Bay was removed by muffling at 400 °C for 8 h (Arnarson and Keil 2000). In order to mix homogeneously, sediment samples with and without being removed of organic matter were mixed with ultra-pure water to produce slurries with a moisture content of 80%. Considering that MeHg content of Taihu Lake sediment slurries was relatively low for analysis, MeHgCl standard solutions were

spiked into the slurries to form $\sim 8 \text{ ng g}^{-1}$ MeHg. TOC content of samples before and after the removal was determined by the elemental analyzer (Schumacher 2002).

Acid volatile sulfide

Fresh sediment samples collected from Meiliang Bay and Zhushan Bay in Taihu Lake were purged with N_2 in order to produce higher levels of AVS (Lee et al.

2000a, b). Specifically, about 50 g sediment slurries were reduced by purging N_2 at 300 mL min^{-1} for 3 days. Sediment samples before and after manipulation were prepared for AVS analysis. As for recovery test, MeHgCl standard solutions were spiked into the slurries to form $\sim 8 \text{ ng g}^{-1}$ MeHg. AVS content in sediment samples was determined using “purge-and-trap” method along with methylene blue spectrophotometry (Allen et al. 1993; Lasorsa and Casas 1996). Then, AVS content in wet sediment samples was normalized to dry sediment weight following Eq. (2).

$$\text{AVS } (\mu\text{g g}^{-1}, \text{ dry weight}) = \text{AVS } (\mu\text{g g}^{-1}, \text{ wet weight}) / (1 - \text{moisture content}) \quad (2)$$

Quality control and statistical analysis

For THg analysis in sediment samples, we used GSD-10 as certified reference material and measured analytical blanks for quality control. The average THg concentration we measured was $279.99 \pm 0.02 \text{ ng g}^{-1}$ (mean \pm SD, $n=6$), which agreed well with the certified value ($0.28 \pm 0.03 \text{ } \mu\text{g g}^{-1}$). The detection limit for THg was 7 ng Hg in terms of absolute mass. For MeHg analysis, the detection limit was 10 pg Hg in terms of absolute mass. Analytical blanks were lower than detection limit. The linear range is from 5 to 800 pg. All glassware used was cleaned with distilled water three times, soaked in 10% (v/v) HNO_3 for at least 48 h, washed with distilled water three times, and finally heated at $500 \text{ }^\circ\text{C}$ for 2 h before use.

Statistical analysis was performed using SPSS 24.0 software. The difference among recovery results of each procedure was assessed by an independent t test. Analysis of variance (ANOVA) was applied to evaluate the significant difference of means. Significance probabilities (p) were calculated and difference was declared significantly for $p < 0.01$ in the current work.

All mercury-containing waste was properly disposed as hazardous waste.

Results and discussion

Optimization of pretreatment procedure using certified reference material

As the pretreatment procedures were to be applied to MeHg analysis in bulk sediment samples, accuracy, operability, and security were considered comprehensively. Therefore, five procedures were selected according to the recent publications involving MeHg analysis in

sediment (Kodamatani et al. 2017a; Wang et al. 2018; Yin et al. 2018). The detailed leaching and extraction procedures of five pretreatment procedures using ERM-CC580 are listed in Table 1 and the recovery results are illustrated in Fig. 2. As shown in Fig. 2, $\text{CuSO}_4/\text{HNO}_3$ -mechanical procedure had the most satisfying recovery ($100.67 \pm 6.75\%$, mean \pm SD) among five procedures tested.

With the same leaching solutions, recovery results of mechanical shaking did not differ significantly from those of manual shaking ($p > 0.1$). Specifically, $\text{CuSO}_4/\text{HNO}_3$ being leaching solvents, extraction efficiency of manual shaking (0.5 h, $116.70 \pm 7.33\%$) was higher than that of mechanical shaking (350 r min^{-1} for 1.5 h) and to some extent exceeded the optimum value (100%). Yet, with $\text{KBr}/\text{H}_2\text{SO}_4/\text{CuSO}_4$ being leaching solvents, mechanical shaking could produce higher and relatively more accurate recovery results ($82.60 \pm 7.87\%$). This fluctuation might be caused by the inadequate stability and repeatability of manual shaking, which usually were the consequences of individual's difference in strength. After considering the accuracy and reproducibility, mechanical shaking was selected instead of manual shaking. In addition, $\text{KOH}/\text{CH}_3\text{OH}$ could produce decent recovery results as well ($86.33 \pm 7.95\%$). However, this procedure required heating as leaching method for 3 h and back-extraction for 6 h, which was rather time-consuming. And all with mechanical shaking, $\text{CuSO}_4/\text{HNO}_3$ as leaching solvents displayed higher leaching efficiency than $\text{KBr}/\text{H}_2\text{SO}_4/\text{CuSO}_4$ and $\text{KOH}/\text{CH}_3\text{OH}$. As a strong oxidizing acid, HNO_3 has a strong ability to destroy the strong embedded sites of MeHg and sediment (Hammerschmidt and Fitzgerald 2001; Liang et al. 2004). The reasons for other procedures producing lower recovery results might be due to their inadequate leaching abilities or interference with sediment matrix (Horvat et al. 1993; Liang et al. 2004; Tseng et al. 1997).

Generally, recovery results of this work accorded with the reported studies (Table 2). Our recovery results using $\text{CuSO}_4/\text{HNO}_3$ ($100.67 \pm 6.75\%$) with mechanical shaking agreed well with He's results ($97.8 \pm 10.2\%$), but the shaking frequency was not described in their work (He et al. 2004). So, this work further clarified and established the pretreatment procedure of $\text{CuSO}_4/\text{HNO}_3$. Moreover, the pretreatment procedure using $\text{KBr}/\text{H}_2\text{SO}_4/\text{CuSO}_4$ as leaching solutions in this work produced a bit lower recovery results than reported results ($\sim 100\%$) (Gu et al. 2013; Kodamatani et al. 2017a). In Kodamatani's both method C and D, they transferred certain amounts of CH_2Cl_2 (in the lower layer of the mixture) to deionized distilled water (Kodamatani et al. 2017a). It was possible to carry

Table 1 Details of five pretreatment procedures tested for determination of methylmercury (MeHg) concentrations in ERM-CC580

Pretreatment procedures	Extraction solutions	Extraction method	Leaching solutions
CuSO ₄ /HNO ₃ -mechanical	1.5 mL 2 mol L ⁻¹ CuSO ₄ 7.5 mL 25% (v/v) HNO ₃	350 r min ⁻¹ , 1.5 h	CH ₂ Cl ₂
CuSO ₄ /HNO ₃ -manual	1.5 mL 2 mol L ⁻¹ CuSO ₄ 7.5 mL 25% (v/v) HNO ₃	Vigorous manual shaking, 0.5 h	CH ₂ Cl ₂
KBr/H ₂ SO ₄ /CuSO ₄ -mechanical	5.0 mL 18% (m/v) KBr in 5% (v/v) H ₂ SO ₄ 1.0 mL 1 mol L ⁻¹ CuSO ₄	350 r min ⁻¹ , 1.5 h	CH ₂ Cl ₂
KBr/H ₂ SO ₄ /CuSO ₄ -manual	5.0 mL 18% (m/v) KBr in 5% (v/v) H ₂ SO ₄ 1.0 mL 1 mol L ⁻¹ CuSO ₄	Vigorous manual shaking, 0.5 h	CH ₂ Cl ₂
KOH/CH ₃ OH	2.5 mL 25% (v/v) KOH/CH ₃ OH	300 r min ⁻¹ , 10 min	CH ₃ OH

inorganic mercury (in the upper layer of the mixture) as well, which could be methylated to MeHg artifacts during back-extraction periods (Bloom et al. 1999). In this work, phase separators were used to avoid inorganic Hg to move into the CH₂Cl₂ phase. Therefore, the difference between the separation methods might result in the minor distinction of the recovery results. As to Gu's pretreatment procedure, even though the average recovery ($104 \pm 15\%$) was similar to the result of the current procedure, the standard deviation of theirs is somewhat higher than other procedures (from 2.56 to 9.09%). This might be related to the potential instability of manual shaking. In addition, pretreatment procedure using KOH/CH₃OH as leaching solutions in this work produced slightly lower recovery results than reported results ($100.18 \pm 2.56\%$) (Liang et al. 1996). Yet, Liang's procedure was dependent on heating process but variations of heating efficiencies of different heaters would bring about difficulty in repeating.

Thus, after comparing the recovery results, efficiency and reproducibility of the five pretreatment procedures, CuSO₄/HNO₃-mechanical procedure was determined as the optimal pretreatment procedure in the present work. And its applicability to sediment samples with different characteristics would be further examined.

Analysis of practical samples

As shown in Table 3, characteristics of surface sediment samples from different sampling sites varied greatly. Sediment samples from reservoirs of Guizhou Province were rich in THg content (especially Qingnian Reservoir), which might be due to their short distance from Wanshan mercury mine area. However, THg concentrations in Meiliang Bay and Zhushan Bay from Taihu Lake were much lower ($< 1/20$) than Guizhou, indicating the mild Hg disturbance by human activities as mentioned before. Moreover, MeHg concentrations in Xingfu

Fig. 2 Recovery of MeHg determination using certified reference material ERM-CC580 by means of five pretreatment procedures (data shown by mean \pm SD, $n = 3$)

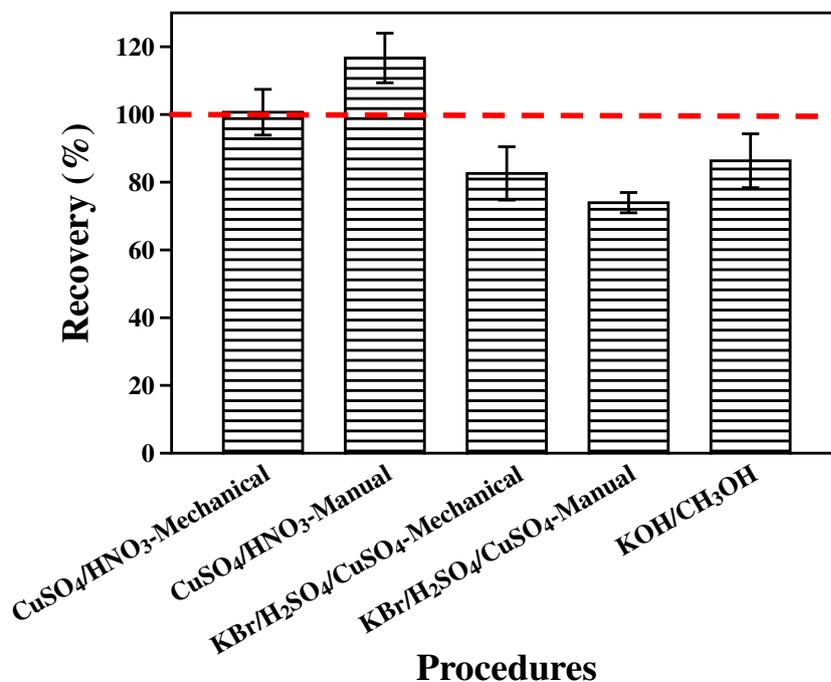


Table 2 Recovery results of relevant pretreatment procedures for determination of MeHg in sediment samples from literature (NG represented “not given”)

Pretreatment procedures	Certified reference materials	Recovery (%)		N	No. of references
		Average	Standard deviation		
CuSO ₄ /HNO ₃	IAEA-405	97.8	10.2	6	He et al. 2004
CuSO ₄ /CH ₃ OH	IAEA-405	96.36	9.09	15	Meng et al. 2015
KBr/H ₂ SO ₄ /CuSO ₄	IAEA-405	100.73	NG	1	Kodamatani et al. 2017
	ERM-CC580	98.93		1	
KBr/H ₂ SO ₄ /CuSO ₄	ERM-CC580	101.87	5.2	3	
KBr/H ₂ SO ₄ /CuSO ₄	ERM-CC580	104	15	3	Gu et al. 2013
KOH/CH ₃ OH	IAEA-356	100.18	2.56	NG	Liang et al. 1996
C ₆ H ₅ CH ₃	BCR-580	111.07	7.33	9	Maggi et al. 2009
	IAEA-405	109.84	3.64	9	

Reservoir and Qingnian Reservoir (over 2 ng g⁻¹) were comparatively higher than those from Meiliang Bay and Zhushan Bay (under 0.8 ng g⁻¹). Therefore, according to the detection limit, lyophilized surface sediment from Xingfu Reservoir and Qingnian Reservoir was suitable for the verification of the application of the optimal pretreatment procedure to sediment with different moisture content, without being spiked with MeHgCl standard solution.

From the analysis of pore water in fresh sediment samples, concentrations of SO₄²⁻, Fe²⁺, and Fe³⁺ were different in Xingfu Reservoir and Qingnian Reservoir (Table 4). Thus, sediment samples from Xingfu Reservoir and Qingnian Reservoir could represent two different water bodies.

In addition, MeHg concentrations in sediment samples from Meiliang Bay and Zhushan Bay were very low and would be under detection limit after mixing with water. They were considered for investigating the influence of OM and AVS on the optimal procedure with spiking MeHgCl. After mixture with water and

subsampling, the final MeHg concentration in the Taihu sediment samples would be under 0.008 ng, which was far lower than the content of spiking standard solution (0.08 ng). Thus, sediment samples in Meiliang Bay and Zhushan Bay were suitable to be analyzed on whether the pretreatment procedure would still apply to sediment samples after the manipulation of OM and AVS.

Application to sediment with various moisture content

Compared with soil (~40%), surface sediment tends to have high levels of moisture content (~70%). However, moisture in sediment might affect MeHg determination unpredictably. On the one hand, Hg methylating microorganisms prefer moist and warm conditions, which might induce higher MeHg content during the pretreatment procedure with heating process included (Kodamatani et al. 2017b). On the other hand, as wet sediment samples were usually more viscous, it is difficult to obtain a homogenous subsample. But whether the moisture content affects the predetermined optimal pretreatment procedure for MeHg analysis remains unclear. In order to evaluate the applicability of CuSO₄/

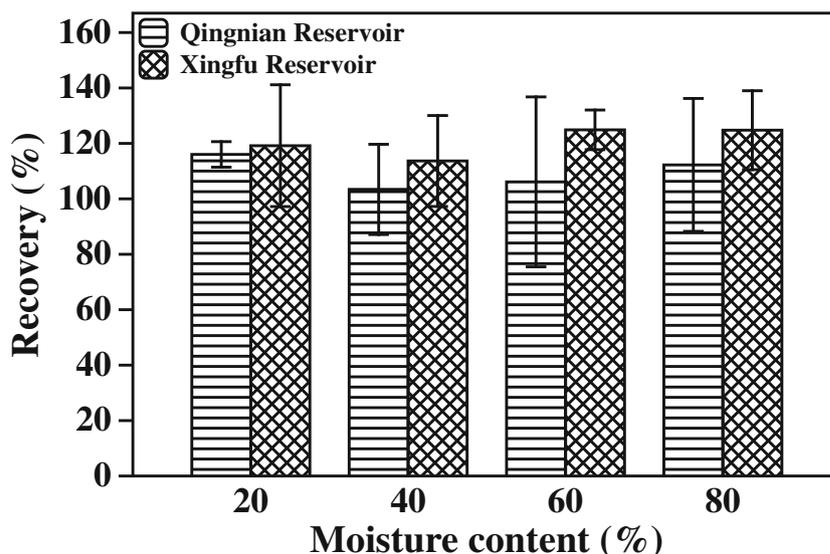
Table 3 Characteristics of surface sediment samples from Xingfu Reservoir, Qingnian Reservoir, Meiliang Bay, and Zhushan Bay. (Data of total mercury (THg), MeHg, and total carbon (TC) were described by mean ± SD, n = 3)

Reservoir/ Lake bay	Moisture content %	THg ng g ⁻¹ , dry weight	MeHg	TC %
Xingfu Reservoir	71.51	976.51 ± 9.41	2.96 ± 0.53	2.36 ± 0.04
Qingnian Reservoir	65.77	6370.96 ± 374.45	2.06 ± 0.53	1.95 ± 0.00
Meiliang Bay	72.27	41.71 ± 12.80	0.45 ± 0.14	1.17 ± 0.01
Zhushan Bay	73.28	176.92 ± 3.92	0.76 ± 0.05	1.71 ± 0.01

Table 4 Concentrations of SO₄²⁻, Fe²⁺, Fe³⁺, and THg in sediment pore water of Xingfu Reservoir and Qingnian Reservoir

Reservoir	SO ₄ ²⁻ mg L ⁻¹	Fe ²⁺	Fe ³⁺	THg μg L ⁻¹
Xingfu Reservoir	0.34	0.22	15.96	0.33
Qingnian Reservoir	0.99	0.00	3.68	1.76

Fig. 3 Recovery of wet sediment samples with different moisture content (data shown by mean \pm SD, $n = 3$). All the results determined in the wet sediment samples were analyzed and illustrated in the ratio with the original MeHg concentration in lyophilized sediment samples according to Eq. (1)



HNO₃-mechanical procedure to wet sediment samples, different aliquots of ultra-pure water were added to lyophilized sediment samples to produce sediment samples with a variety of moisture content.

The recovery results of wet sediment samples with various moisture content are illustrated in Fig. 3. With moisture content varying from 20 to 80%, recovery results in both reservoirs were mostly within acceptable range (100~125%). Specifically, sediment samples from Xingfu Reservoir could produce slightly higher recovery results than Qingnian Reservoir. Somehow, all the recovery results were above 100%, which might be caused by the original deviation in MeHg determination of lyophilized sediment samples. The reason for CuSO₄/HNO₃-mechanical procedure producing satisfying recovery might be that HNO₃ could destroy the bond of MeHg and moisture in wet sediment samples. So it would enable MeHg to be leached out (Liang et al. 2004).

Generally according to the results, CuSO₄/HNO₃-mechanical procedure could be applied to MeHg determination in wet sediment samples with various moisture content.

Table 5 Content (%) of total organic carbon (TOC) and total nitrogen (TN) before and after the removal of organic matter in lyophilized Taihu surface sediment samples

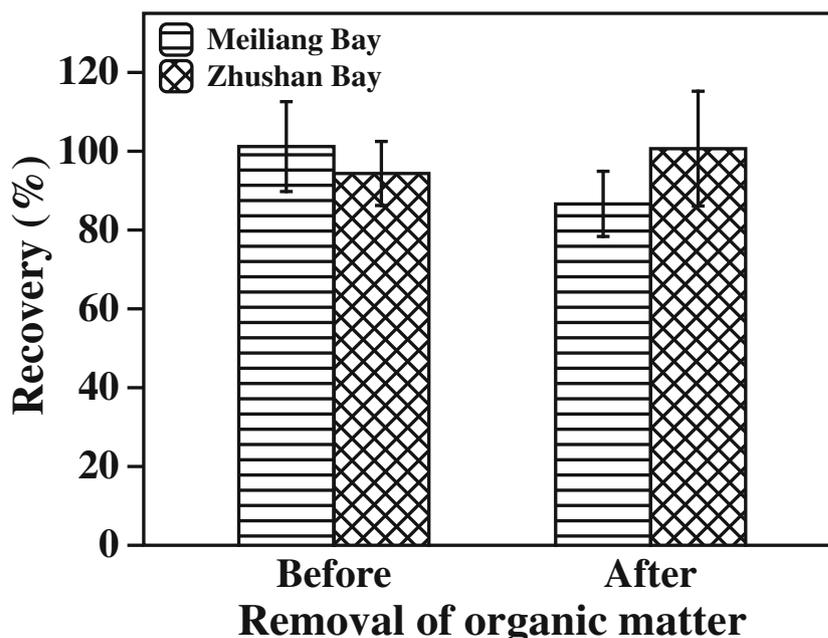
Lake regions	Before removal		After removal	
	TOC	TN	TOC	TN
Meiliang Bay	1.02 \pm 0.01	0.11 \pm 0.00	0.30 \pm 0.00	0.09 \pm 0.00
Zhushan Bay	1.56 \pm 0.01	0.16 \pm 0.00	0.41 \pm 0.01	0.13 \pm 0.01

Application to sediment with distinct organic matter content

Sediment organic matter, like humic substances, was able to bind MeHg so strongly that MeHg became difficult to be leached out completely (Caricchia et al. 1997; Schartup et al. 2012). As organic matter in sediment or soil samples are difficult to be measured directly, we used the content of TOC to represent the level of organic matter. Sediment samples with low or high organic matter were achieved by heating or not. Content of TOC and TN before and after the removal is shown in Table 5. After being muffled for 8 h, the TOC content in surface sediment samples dropped markedly (from over 1% to less than 0.5%) compared with TN content. The results indicated that after the removal, sediment samples could be used as contrasts containing low organic matter in comparison with the original sediment samples. The MeHgCl spiking recovery results in these comparison groups were analyzed to determine whether the predetermined optimal pretreatment procedure could apply to sediment samples rich or lacking in organic matter.

The recovery results are illustrated in Fig. 4, and all the results in wet sediment samples were converted to the ratio of MeHg content in lyophilized samples following Eq. (1). As shown in Fig. 4, despite the variation in TOC content, the spiking recoveries of MeHg did not differ a lot. Before the removal of organic matter, while the TOC content was higher than 1%, the recovery results in both surface sediment samples of Taihu Lake were near 100%. Then, after the removal, as the TOC content dropped sharply, the recoveries in Meiliang Bay dropped a little but were still within acceptable limit (> 85%). In Zhushan Bay sediment

Fig. 4 MeHg spiking recovery before and after the removal of organic matter using the optimal pretreatment procedure in Taihu surface sediment samples (data shown by mean ± SD, *n* = 3)



samples, the recovery results increased to around 100% after the removal. Overall speaking, the removal of the organic matter did not influence the spiking recovery results of Taihu surface sediment samples remarkably.

According to the spiking recovery results above, the pretreatment procedure using CuSO₄/HNO₃ as leaching solutions with mechanical shaking as extracting method applies to sediments samples with distinct content of organic matter.

Application to sediment with distinct acid volatile sulfide content

Sulfide, especially AVS, is the most reactive phase for most metals in sediment, Hg and MeHg included (Lee et al. 2000b; Rickard and Morse 2005). The content of AVS is able to reflect the sulfide that can bond with MeHg tightly (Zhu et al. 2017). After the manipulation of AVS, the reduced sediment samples had the AVS content over 2 μg g⁻¹, while the content of the original sample were lower than 1.2 μg g⁻¹ (dry weight)

Table 6 Concentrations of acid volatile sulfide (AVS) in Taihu surface sediment samples before and after the manipulation by purging N₂. AVS content in wet sediment was corrected to dry weight according to Eq. (2)

Lake regions	AVS (μg g ⁻¹ , dry weight)	
	Non-treated	Purged with N ₂
Meiliang Bay	1.13	2.71
Zhushan Bay	1.16	2.01

(Table 6). The increase of AVS content in reduced sediment samples was consistent with the trend of Lee’s (Lee et al. 2000b). As a result, sediment samples purged with N₂ could be used as contrasts to the original sediment samples which are rich in sulfide.

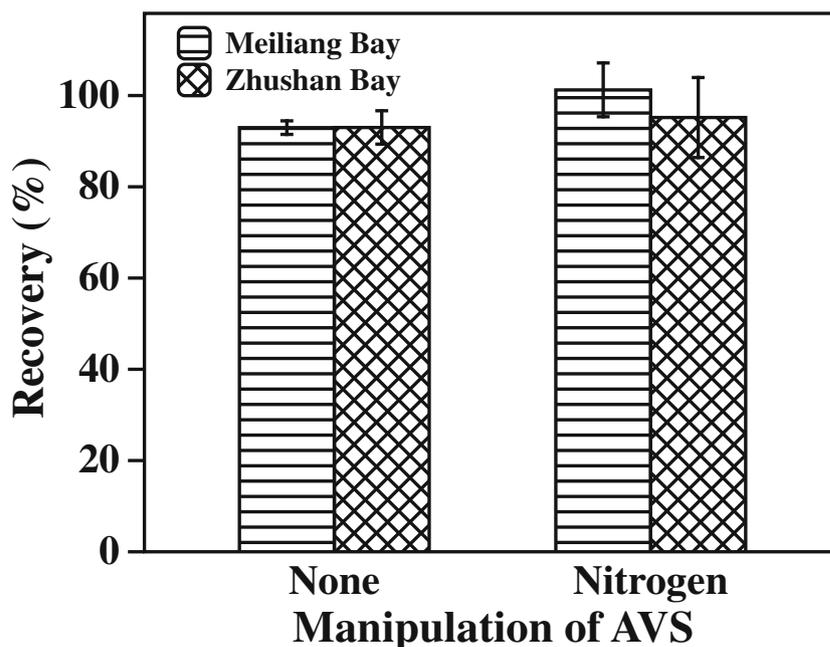
After spiking the slurries with MeHgCl standard solution, the recovery results can help decide whether the optimal pretreatment procedure could apply to sediment samples with different AVS content. The recovery results are illustrated in Fig. 5. Before manipulation, while the AVS content was comparatively low, the recovery results were around 92%. After the manipulation, the recoveries increased a little (by the ratio of 8.9% and 2.3% respectively) with the increase of AVS content. Generally speaking, all the recovery results were near 100% within the mentioned range of AVS content.

Therefore, the pretreatment procedure using CuSO₄/HNO₃ as leaching solutions with mechanical shaking as extraction method could accurately determine MeHg content in sediment samples with a variety of AVS content.

Conclusions

The current work compared recovery results of five pretreatment procedures for MeHg analysis in sediment samples using ERM-CC580. And the procedure using CuSO₄/HNO₃ as leaching solutions with mechanical shaking as extraction method produced the most satisfying recovery result, which was 100.67 ± 6.75% in

Fig. 5 MeHg spiking recovery before and after the manipulation of AVS using the optimal pretreatment procedure in Taihu surface sediment samples (data shown by mean \pm SD, $n = 3$)



average. In addition, moisture content in sediment samples (from 20 to 80%) had little influence on the analytical performance of the optimal pretreatment procedure. Considering the strong complexation ability of organic matter and sulfide with MeHg, sediment samples were manipulated physically to produce a relatively wide range of TOC (from ~ 0.3 to $\sim 1.5\%$) and AVS (from ~ 1.1 to $\sim 2.7 \mu\text{g g}^{-1}$, dry weight) content. And the spiking recovery results were mostly around 100%, indicating that the optimal pretreatment procedure was able to produce satisfactory results for MeHg determination in sediment samples with various properties.

There is possibility that spiking recovery tests may not entirely reflect the real performance of MeHg in sediment samples. It is likely that natural compounds may bind with MeHg so tightly that they cannot be extracted easily (Qian et al. 2002). Even so, spiking recovery tests have been carried out in plenty of work to prove the accuracy of their analytical methods when the background MeHg concentration was under detection limit (Heyes et al. 2004; Horvat et al. 1993; Liang et al. 2004). Also, inevitably, the manipulation of one characteristic in sediment may bring about changes to other characteristics as well. However, considering the manipulation methods used are mainly through physical instead of chemical means, the procedures can be well-controlled (Lee et al. 2000b). And using the same sediment samples in MeHg recovery tests could eliminate the influence of other irrelevant characteristics, like background MeHg content and particle size. Still, if time and energy permit, researchers should collect sediment samples as various as possible.

Hopefully, this work can provide a feasible approach to evaluate environmental MeHg risks and thereby reduce human's exposure to pollution sources and finally alleviate health risks.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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