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 hasn't 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 $CuSO_4/HNO_3$ as leaching solutions and mechanical shaking as extraction method was proved to produce the most satisfactory recovery results ($100.67 \pm 6.75\%$, mean $\pm$ standard deviation). And when moisture contents 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 $CuSO_4/HNO_3$-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

1. Introduction

Mercury (Hg) , a toxic metal, is highly persistent and able to travel globally (Poulain 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.

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 ng 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're still others using wet samples directly for analysis (Lambertsson and Nilsson 2006; Mikac et al. 1999; Yu et al. 2012). Also, owing to their different moisture contents 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

87 with distinct characteristics merits further research. Typically, researchers would collect
88 worldwide sediment samples to testify their analytical methods, which is quite demanding and
89 lacks truth values (Kodamatani et al. 2017a; Liang et al. 2004; Maggi et al. 2009). In this work,
90 instead of collecting a diversity of sediment samples, we manipulated certain chemical
91 compositions of sediment physically to present different features. Then we spiked
92 methylmercury chloride (MeHgCl) standard solution to the manipulated sediment and
93 analyzed the recovery results.

94   The objectives of this work are to evaluate the optimal pretreatment procedure for MeHg
95 determination in sediment and then to study the applicability of this procedure to practical
96 sediment samples with different characteristics relative to MeHg determination. For this aim,
97 five pretreatment procedures were compared, including $CuSO_4/HNO_3$ as leaching solutions
98 with mechanical shaking or manual shaking as extraction methods (short as $CuSO_4/HNO_3$-
99 mechanical and $CuSO_4/HNO_3$-manual), $KBr/H_2SO_4/CuSO_4$ as leaching solutions with
100 mechanical shaking or manual shaking as extraction methods (short as $KBr/H_2SO_4/CuSO_4$-
101 mechanical and $KBr/H_2SO_4/CuSO_4$-manual) and KOH/CH_3OH as leaching solutions with
102 mechanical shaking as extraction method (short as KOH/CH_3OH). And practical sediment
103 samples were manipulated physically to achieve different contents of moisture, organic matter
104 (OM) and acid volatile sulfide (AVS). Recovery results of MeHg analysis using the optimal
105 pretreatment procedure were compared in sediment samples with and without manipulation.
106

2. Materials and methods

2.1. Reagents

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

117   Certified reference materials included ERM-CC580 (MeHg content: 75.5 ± 3.7 ng g^{-1} Hg,
118 European Reference Materials, Institute for Reference Materials and Measurements, Belgium)
119 and GSD-10 (THg content: 0.28 ± 0.03 µg g^{-1}, GBW07310, IGGE, China). Standard solutions

120   included methylmercury chloride standard solution (65.5 ± 2.5 µg g^{-1} Hg, GBW08675,

121   National Institute of Metrology, China). ERM-CC580 and GSD-10 were kept at 4 °C in dark.

122   MeHgCl standard solution was diluted to 10.0 mg L^{-1} Hg by ultra-pure water (18.2 MΩ,

123   Millipore, Darmstadt, Germany) and stored in dark. MeHgCl working solution was stepwise

124   diluted by ultra-pure water when it would be used.

125

2.2. Sediment pretreatment procedures

127   Around 0.25 g of ERM-CC580 were weighed into a 50 mL polypropylene centrifuge tube

128   (Corning, USA) for each treatment. The pretreatment procedures evaluated were as follows.

129   All experiments were performed in triplicate.

130

2.2.1. CuSO$_4$/HNO$_3$ leaching, CH$_2$Cl$_2$ extraction, mechanical/manual shaking

132   1.5 mL of 2 mol L^{-1} CuSO$_4$ and 7.5 mL of 25% (v/v) HNO$_3$ were added to the 50 mL

133   centrifuge tubes with ERM-CC580 and waited 1 h for MeHg to be leached out thoroughly. To

134   realize extraction, 10.0 mL of CH$_2$Cl$_2$ were added to each tube and the mixture was shaken

135   mechanically (with a reciprocating shaker) at 350 r min^{-1} for 1.5 h (He et al. 2004) or manually

136   for 0.5 h (Gu et al. 2013). Different lengths of the extraction time were applied according to

137   the reported procedures. After leaching and extraction, these tubes were centrifuged at 3000 r

138   min^{-1} for 15 min. Then the mixture was filtered with phase separators (Whatman, GE

139   Healthcare Life Sciences, UK) and the organic phase with MeHg was kept. 4.0 mL of the

140   organic phase were added to tubes with around 20 mL distilled water and 2~3 pieces of boiling

141   stones (Saint-Gobain Performance Plastics, France) in them. These tubes were heated at 65 °C

142   for 6 h to remove organic solvent. After heating, each sample was brought to 20.0 mL with

143   ultra-pure water. 200.0 µL of the extract were pipetted into 40 mL amber glass vials (Agilent

144   Technologies, USA) for MeHg analysis by the MERX-M Automatic Methylmercury System

145   (Brooks Rand Laboratories, USA) following USEPA method 1630 (USEPA 2001).

146

2.2.2. KBr/H$_2$SO$_4$/CuSO$_4$ leaching, CH$_2$Cl$_2$ extraction, mechanical/manual shaking

148   5.0 mL of 18% (m/v) KBr dissolved in 5% (v/v) H$_2$SO$_4$ and 1.0 mL of 1 mol L^{-1} CuSO$_4$

149   were added to centrifuge tubes with about 0.25 g ERM-CC580 and waited 1 h. Then 10.0 mL

150   of CH$_2$Cl$_2$ were added to each tube and the mixture was shaken mechanically at 350 r min^{-1} for

151   1.5 h or manually for 0.5 h. The following procedure and analytical method were the same as

152   CuSO$_4$/HNO$_3$ procedure.

2.2.3. 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/CH$_3$OH were added to centrifuge tubes with around 0.25 g ERM-CC580. After heating at 75 °C for 3 h, 10.0 mL CH$_2$Cl$_2$ 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 CuSO$_4$/HNO$_3$ procedure.

2.3. 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 was 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 high organic matter contents (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.

2.3.1. Analysis of pore water samples

Sulfate (SO$_4^{2-}$) 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 mmID × 250 mm) with the eluent (3.6 mmol L^{-1} Na$_2$CO$_3$) 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 (Fe^{2+}) were determined using 1,10-phenantroline method with a UV-visible spectrophotometer (Shanghai Sunny Hengping, 756PC, China) (Tamura et al. 1974).

186 Concentration of ferrous iron (Fe^{3+}) could be obtained by subtracting the concentration of Fe^{2+}

187 from total iron. Total mercury (THg) concentrations in pore water samples were determined

188 with MERX-T Automatic Total Mercury System (Brooks Rand Laboratories, USA) following

189 USEPA 1631, Revision E (USEPA 2002).

190

191 **2.3.2. Analysis of sediment samples**

192 The concentrations of total carbon (TC) and total nitrogen (TN) in sediment samples were

193 determined with an elemental analyzer (Elementar, Vario EL III, Germany). The determination

194 of TC and TN was performed in triplicate. Sediment moisture contents were measured using a

195 weight loss method with a lyophilizer. THg contents of lyophilized sediment samples were

196 determined by Leeman mercury analyzer (Leeman Labs Hydra II C, USA) according to the

197 USEPA 7473 (USEPA 2007). MeHg concentrations of the samples were determined according

198 to the predetermined optimal pretreatment procedure. Analysis of MeHg contents was

199 performed in triplicate.

200

201 ## 2.4. Manipulation of sediment characteristics

202 The lyophilized surface sediment samples with different concentrations of moisture,

203 organic matter and acid volatile sulfide were achieved through physical methods. Then we

204 immediately determined the MeHg concentrations in the manipulated samples using the

205 predetermined optimal pretreatment procedure. The MeHg concentration analysis experiments

206 were performed in triplicate.

207

208 **2.4.1. Moisture content**

209 Considering the high background concentrations of MeHg in Xingfu Reservoir and

210 Qingnian Reservoir, sediment samples there were suitable for investigating whether the

211 optimal pretreatment procedure could apply to sediment with different moisture contents.

212 Lyophilized surface sediment samples from Xingfu Reservoir and Qingnian Reservoir were

213 mixed with different aliquots of ultra-pure water to produce wet samples with different

214 moisture contents. After homogeneous mixing, we determined the MeHg contents in these wet

215 samples directly. In detail, 0.25 g wet sediment samples with 20, 40, 60 and 80% moisture

216 content were spiked with MeHgCl working standard solution containing 2.0 ng Hg. During the

217 pretreatment, 4.0 mL in 10.0 mL extraction solutions were pipetted in the filtration and 2.0 mL

218 in 20.0 mL back-extraction solutions were brought for MeHg analysis after heating. For

convenience, all the recovery results in wet sediment samples were converted to the ratio of
MeHg contents in lyophilized samples following equation (1):

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

2.4.2. 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 moisture content of 80%. Considering that
MeHg contents of Taihu Lake sediment slurries were relatively low for analysis, MeHgCl
standard solutions were spiked into the slurries to form ~8 ng g^{-1} MeHg. TOC contents of
samples before and after the removal were determined by the elemental analyzer (Schumacher
2002).

2.4.3. 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; Lee et al.
2000b). Specifically, about 50 g sediment slurries were reduced by purging N$_2$ at 300 mL min^{-1}
for 3 d. 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 ~8 ng g^{-1}
MeHg. AVS contents in sediment samples were determined using "purge-and-trap" method
along with methylene blue spectrophotometry (Allen et al. 1993; Lasorsa and Casas 1996).
Then AVS contents in wet sediment samples were normalized to dry sediment weight
following equation (2).

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

2.5. 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 ± 0.02 ng g^{-1} (mean ± SD, n=6), which agreed well with the certified
value (0.28 ± 0.03 μg 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 pg to 800 pg. All glass ware

250   used was cleaned with distilled water three times, soaked in 10% (v/v) HNO_3 for at least 48 h,

251   washed with distilled water three times and finally heated at 500 °C for 2 h before use.

252   Statistical analysis was performed using SPSS 24.0 software. The difference among

253   recovery results of each procedure was assessed by an independent *t* test. Analysis of variance

254   (ANOVA) was applied to evaluate the significant difference of means. Significance

255   probabilities (*p*) were calculated and difference was declared significantly for $p < 0.01$ in the

256   current work.

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

258

3. Results and Discussion

3.1. Optimization of pretreatment procedure using certified reference material

262   As the pretreatment procedures were to be applied to MeHg analysis in bulk sediment

263   samples, accuracy, operability and security were considered comprehensively. Therefore, five

264   procedures were selected according to the recent publications involving MeHg analysis in

265   sediment (Kodamatani et al. 2017a; Wang et al. 2018; Yin et al. 2018). The detailed leaching

266   and extraction procedures of five pretreatment procedures using ERM-CC580 were listed in

267   Table 1 and the recovery results were illustrated in Fig. 2. As shown in Fig. 2, $CuSO_4/HNO_3$-

268   mechanical procedure had the most satisfying recovery ($100.67 \pm 6.75\%$, mean $\pm$ SD) among

269   five procedures tested.

270   With the same leaching solutions, recovery results of mechanical shaking didn't differ

271   significantly from those of manual shaking ($p > 0.1$). Specifically, $CuSO_4/HNO_3$ being

272   leaching solvents, extraction efficiency of manual shaking (0.5 h, $116.70 \pm 7.33\%$) was higher

273   than that of mechanical shaking (350 r min^{-1} for 1.5 h) and to some extent exceeded the

274   optimum value (100%). Yet, with $KBr/H_2SO_4/CuSO_4$ being leaching solvents, mechanical

275   shaking could produce higher and relatively more accurate recovery results ($82.60 \pm 7.87\%$).

276   This fluctuation might be caused by the inadequate stability and repeatability of manual

277   shaking, which usually were the consequences of individual's difference in strength. After

278   considering the accuracy and reproducibility, mechanical shaking was selected instead of

279   manual shaking. In addition, KOH/CH_3OH could produce decent recovery results as well

280   ($86.33 \pm 7.95\%$). However, this procedure required heating as leaching method for 3 h and

281   back-extraction for 6 h, which was rather time-consuming. And all with mechanical shaking,

CuSO$_4$/HNO$_3$ as leaching solvents displayed higher leaching efficiency than KBr/H$_2$SO$_4$/CuSO$_4$ and KOH/CH$_3$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 CuSO$_4$/HNO$_3$ (100.67 ± 6.75%) with mechanical shaking agreed well with He's results (98.94 ± 6.62%), 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 CuSO$_4$/HNO$_3$. Moreover, the pretreatment procedure using KBr/H$_2$SO$_4$/CuSO$_4$ as leaching solutions in this work produced a bit lower recovery results than reported results (~100%) (Gu et al. 2013; Kodamatani et al. 2017a). In Kodamatani's both Method C and D, they transferred certain amounts of CH$_2$Cl$_2$ (in the lower layer of the mixture) to deionized distilled water (Kodamatani et al. 2017a). It was possible to carry 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 CH$_2$Cl$_2$ 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 ± 15%) was similar to the result of 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/CH$_3$OH as leaching solutions in this work produced slightly lower recovery results than reported results (100.18 ± 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, CuSO$_4$/HNO$_3$-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.

3.2. 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 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 were 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 SO_4^{2-}, Fe^{2+}, Fe^{3+} 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.

3.3. Application to sediment with various moisture contents

Compared to 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 contents 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's 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 $CuSO_4/HNO_3$-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 contents.

The recovery results of wet sediment samples with various moisture contents were illustrated in Fig. 3. With moisture contents 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 the original deviation in MeHg determination of lyophilized sediment samples. The reason for $CuSO_4/HNO_3$-mechanical procedure producing satisfying recovery might be that HNO_3 could destroy the strong 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, $CuSO_4/HNO_3$-mechanical procedure could be applied to MeHg determination in wet sediment samples with various moisture contents.

3.4. Application to sediment with distinct organic matter contents

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 is 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. Contents of TOC and TN before and after the removal were shown in Table 5. After being muffled for 8 h, the TOC contents in surface sediment samples dropped markedly (from over 1% to less than 0.5%) compared to TN contents. 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 were illustrated in Fig. 4, and all the results in wet sediment samples were converted to the ratio of MeHg contents in lyophilized samples following equation (1). As shown in Fig. 4, despite the variation in TOC contents, the spiking recoveries of MeHg didn't differ a lot. Before the removal of organic matter, while the TOC contents were higher than 1%, the recovery results in both surface sediment samples of Taihu Lake were near 100%. Then after the removal, as the TOC contents dropped sharply, the recoveries in Meiliang Bay

dropped a little but were still within acceptable limit (>85%). In Zhushan Bay sediment samples, the recovery results increased to around 100% after the removal. Overall speaking, the removal of the organic matter didn't influence the spiking recovery results of Taihu surface sediment samples remarkably.

According to the spiking recovery results above, the pretreatment procedure using $CuSO_4/HNO_3$ as leaching solutions with mechanical shaking as extracting method applies to sediments samples with distinct contents of organic matter.

3.5. Application to sediment with distinct acid volatile sulfide contents

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 contents over 2 µg g^{-1}, while the contents of the original sample were lower than 1.2 µg g^{-1} (dry weight) (Table 6). The increase of AVS contents in reduced sediment samples was consistent with the trend of Lee's (Lee et al. 2000b). As a result, sediment samples purged with N_2 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 contents. The recovery results were illustrated in Fig. 5. Before manipulation, while the AVS contents were 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 contents. Generally speaking, all the recovery results were near 100% within the mentioned range of AVS content.

Therefore, the pretreatment procedure using $CuSO_4/HNO_3$ as leaching solutions with mechanical shaking as extraction method could accurately determine MeHg contents in sediment samples with a variety of AVS contents.

4. Conclusions

The current work compared recovery results of five pretreatment procedures for MeHg analysis in sediment samples using ERM-CC580. And the procedure using $CuSO_4/HNO_3$ as leaching solutions with mechanical shaking as extraction method produced the most satisfying recovery result, which was 100.67 ± 6.75% in average. In addition, moisture contents in

413   sediment samples (from 20 to 80%) had little influence on the analytical performance of the

414   optimal pretreatment procedure. Considering the strong complexation ability of organic matter

415   and sulfide with MeHg, sediment samples were manipulated physically to produce a relatively

416   wide range of TOC (from ~0.3 to ~1.5%) and AVS (from ~1.1 to ~2.7 μg g^{-1}, dry weight)

417   contents. And the spiking recovery results were mostly around 100%, indicating that the

418   optimal pretreatment procedure was able to produce satisfactory results for MeHg

419   determination in sediment samples with various properties.

420   There is possibility that spiking recovery tests may not entirely reflect the real

421   performance of MeHg in sediment samples. It's likely that natural compounds may bind with

422   MeHg so tightly that they cannot be extracted easily (Qian et al. 2002). Even so, spiking

423   recovery tests have been carried out in plenty of work to prove the accuracy of their analytical

424   methods when the background MeHg concentration was under detection limit (Heyes et al.

425   2004; Horvat et al. 1993; Liang et al. 2004). Also, inevitably, the manipulation of one

426   characteristic in sediment may bring about changes to other characteristics as well. However,

427   considering the manipulation methods used are mainly through physical instead of chemical

428   means, the procedures can be well-controlled (Lee et al. 2000b). And using the same sediment

429   samples in MeHg recovery tests could eliminate the influence of other irrelevant characteristics,

430   like background MeHg content and particle size. Still, if time and energy permit, researchers

431   should collect sediment samples as various as possible.

432   Hopefully, this work can provide a feasible approach to evaluate environmental MeHg

433   risks and thereby reduce human's exposure to pollution sources and finally alleviate health risks.

434

Conflict of Interest Statement

436   The authors declare that there are no conflicts of interest.

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