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Speciation of mercury in soil and sediment by selective solvent and acid extraction

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Abstract In order to characterize the mercury hazard in soil, a sequential extraction scheme has been developed to classify mercury species based on their environmental mobility and/or toxicity for either routine lab analysis or on-site screening purposes. The alkyl mercury species and soluble inorganic species that contribute to the major portion of potential mercury toxicity in the soil are extracted by an acidic ethanol solution (2% HCl+10% ethanol solution) from soil matrices as "mobile and toxic" species. A High-Performance Liquid Chromatography (HPLC) system coupled with Inductively Coupled Plasma Mass Spectrometry (ICP–MS) detection has been developed to further resolve the species information into soluble inorganic species (Hg^{2+}), methylmercury(II) ($MeHg^{+}$) and ethylmercury(II) ($EtHg^{+}$) species. Alternatively, these species can be separated into "soluble inorganic mercury" and "alkyl mercury" sub-categories by Solid-Phase Extraction (SPE). A custom Sulfydryl Cotton Fiber (SCF) material is used as the solid phase medium. Optimization of the SCF SPE technique is discussed. Combined with a direct mercury analyzer (DMA-80), the SCF SPE technique is a promising candidate for on-site screening purposes. Following the ethanol extraction, the inorganic mercury species remaining in soil are further divided into "semi-mobile" and "non-mobile" sub-categories by sequential acid extractions. The "semi-mobile" mercury species include mainly elemental mercury (Hg) and mercury-metal amalgams. The non-mobile mercury species mainly include mercuric sulfide (HgS) and mercurous chloride (Hg_2Cl_2).

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Electronic supplementary material Evaluation of the KCl+MeOH+HAc extract solution for mercury species; optimization of the 2% HCl+10% ethanol extraction procedure; comparison of the proposed method with EPA Method 1311 Toxicity Characteristic Leaching Procedure (TCLP); evaluation and optimization of eluents and flow rate for the SCF SPE separation. This material is available free of charge via the Internet at http://link.springer.de/journals/abc.

Keywords Mercury · Speciation · Extraction · Soil

Introduction

The toxicity, bioaccumulation, and environmental mobility of mercury in soil depend on the species present. Alkyl mercury species, such as methylmercury(II) ($MeHg^{+}$) and ethylmercury(II) ($EtHg^{+}$), are at least an order of magnitude more mobile than inorganic mercury species, and thus are more toxic and more readily bio-accumulated [1]. Soluble inorganic mercury species, such as mercuric chloride ($HgCl_2$), are more easily transported by natural processes than the other inorganic mercury species, and typically serve as the substrate for the mercury methylation process [2]. These alkyl mercury species and soluble inorganic species contribute to the major portion of potential mercury toxicity in soils. Mercury species such as elemental mercury (Hg) and mercury–metal amalgams, are less toxic than soluble inorganic mercury species because they are less mobile in environmental processes. Mercury species such as mercuric sulfide (HgS) are chemically stable in the soil over geologic time periods and, thus, are the least toxic mercury species.

Typically, alkyl mercury species are determined by acid digestion with solvent extraction, such as the Westöö [3] method and its modifications [4, 5, 6, 7, 8, 9, 10, 11, 12], or by acidic vapor distillation [13]. In the Westöö method and its modifications the alkyl mercury species from the acidic digestate are extracted into an organic solvent phase, followed by several cleanup steps [3] and then

Table 1 Operationally defined mercury fractions in this study

Operationally-defined mercury fractions Total mercury		Individual mercury species All mercury-containing species
"Mobile and toxic" mercury	Alkyl mercury	MeHgCl
		EtHgCl
	Soluble inorganic mercury	$HgCl_2$
		$Hg(OH)_2$
		$Hg(NO_3)_2$
		$HgSO_4$
		HgO
		Hg^{2+} complexes[a]
Non-extractable mercury	Semi-mobile mercury	Hg or Hg–M[b]
		Hg^{2+} complexes[a]
		Hg_2Cl_2 (minor)
	Non-mobile mercury	Hg_2Cl_2 (major)
		HgS
		HgSe

[a]Certain inorganic mercury complexes may be present in both fractions
[b]This represents a mercury–metal amalgam

back-extracted into an aqueous phase [12]. This extraction scheme normally involves toxic solvents such as benzene [9, 10], toluene [11, 12], chloroform [14], or dichloromethane [15], and relies heavily on the efficiency of cleanup and back-extraction steps. It has been reported that acidic vapor distillation methods in the presence of organic substances may artificially generate $MeHg^+$ [13, 16]. Since strong acid is normally used in such extractions or distillations to liberate alkyl mercury from solid matrices, sequential extraction is impossible, and the inorganic speciation has to be carried out in a different portion of the sample.

There are two major analysis methods for extracted mercury species. The first one is based on ethylation, purge-and-trap collection, gas chromatography (GC) separation with Cold-Vapor Atomic Fluorescence (CVAFS) detection [7, 13, 15, 16, 17, 18, 19, 20], or with Microwave Induced Plasma Atomic Emission Spectrometry (MIP–AES) detection [5, 8, 19, 21, 22, 23]. The other major technique is based on HPLC separation with ICP–MS detection [24, 25, 26]. Much effort has been used to improve the detection limits and separation efficiency of both methods to meet the requirements of routine laboratory analysis. However, instrumentation limitations make it difficult, if not impossible, to apply these methods for on-site screening for the remediation of mercury contaminated sites. Recently, a new direct mercury analyzer, DMA-80, has proven to have a great on-site capacity for the direct analysis of total mercury in a variety of solid and aqueous matrices [27]. EPA Method 7473 has been developed based on this instrument for the analysis of total mercury in various matrices [28]. Therefore, by combining proper extraction and separation methods, the DMA-80 can be potentially applied for on-site mercury speciation.

In the present study, a new extraction scheme using acidic ethanol solution has been investigated to extract alkyl mercury and soluble inorganic mercury from soil and sediment matrices. Following the extraction, a custom Sulfydryl Cotton Fiber Solid Phase Extraction (SCF SPE) column is applied to further separate extractable alkyl mercury species from extractable inorganic species. The DMA-80 mercury analyzer is used to analyze each separated fraction to obtain mercury species information. The procedure that integrates ethanol extraction, SCF SFE separation, and DMA-80 detection has a great potential for on-site mercury species screening purpose. Alternatively, a method based on ethanol extraction and HPLC–ICP–MS analysis has also been established and is ideal for routine laboratory mercury speciation. Following ethanol extraction a sequential acid extraction procedure can be used to further divide the remaining inorganic mercury species into two categories: the "semi-mobile" and "non-mobile" species. Operationally defined mercury fractions are summarized in Table 1.

Experimental

Instrumentation

The direct mercury analyzer used for this work was a Milestone DMA-80 Direct Mercury Analyzer (Milestone GmbH, Germany). Its unique processing of the sample by thermal decomposition, amalgamation, and atomic absorption spectrometry allows for direct analysis of the sample matrices [27]. The operation conditions for DMA-80 used throughout this work were based on the guidelines of EPA Method 7473 protocol [28].

The ICP–MS instrument used for this work was a Hewlett–Packard HP-4500 (Agilent Technologies, USA and Yokogawa Analytical System, Japan). The instrument was tuned using a 10 $\mu g\,kg^{-1}$ multi-element tuning solution in 30% methanol. Time Resolved Analysis (TRA) was engaged. The operation conditions used throughout this work are summarized as follow: RF power: 1450 W; plasma gas flow: 15 $L\,min^{-1}$; auxiliary gas: 1 $L\,min^{-1}$; carrier gas: 0.91 $L\,min^{-1}$; blend gas: 0.07 $L\,min^{-1}$; S/C temperature: –5 °C; concentric nebulizer; nickel sampling and skimmer cone.

The HPLC system used for this work was a ConstaMetric 4100 Pump (Thermo Separation Products, USA) and a 5 µm Supelcosil LC-18 HPLC column with a Pelliguard LC-18 guard column (Supelco, PA, USA). The mobile phase was buffered 30% methanol (refer to Reagent Section).

The SPE apparatus used for this work was an SPE manifold (Supelco, PA, USA) with the custom SCF SPE column prepared as follows:

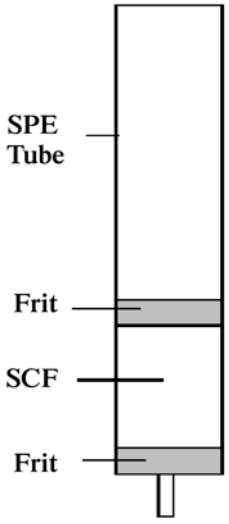

Fig. 1 Diagram of custom SCF SPE column

Place a PTFE frit at the bottom of a 6 mL SPE tube (glass, 1.1 cm i.d.). Add a 0.2 g portion of the SCF (refer to Reagent Section) along with 3 mL Distilled De-Ionized (DDI) water. Place a second PTFE frit on the top of the SCF. Apply pressure to the top frit to compact the SCF into a homogeneous disk between the two frits; use care to avoid channeling. The bed volume of the SCF disk is about 0.8 mL per 0.1 g of SCF medium. A diagram of the prepared SCF column is illustrated in Fig. 1. Condition the SCF column by passing 10 mL DDI water, then 10 mL 6 mol L^{-1} HCl, and finally 15 mL DDI water sequentially through the column using a flow rate of 1 mL min^{-1}.

Reagents and standards

ACS grade HCl, HNO_3, H_2SO_4, NaCl, $CuCl_2$, and NaOH were obtained from Fisher Scientific (Pittsburgh, PA, USA). The reagent grade acetic acid, acetic anhydrate, ammonium acetate, 2-mercaptoethanol and Optima grade methanol were also obtained from Fisher Scientific. Ethanol (USP, 200 proof) was obtained from McCormick Distilling Co. (Weston, MO, USA). 97% mercaptoacetic acid was obtained from Aldrich (WI, USA). 18 MΩ cm DDI water was purified with a Milli-Q system (Millipore, USA). 2% (v/v) HCl+10% (v/v) ethanol extraction solution was prepared by dilution of the proper amount of concentrated HCl and ethanol in DDI water.

Standard solutions containing 1 mg mL^{-1} mercury chloride and methylmercury(II) chloride in water were commercially available from Alfa Aesar (Ward Hill, MA, USA). High purity mercury, mercuric oxide (red), mercuric sulfide (red) and zinc (powder), as well as 95% ethylmercury(II) chloride were also obtained from Alfa Aesar. Ethylmercury(II) chloride solution (1 mg mL^{-1}) was prepared by dilution of the proper amount of ethylmercury(II) chloride in 0.01% (w/v) $K_2Cr_2O_4$+5% (v/v) HNO_3 +50% (v/v) methanol solution [29]. (Caution: Mercury species, especially organo-mercury species, are particularly toxic and are contact and inhalation hazards.) The prepared mercury standard solutions were stored in brown glass bottles with TFE-lined closures at 4 °C in a dark environment. The mercury–zinc amalgam was prepared by mixing 1:4 (w/w) mercury:zinc together and grinding thoroughly.

The preparation of SCF as an SPE medium was modified from Liu's procedure [30] and described as follows: prepare a mixture containing 50 mL of mercaptoacetic acid, 35 mL of acetic anhydrate, 16 mL of acetic acid, 0.15 mL of H_2SO_4 and 5 mL of DDI water in a clean vessel. Immerse a 15 g portion of cotton in this mixture. Cover the vessel and place it in a hot-water bath at 40±2 °C for four days. Remove the product from the reagent mixture and place it in a filter funnel and rinse with water until the pH of the washings is neutral. Dry the product at 40±2 °C for two days.

SCF eluent 1 (1 mol L^{-1} HCl+1 mol L^{-1} NaCl) for alkyl mercury species was prepared by diluting 20.7 mL of concentrated HCl to 250 mL in DDI water, then dissolving 14.6 g of solid NaCl in the 1 mol L^{-1} HCl.

SCF eluent 2 (6 mol L^{-1} HCl+saturated NaCl+0.1% $CuCl_2$) for inorganic mercury species was prepared by diluting 124 mL of concentrated HCl to 250 mL in DDI water, then adding 0.25 g of solid $CuCl_2$ and 11.0 g of solid NaCl.

HPLC speciation mobile phase, (30% (v/v) methanol+0.001% (v/v) 2-mercaptoethanol+0.2 mol L^{-1} ammonium acetate), modified from Wilken's procedure [31], was prepared by diluting 150 mL methanol, 25 µL 2-mercaptoethanol and 2.4 g ammonium acetate in 350 mL DDI water.

HNO_3:DDI, water extraction solution 1:2 (v/v), was prepared by dilution of the proper amount of concentrated HNO_3 in DDI water.

HCl:HNO_3:DDI water, 1:6:7 (v/v/v), was prepared by dilution of the proper amount of concentrated HCl and concentrated HNO_3 in DDI water.

Mercury standard reference material NIST SRM 2709 (San Joaquin Soil) was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Mercury certified reference material BCR CRM 580 (Estuarine Soil) was obtained from Institute for Reference Materials and Measurements (Retieseweg, 2440 Geel, Belgium).

The extraction of "mobile and toxic" mercury species

This extraction involves the use of a solution of 2% HCl+10% ethanol to extract the "mobile and toxic" mercury species from the soil or sediment samples. The target mercury species include toxic alkyl mercury species, such as $MeHg^+$ and $EtHg^+$ species, as well as inorganic mercury species that have great mobility in the environment, such as soluble Hg^{2+} ions and mercuric oxide (HgO).

A sample (1.0–2.0 g) was weighed and added to a 10-mL centrifuge tube with 2.5 mL of the extract solution. The sample and the extract solution were mixed well by vortex mixing for 2 min. If necessary, concentrated HCl was added drop-wise until the pH of the mixture was between 1.5 and 3. The sample was sonicated at 60±2 °C for 7 min. A centrifuge was applied to separate the supernatant and soil matrix. The extraction was repeated three additional times. DDI water (2.5 mL) was then added to the sample residue. The sample was vortex mixed, then centrifuged. All supernatants and water rinses were combined. This solution contains the "mobile and toxic" mercury species and can be divided for further speciation.

SPE speciation procedure

This speciation procedure involves the use of SCF as the separation medium, and separates the "mobile and toxic" mercury fraction into soluble inorganic and alkyl mercury fractions. The preparation and the conditioning of an SCF SPE column are described in the Reagent Section.

Following the ethanol extraction, the pH of the extract was adjusted to a value in the range of 5–7. The solution was filtered to retain particles larger than 10 µm. Retained particles were rinsed with 3 mL 0.1% HCl. The filtered solution and the rinse solution were combined (Refer to Results and discussion section). The pH of the filtered solution was adjusted to a value in the range 2–4 with 6 mol L^{-1} HCl. The solution was then passed through the SCF SFE column using a flow rate of 1 mL min^{-1}. The "mobile and toxic" mercury species were retained on the SCF medium.

The alkyl mercury species were eluted from the SCF SFE column by passing 8 mL SCF eluent 1 (1 mol L^{-1} HCl+1 mol L^{-1} NaCl) followed by 2 mL DDI water through the column at a flow rate of 1 mL min^{-1}. The eluate was analyzed by either the DMA-80 or the ICP–MS to determine the amount of alkyl mercury that had been extracted. If the alkyl mercury in the eluate was too dilute to be analyzed by the DMA-80, such eluate could be passed through

a second SCF SFE column (for pre-concentration) after its pH was adjusted to 2–4. The second SCF medium could be analyzed directly by the DMA 80.

The "mobile and toxic" inorganic mercury fraction would remain on the first SCF medium and could be analyzed directly by the DMA-80. Alternatively, the remaining inorganic mercury fraction could be eluted from the SCF medium by passing 8 mL eluent 2 (6 mol L^{-1} HCl+saturated NaCl+0.1% $CuCl_2$) followed by 2 mL DDI water through the SCF column at a flow rate of 1 mL min^{-1}. This eluate was then analyzed using the DMA-80 or the ICP–MS to determine the amount of soluble inorganic mercury species extracted from the sample.

LC–ICP–MS speciation procedure

An HPLC system directly coupled with an ICP–MS detector has been applied for the study of mercury speciation by many research groups [24, 25, 26]. The advantages of this hyphenated technique over other mercury speciation techniques, such as GC–CVAAS, include direct separation with preserved species integrity, high sensitivity, broad linear dynamic range and multi-isotopic capacity [32]. In the present study, an HPLC–ICP–MS speciation procedure was developed for the separation of the "mobile and toxic" mercury fraction into individual inorganic, methylmercury(II) and ethylmercury(II) species.

Following the ethanol extraction, the pH of the extract was adjusted to a value in the range 3–7. A portion of 50 µL filtered extract was injected into the HPLC system. The column and mobile phase for the HPLC system, as well as the ICP–MS instrument, are described in the Instrument and reagent sections. A sample chromatogram of the separation of Hg^{2+}, $MeHg^{+}$ and $EtHg^{+}$ is illustrated in Fig. 2. If necessary, the SCF medium described above could be applied as a pre-separation technique prior to the HPLC separation to reduce interference resulting from the huge amount of soluble inorganic mercury in relation to the trace amount of methylmercury(II) species.

Sequential extraction of remaining inorganic species

The matrix material remaining after the ethanol extraction may be further divided into "semi-mobile" and "non-mobile" mercury fractions. The "semi-mobile" mercury species include mainly Hg and mercury–metal amalgam. The "non-mobile" mercury species mainly include HgS and Hg_2Cl_2.

The sample portion remaining after the ethanol extraction was first sonicated with 5 mL DDI water at 60±2 °C for 5 min. The sample was centrifuged and the supernatant was discarded to remove chlorine ions. Then, 5 mL 1:2 (v/v) HNO_3:DDI water extraction solution was added. The sample was mixed with the extract solution by vortex mixing. The mixture was heated to 95±2 °C for 20 min in a water bath and then centrifuged. The supernatant was separated and the extraction was repeated. The remaining soil sample portion was washed with 5 mL DDI water. The rinse water was combined with both supernatants. This solution containing the "semi-mobile" mercury species was analyzed by the DMA-80.

The rest of the matrix material could be directly analyzed by the DMA-80 for "non-mobile" mercury species. Alternatively, it could be further extracted with 5 mL 1:6:7 (v/v/v) HCl:HNO_3:DDI water at 95±2 °C for 20 min two times in the water bath, then washed with 5 mL DDI water. The rinse water was combined with both supernatants and analyzed by the DMA-80.

Results and discussion

Solubility of mercury species

In this study, a new extraction system based on dilute ethanol and HCl solution is developed. The target mercury species are alkyl mercury species (most toxic) and soluble inorganic mercury species (most mobile). Ethanol can dissolve both alkyl mercury species and soluble inorganic mercury species. Dilute HCl is used to liberate alkyl mercury species from the matrices and to dissolve the HgO species. Since the extraction conditions are mild, sequential acid extraction for further speciation of inorganic mercury species such as Hg and HgS becomes possible.

Each pure mercury species (10 mg) underwent preliminary testing for solubility under the following extraction conditions:

1. 10 mL 2% HCl+10% ethanol, sonicated at 60±2 °C for 30 min;
2. 10 mL 1:2 HNO_3: DDI water, heated in 95±2 °C water-bath for 40 min; and
3. 10 mL 1:6:7 HCl: HNO_3: DDI water, heated in 95± 2 °C water-bath for 40 min.

The solubility of each species is defined as the portion of 10 mg of each species dissolved in 10 mL of each extrac-

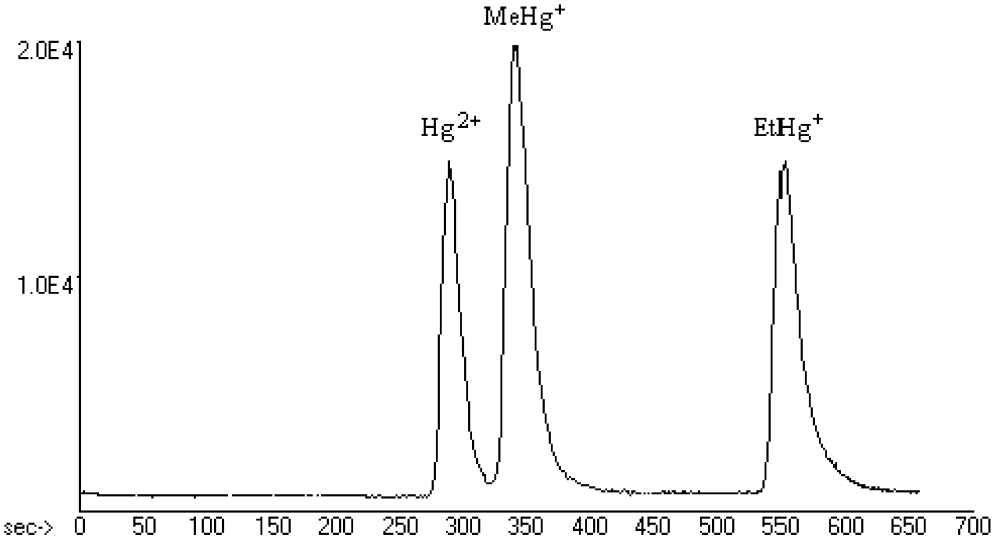

Fig. 2 Chromatogram obtained by using HPLC–ICP–MS to separate Hg^{2+}, $MeHg^{+}$ and $EtHg^{+}$ species

Table 2 Solubility (%) determined by percent recovered of 10 mg of each species in 10 mL of each extraction solution

Species	2% HCl+ 10% ethanol, sonicated at 60±2 °C for 30 min	1:2 HNO_3:DDI water, heated at 95±2 °C in a waterbath for 40 min	1:6:7 HCl:HNO_3:DDI water, heated at 95±2 °C in a water-bath for 40 min
MeHgCl	98	99	104
EtHgCl	96	94	93
$HgCl_2$	96	99	99
HgO	97	97	102
Hg	4	95	102
Hg_2Cl_2	1.6	11	96
HgS	0.15	0.04	97

The solubility of each species is defined as the portion of 10 mg of each species dissolved in 10 mL of each extraction solution

tion solution, and was determined with analysis of the extraction solution by the DMA-80. Results are summarized in Table 2. The results indicate that 2% HCl+10% ethanol extraction solution can dissolve most of the alkyl mercury, soluble mercury and HgO. This extraction solution has little ability to dissolve Hg and HgS. Hg can be dissolved mainly in 1:2 HNO_3:DDI water at 95 °C. HgS and Hg_2Cl_2 are mostly soluble in 1:6:7 HCl:HNO_3:DDI water at 95 °C.

Performance evaluation of the 2% HCl+10% ethanol extraction procedure

The 2% HCl+10% ethanol extraction procedure as specified in the Experimental section was evaluated using spike recovery tests on several natural matrices. The natural matrices selected for spiking were pure silica (SiO_2), NIST SRM 2709 (San Joaquin Soil), and the soil matrix discussed in supplementary material. The mercury species were spiked at 25 µg mercury per gram of sample. The extracts were analyzed by the DMA-80 and/or LC–ICP–MS. Results are summarized in Table 3. Recoveries of better than 87% were obtained for all materials tested. This extraction procedure only requires heated sonication, a vortex mixer, and a centrifuge. Therefore, it can be easily performed in the field.

Performance evaluation of the SCF SPE procedure

SCF material, produced by introducing the sulfydryl functional group into natural cotton fibers, was developed to pre-concentrate trace amount of mercury species in water [33, 34]. It has been established by X-ray Photo Spectrometry (XPS) [35, 36] that the active functional group responsible for mercury retention in SCF is the sulfydryl group (R–SH). This group has a high affinity for the mercury species. The most commonly used reagent in preparation of SCF is mercaptoacetic acid (HS–CH_2–COOH). The word "mercaptan" is derived from "mercury capturer" [32] and hence, it is not surprising that these thiols have a strong affinity for mercury species, especially when they lose protons [37, 38]. The mechanism by which the sulfydryl functional group retains Hg^{2+}, $MeHg^+$ and $EtHg^+$ species is proposed:

$$2R-SH + Hg^{2+} \Leftrightarrow R-S-Hg-S-R + 2H^+ \quad (1)$$

$$R-SH + MeHg^+ \Leftrightarrow R-S-Hg-Me + H^+ \quad (2)$$

$$R-SH + EtHg^+ \Leftrightarrow R-S-Hg-Et + H^+ \quad (3)$$

Since Hg^{2+} can complex with two S-donor ligands (sulfydryl functional groups), it has stronger affinity than either $MeHg^+$ or $EtHg^+$, which can only complex with one sulfydryl functional group. The retention and desorption of mercury species on the SCF material can be controlled by altering $[H^+]$ in the solution. When SCF is exposed to an environment with pH ranging from 1 to 8 the sulfydryl functional group loses a proton. It is active as "R–S^-" and "captures" mercury species "passing" by. The equilibria shown in the above equations shift towards the right and the products R–S–Hg–S–R or R–S–Hg–R′ (R′ is either Me or Et group) are formed. When SCF is exposed to a strong acid environment, the equilibria shown above shift towards the left hand side and mercury species can be eluted from the SCF material into the eluents. Since $MeHg^+$ or $EtHg^+$ species only complex with one sulfydryl functional group, it will be eluted from the SCF material as $MeHg^+$ or $EtHg^+$ in a moderate acid environment (1–2 mol L^{-1} $[H^+]$). This moderate acid environment is not efficient enough to elute inorganic mercury species associated with sulfydryl functional group. When the acid concentration is increased to 6 mol L^{-1} or higher, the inorganic mercury can be eluted from the SCF medium as Hg^{2+}. Furthermore, the presence of a large amount of $[Cl^-]$ will complex with Hg^{2+} to form $[HgCl_4]^{2-}$, which is much more easily dissolved in aqueous solution compared to $HgCl_2$. The elution of Hg^{2+} from SCF medium is enhanced. Therefore, SCF is a promising candidate for preconcentration and separation of mercury species.

In this study, the SCF medium was packed into a SPE column to trap the "mobile and toxic" mercury species extracted with 2%HCl+10% ethanol from the soil matrices. The alkyl mercury species and soluble inorganic mercury species were sequentially released from the SCF SPE column using 1 mol L^{-1} HCl+1 mol L^{-1} NaCl solution and 6 mol L^{-1} HCl+saturated NaCl+0.1% $CuCl_2$ solution. Combining the custom SCF SPE column with a commercially available SFE manifold, this procedure is simple and fast, and can be performed on-site easily.

The following conditions were determined to be optimal for the SCF SPE procedure: mass of SCF medium:

Table 3 Spike recoveries of mercury species from various matrices

Species recovery	Silica[a]	Soil	SRM 2709
Hg^{2+} (%)	94–98	106±15	98±7
$MeHg^+$ (%)	92–105	104±10	92±17
$EtHg^+$ (%)	87–102	92±18	92±17
HgO (%)	94–100	92±13	N/A

Uncertainties expressed as 95% confidence interval with n=3
[a]two replicates

Table 4 Recovery of "mobile and toxic" mercury species in the SCF SPE procedure

Eluent	$MeHg^+$	$EtHg^+$	Hg^{2+}
Unretained	<0.5%	<0.5%	<0.5%
1 mol L^{-1} HCl+1 mol L^{-1} NaCl	96±3%	99±5%	<0.1%
6 mol L^{-1} HCl+saturated NaCl+0.1% $CuCl_2$	<3%	<3%	98±4%
Residue (in SCF)	<DL	<DL	<DL

Uncertainties expressed as 95% confidence interval with n=3
<DL: below the detection limit of the DMA-80

0.2 g; bed volume of 0.1 g SCF disk: 0.7 mL; pH range for initial solution: 2 to 5; eluent flow rate: 1 mL min^{-1}; eluent 1: 1 mol L^{-1} HCl+1 mol L^{-1} NaCl solution; eluent 2: 6 mol L^{-1} HCl+saturated NaCl+0.1% $CuCl_2$. The overall performance of the SCF SPE procedure was tested. $MeHg^+$, $EtHg^+$ and Hg^{2+} (25 µg of each) were spiked into 10 mL portions of 10% ethanol solution. These spiked solutions were passed through SCF SFE column. The retained mercury species were eluted from the column under the optimal conditions. Percentage recoveries of mercury species in each portion of the eluents were analyzed by the DMA-80. Results are summarized in Table 4. The results suggest that $MeHg^+$ and $EtHg^+$ are quantitatively separated from Hg^{2+} using the SCF SPE procedure.

Evaluation of pH-dependence on the SCF SPE column

The pH value of 10% ethanol solution was adjusted to <1, 2, 3, 4, 5, 6, 8, and 11 with 6 mol L^{-1} HCl or 5 mol L^{-1} NaOH. A mixture of 25 µg each of Hg^{2+}, $MeHg^+$ and $EtHg^+$ was spiked into 10 mL 10% ethanol solutions of different pH. The spiked solutions were passed through 0.1 g SCF SPE column. The alkyl mercury species were eluted using 8 mL 1 mol L^{-1} HCl+1 mol L^{-1} NaCl and were analyzed using the LC–ICP–MS. The inorganic mercury species were eluted by 8 mL 6 mol L^{-1} HCl+saturated NaCl+0.1% $CuCl_2$ and analyzed by the DMA-80. As indicated in Table 5, a pH range of 2 to 5 can be used to obtain satisfactory SCF SPE performance.

Evaluation of retaining ability of the SCF medium

In order to determine the retaining ability of the SCF medium, 100 µg mL^{-1} $HgCl_2$ prepared in two different matrices was passed through a 0.1 g SCF SPE column using optimum conditions. The two matrices were:

1. 10% ethanol at pH=3; and
2. salt solution in 10% ethanol with pH=3, which contains 1% Na^+, 300 mg kg^{-1} Ca^{2+}, 200 mg kg^{-1} Al^{3+}, 100 mg kg^{-1} Fe^{3+} and Mg^{2+}, 50 mg kg^{-1} Mn^{2+} and K^+, 10 mg kg^{-1} Ba^{2+}, and 1 mg kg^{-1} Ni^{2+}, Cu^{2+} and Zn^{2+}.

The components and their concentrations in the salt solution were determined based on the components extracted from SRM 2709 and the soil matrix described in supplementary material by 2% HCl+10% ethanol and whose concentrations were above 1 mg kg^{-1} in final extracts. The retaining ability (>99.5% mercury retained) of 0.1 g SCF medium was about 1000 µg Hg^{2+} in 10% ethanol solution and 500 µg Hg^{2+} in the salt solution. The cumulative amount of mercury species passed through the SCF medium and the cumulative mercury retained on the SCF medium are summarized in Table 6.

Table 5 Recoveries of mercury species in matrices of different pH for the SCF SPE separation

pH	<1	2	3	4	5	6	8	11
$MeHg^+$ (%)	101	100	97	98	96	97	93	68
$EtHg^+$ (%)	90	102	103	102	103	93	102	68
Hg^{2+} (%)	104	102	99	105	100	106	107	93

Table 6 Mercury retention ability of 0.1 g SCF disk in different solutions

10% ethanol		Salt solution	
Total Hg passed (µg)	Cumulative retention (%)	Total Hg passed (µg)	Cumulative retention (%)
500	100	200	99.9
1000	100	400	99.6
1200	99.3	600	99.3
1400	98.6	800	99.0
N/A	N/A	1000	98.0

The components and their concentrations in the salt solution were determined based on the components extracted from SRM 2709 and the soil matrix described in supplementary material by 2% HCl+10% ethanol, and whose concentrations were above 1 mg kg^{-1} in final extracts

Minimizing the bias introduced by inorganic mercury species on the measurement of alkyl mercury species

The SCF SPE procedure was optimized to minimize the amount of inorganic mercury species eluted as alkyl mercury species. Such bias must be considered when a trace amount of alkyl mercury species co-exists with a large amount of inorganic mercury species. $MeHg^+$ (25 µg) and $HgCl_2$ (25 µg) in 10% ethanol at pH 3 were passed through 0.1 g SCF SPE column and eluted with 8 mL SCF eluent. As indicated in Table 7, 1 mol L^{-1} HCl+1 mol L^{-1} NaCl can reduce the bias by an order of magnitude compared with 1.5 mol L^{-1} HCl+1.5 mol L^{-1} NaCl. However, further reducing the eluent 1 concentration may sacrifice $MeHg^+$ recovery. In Table 8, 25 µg $MeHg^+$ with 25 µg or 500 µg Hg^{2+} in the 10% ethanol solution were passed through SCF SPE column at pH 3 and eluted with 8 mL 1 mol L^{-1} HCl+1 mol L^{-1} NaCl. More than 95% spiked $MeHg^+$ was recovered in all cases. Compared to 0.1 g SCF medium, 0.2 g SCF medium can reduce Hg^{2+} interference signifi-

Table 7 Optimization of eluent 1 to minimize 25 µg Hg^{2+} interference for the recovery of 25 µg $MeHg^+$ in the SCF SPE procedure

Eluent	$MeHg^+$ recovery (%)	Hg^{2+} interference (%)
1.5 mol L^{-1} HCl+1.5 mol L^{-1} NaCl	99	2
1.5 mol L^{-1} HCl+1 mol L^{-1} NaCl	97	2
1 mol L^{-1} HCl+1 mol L^{-1} NaCl	97	0.3
0.5 mol L^{-1} HCl+0.5 mol L^{-1} NaCl	94	0.4

Table 8 Optimization of the amount of SCF medium to minimize Hg^{2+} interference for the recovery of 25 µg $MeHg^+$ in the SCF SPE procedure

SCF medium (g)	Hg^{2+} amount (µg)	$MeHg^+$ recovery (%)	Hg^{2+} interference (%)
0.1	25	97	0.3
0.2	25	96	<DL
0.1	500	95	1.8
0.2	500	95	0.1

<DL: below the detection limit of the DMA-80

cantly when 25 µg $MeHg^+$ species co-exists with 500 µg Hg^{2+} species.

Species transformation during the SCF SPE procedure

Species transformation was observed from alkyl mercury (both methyl and ethyl) to inorganic mercury when alkyl mercury species were spiked in 1 mol L^{-1} HCl+1 mol L^{-1} NaCl solution with 500 mg kg^{-1} Fe^{3+}. The transformations (decompositions) of 50 µg $MeHg^+$ and 50 µg $EtHg^+$ to Hg^{2+} were observed in 8 h. Approximately 60% $MeHg^+$ and 100% $EtHg^+$ transform to Hg^{2+}, as illustrated in Figs. 3 and 4, respectively. No transformations were observed when alkyl mercury species were spiked in 1 mol L^{-1} HCl+1 mol L^{-1} NaCl solution without 500 mg kg^{-1} Fe^{3+}, or in 500 mg kg^{-1} Fe^{3+} solution without 1 mol L^{-1} HCl+1 mol L^{-1} NaCl. No transformation was observed when alkyl mercury species were spiked in 1 mol L^{-1} HCl+1 mol L^{-1} NaCl solution with 500 mg kg^{-1} Ni^{2+}, Cu^{2+}, Zn^{2+}, Sn^{2+} and Al^{3+} in 8 h. The experimental results suggest that the transformations (decompositions) only happen under strongly acidic conditions with the co-existence of Fe^{3+}. The mechanism of such transformation needs to be further investigated.

To minimize the possible on-column transformation of $MeHg^+$ to Hg^{2+} during SCF SPE elution for $MeHg^+$, Fe^{3+} was removed from the sample extracts before passing through the SPE column. The pH values of the sample extracts were adjusted to 4–6. At this pH, the Fe^{3+} was precipitated as $Fe(OH)_3$ and filtered using a 10 µm filter paper. The removal of the majority of Fe^{3+} was achieved. To minimize potential co-precipitation of $MeHg^+$ species during the filtration, 5 mL 0.1% HCl was tested to rinse the filtered $Fe(OH)_3$ precipitate on the filter paper. The rinse solution was then combined with the filtered solution that contained $MeHg^+$ species. Recovery of 95% of 50 ng $MeHg^+$ was achieved after the filtration of 10 mL 10% ethanol solution with 500 mg kg^{-1} Fe^{3+}, minimizing the possibility of $MeHg^+$ loss during the filtration.

Performance evaluation of the sequential extraction procedure

The ethanol extraction procedure, followed by two-step acid extractions for "semi-mobile" and "non-mobile" inorganic mercury species (as specified in the Experimental section) were evaluated by spike recovery on the soil matrices. The sample was spiked with individual mercury species at 25 µg mercury per gram sample. Each extraction fraction was analyzed by the DMA-80. The results are summarized in Table 9. Satisfactory recoveries for the target mercury species were achieved in each extraction

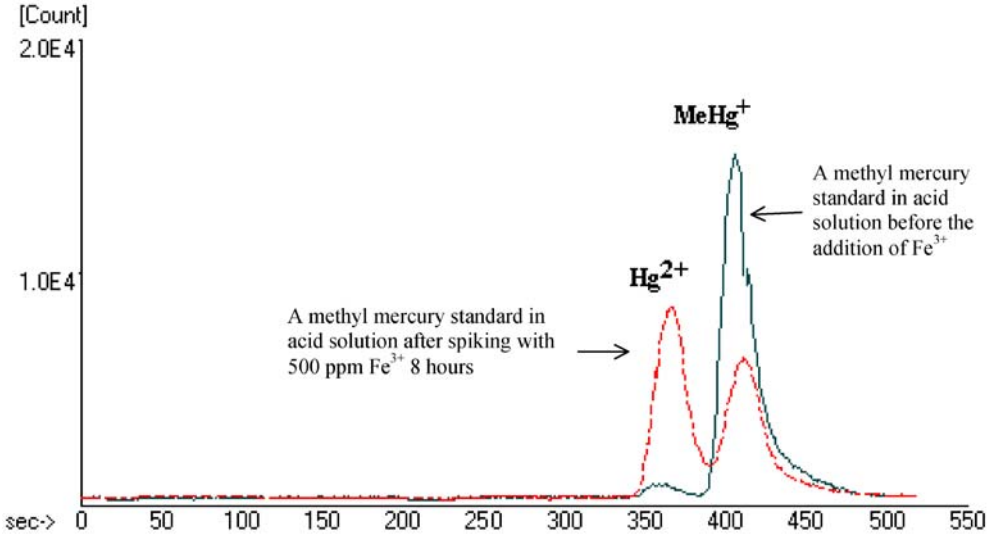

Fig. 3 Methylmercury(II) species transferred to inorganic mercury in 1 mol L^{-1} HCl+1 mol L^{-1} NaCl solution with 500 mg kg^{-1} Fe^{3+} in 8 h

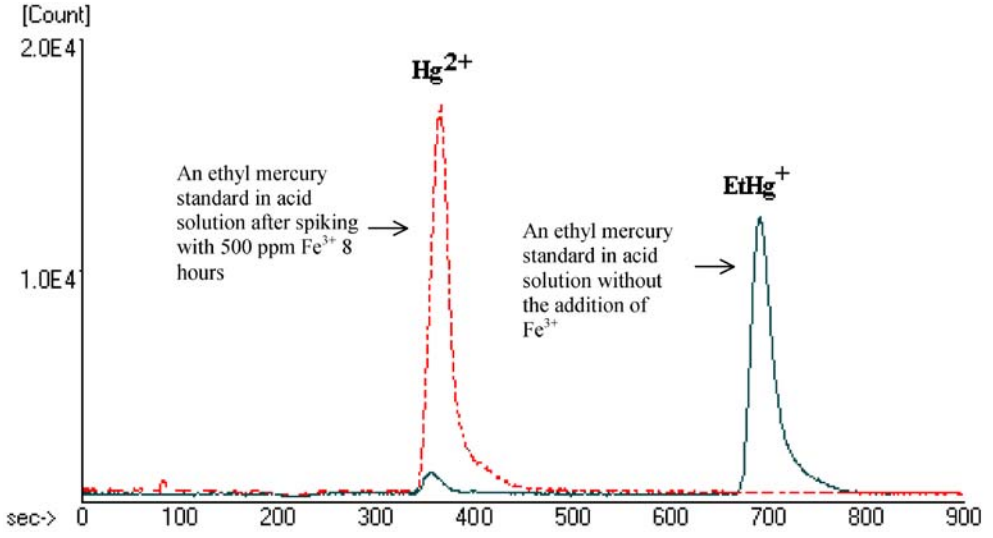

Fig. 4 Ethylmercury(II) species transferred to inorganic mercury in 1 mol L^{-1} HCl+ 1 mol L^{-1} NaCl solution with 500 mg kg^{-1} Fe^{3+} in 8 h

Table 9 Recoveries of mercury species in sequential extractions in soil sample

Species recovery (%)	2% HCl+10% ethanol extraction	1:2 HNO_3:DDI water extraction	1:6:7 HCl:HNO_3:DDI water extraction
$HgCl_2$	97±8	<1	<1
HgO	99±11	4±3	<1
Hg–(Zn)	5±3	94±9	<1
HgS	<1	5±3	97±7

Hg–(Zn): mercury–zinc amalgam
Uncertainties expressed as 95% confidence interval with n=3

step. Sub-speciation of "semi-mobile" and "non-mobile" mercury species by alternative techniques, such as EPA Method 3052 [39] or other acid leaching and digestion procedures [40, 41, 42, 43, 44], may also be appropriate.

Validation of the method

A portion of 1 g BCR CRM 580 Estuarine Soil, which contains 132±3 mg kg^{-1} total mercury and 70.2±3.4 µg kg^{-1} $MeHg^+$ as mercury, was extracted using the 2%HCl+10% ethanol extraction procedure. The inorganic mercury species in the remaining sample residue were measured by the DMA-80 directly. The final results are summarized in Table 10. To minimize inorganic Hg interference in the HPLC–ICP–MS measurement of $MeHg^+$, the SCF SPE procedure was used to pre-separate Hg^{2+} and $MeHg^+$. As indicated in the second row of Table 10, the amount of $MeHg^+$ determined as mercury by this SPE–HPLC–ICP–MS procedure is 73.6±6.3 µg kg^{-1} (95% confidence interval with n=6) which is consistent with the certified value 70.2±3.4 µg kg^{-1}. The inorganic Hg extracted by the ethanol solution is 1.4±0.4 mg kg^{-1} (95% confidence interval with n=3). The total Hg determined by the entire protocol is 134±10 mg kg^{-1}, consistent with the certified value 132± 3 mg kg^{-1}.

Alternatively, after the ethanol extraction, the SCF SPE procedure was applied to separated Hg^{2+} and $MeHg^+$. Then, 1 mol L^{-1} HCl+1 mol L^{-1} NaCl eluate that contains eluted $MeHg^+$ species was neutralized to pH 3, and passed through a second SCF medium for post-concentration. The second SCF medium was directly analyzed by the DMA-80. As indicated in the third row of Table 10, 78±27 µg kg^{-1} $MeHg^+$ was extracted and the total Hg determined is 128±7 mg kg^{-1} (95% confidence interval with n=3). The results are consistent with the certified values and also comparable with the results obtained by the SPE–HPLC–ICP–MS method. The results demonstrate the potential of using the combination of the ethanol extraction with SCF SPE separation and DMA-80 measurement for on-site screening purposes.

Conclusion

A sequential extraction method for mercury speciation in a soil matrix has been established based on the mobility and toxicity of different mercury species. The most mo-

Table 10 Validation of the method using BCR CRM 580 Estuarine Soil

	$MeHg^+$as Hg (µg kg^{-1})	Soluble inorganic Hg (mg kg^{-1})	Non-extractable Hg (mg kg^{-1})	Total Hg (mg kg^{-1})
SPE–HPLC–ICP–MS	73.6±6.3[1]	1.4±0.4[2]	133±9.62[2]	134±10
SPE–SPE–DMA-80	78±27[2]	0.9±0.3[2]	127±7[2]	128±7
Certified	70.2±3.4	N/A	N/A	132±3

[1]Uncertainties expressed as 95% confidence interval with n=6
[2]Uncertainties expressed as 95% confidence interval with n=3

bile and toxic mercury species, including alkyl mercury and soluble inorganic mercury species, are extracted using an acidic ethanol solution. The extracts can be further separated into alkyl mercury and soluble inorganic mercury by SCF SPE–DMA-80 or, into $MeHg^+$, $EtHg^+$ and Hg^{2+} by HPLC–ICP–MS or other analysis procedures. The inorganic mercury remaining after the ethanol extraction can be separated into "semi-mobile" and "non-mobile" fractions by 1:2 HNO_3:DDI water hot acid extraction and 1:6:7 HCl:HNO_3:DDI water hot acid extraction, respectively. The instrumentation involved in the sequential extractions, SCF SPE separation and DMA-80 detection are simple and portable. The procedures of ethanol extraction, SCF SPE separation, and DMA-80 measurement are easy and fast. Therefore, this combination is a promising candidate for on-site screening purposes as well as laboratory quantitation. Future research will be focused on the expansion of such extraction protocols to very different matrices, such as biological tissues and botanical matrices, and the further validation of the developed method [45] by speciated isotope dilution mass spectrometry [46, 47], as well as on-site measurement at mercury-contaminated sites.

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