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Occurrence of monoethylmercury in the Florida Everglades: Identification and verification

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A combination of various analytical techniques and stable isotope tracer experiments confirms monoethylmercury is present in Everglades soil.

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ABSTRACT

A few studies have reported the occurrence of monoethylmercury ($CH_3CH_2Hg^+$) in the natural environment, but further verification is needed due to the lack of direct evidence and/or uncertainty in analytical procedures. Various analytical techniques were employed to verify the occurrence of $CH_3CH_2Hg^+$ in soil of the Florida Everglades. The identity of $CH_3CH_2Hg^+$ in Everglades soil was clarified, for the first time, by GC/MS. The employment of the recently developed aqueous phenylation-purge-and-trap-GC coupled with ICPMS confirmed that the detected $CH_3CH_2Hg^+$ was not a misidentification of CH_3SHg^+. Stable isotope-tracer experiments further indicated that the detected $CH_3CH_2Hg^+$ indeed originated from Everglades soil and was not an analytical artifact. All these evidence clearly confirmed the occurrence of $CH_3CH_2Hg^+$ in Everglades soil, presumably as a consequence of ethylation occurring in this wetland. The prevalence of $CH_3CH_2Hg^+$ in Everglades soil suggests that ethylation could play an important role in the biogeochemical cycling of Hg.

1. Introduction

Mercury is a global pollutant that originates from both natural and anthropogenic sources. Speciation of mercury determines both its toxicity and biogeochemistry (Craig et al., 2003). One of the organomercury species, monomethylmercury (CH_3Hg^+), has been intensively studied due to its prevalence in the environment, high toxicity, and high enrichment factor through food webs (Mason and Benoit, 2003). It has been widely recognized that microbial activities play an important role in the formation and degradation of CH_3Hg^+ in the environment (Mason and Benoit, 2003; Morel et al., 1998). However, little is known about the occurrence of other organomercury species, such as monoethylmercury ($CH_3CH_2Hg^+$), in the natural environment. As a short-chain alkyl mercury species, $CH_3CH_2Hg^+$ is also highly toxic, although its toxicological mechanisms and effects may differ from those of CH_3Hg^+ (Clarkson, 2002; Gerstner and Huff, 1977; Magos, 2001). The lack of $CH_3CH_2Hg^+$ information could be partially attributed to the fact that sensitive and reliable methods for the analysis of trace level $CH_3CH_2Hg^+$ in environmental matrices were not available until recently. The conventional and widely used mercury speciation method, aqueous ethylation followed by purge-and-trap-gas chromatography (GC)-atomic fluorescence spectrometry (AFS) method cannot distinguish $CH_3CH_2Hg^+$ from Hg^{2+} because the ethylation products of both compounds are diethylmercury ($Hg(CH_3CH_2)_2$) (Cai et al., 2000).

In the past half-century, a few studies have reported the occurrence of $CH_3CH_2Hg^+$ in the environment. Using a conventional GC with an electron capture detection (ECD) method, $CH_3CH_2Hg^+$ was detected in fish collected from the polluted Jinzhu River in Japan (Yamanaka and Ueda, 1975) and in the sediment from the polluted areas of St. Clair system (Jerneloev and Wennergren, 1980). By using various isolation/preconcentration methods combing with GC-ECD, Horvat detected $CH_3CH_2Hg^+$ in different biological reference materials, such as mussel, copepod, shrimps and tuna fish, certified by the International Atomic Energy Agency (IAEA) (Horvat, 1991). In all these cases, the detected $CH_3CH_2Hg^+$ was present at mg/Kg or sub mg/Kg levels. Accompanying with the advances in analytical techniques, reports on the finding of $CH_3CH_2Hg^+$ at lower levels appeared. In 1993, a study (Hintelmann and Wilken, 1993), in the course of developing an HPLC-AFS method for organomercury speciation analysis, detected $CH_3CH_2Hg^+$ at μg/Kg level in sediment of a polluted river in Germany. Two years later (Hintelmann et al., 1995), the same group reported the occurrence of $CH_3CH_2Hg^+$ and some other

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organomercury species, such as phenylmercury and methoxyethylmercury compounds, in soils/sediments of some industrially contaminated sites in Germany. In addition to the finding of $CH_3CH_2Hg^+$ in polluted areas, the occurrence of $CH_3CH_2Hg^+$ in some relatively pristine environments was also reported. During the development of a GC-AFS method for mercury speciation analysis, a study (Alli et al., 1994) observed the presence of $CH_3CH_2Hg^+$ in soil of the Florida Everglades. Preliminary study was carried out (Cai et al., 1997) using GC-AFS with two GC columns and different derivatization reactions in an effort to verify the occurrence of $CH_3CH_2Hg^+$ in Everglades soil. Using a similar GC-AFS method, the presence of $CH_3CH_2Hg^+$ in the soils of some Canadian wetlands was reported (Siciliano et al., 2003), and the prevalence of $CH_3CH_2Hg^+$ in Canadian wetlands was revealed in follow-up studies (Holmes and Lean, 2006). In the soil of these wetlands, including the Florida Everglades (Cai et al., 1997) and Canadian wetlands (Holmes and Lean, 2006), $CH_3CH_2Hg^+$ was determined at μg/Kg level, similar to the concentration of CH_3Hg^+ in the corresponding sites.

In the aforementioned studies, $CH_3CH_2Hg^+$ was determined in either polluted (by industry) or relatively natural environment (without apparent anthropogenic pollution sources). If the occurrence of $CH_3CH_2Hg^+$ in the environment is confirmed, the corresponding environmental implications and risk of exposure should be assessed accordingly. As a consequence, our understanding of the biogeochemistry of mercury could be altered. However, uncertainty remains in the above mentioned studies where occurrence of $CH_3CH_2Hg^+$ was reported. First, $CH_3CH_2Hg^+$ was identified according to its retention time on either GC or HPLC columns. It is possible that other compounds bearing the same retention times with $CH_3CH_2Hg^+$ under the same experimental conditions would have been wrongly recognized as $CH_3CH_2Hg^+$. For example, it was found that one sulfur-containing species, CH_3SHg^+, showed the same retention time as $CH_3CH_2Hg^+$ standard when an HPLC-inductively coupled plasma mass spectrometry (ICPMS) method was used for mercury speciation (Wilken et al., 2003). Results are questionable in studies where ECD was used, because ECD is not an element-specific detector. Mass spectra of $CH_3CH_2Hg^+$ present in environmental samples have never been obtained. Second, no solid evidence has confirmed that detected $CH_3CH_2Hg^+$ originated from the environmental samples. The $CH_3CH_2Hg^+$ could, in fact, be an artifact that was formed during sample preparation and/or analytical processes. Therefore, direct and solid evidence is needed to verify the occurrence of $CH_3CH_2Hg^+$ in the environment.

The purpose of this study was to further verify the natural occurrence of $CH_3CH_2Hg^+$ in the soil of the Florida Everglades. The Everglades was selected because it is one of the few wetlands where the presence of $CH_3CH_2Hg^+$ in soil was reported and because extensive mercury data exist for this ecosystem, which could provide us with additional information for future study. Various analytical techniques, including the recently developed aqueous phenylation-purge-and-trap-GC-ICPMS (Mao et al., 2008), HPLC-AFS (Gao et al., 2008), and in particular GC/MS which is able to provide the molecular and structural information, were used to confirm the identity of $CH_3CH_2Hg^+$. Stable isotope tracer experiments were conducted to exclude the possibility of artifact formation of $CH_3CH_2Hg^+$ during sample preparation and/or analytical processes.

2. Experimental section

2.1. Chemicals and materials

All mercury standards were purchased from Ultra Scientific (N. Kingstown, RI, USA). Standard solutions of methylmercury chloride (CH_3HgCl) and ethylmercury chloride (CH_3CH_2HgCl) were prepared by dissolving the standards in methanol. Enriched ^{199}HgO (atomic percentage 91.09 ± 0.05) was purchased from Oak Ridge National Laboratory (Oak Ridge, Tennessee) and dissolved in 10% HCl. Other reagents used were of reagent grade or higher. All the gases used were passed through activated charcoal traps to remove mercury background.

2.2. Instrumentation

Mercury speciation was performed using a modified PerkinElmer AutoSystem XL GC (PerkinElmer) coupled with an Elan DRC-e ICPMS (PerkinElmer) following a procedure reported recently (Mao et al., 2008). Aqueous phenylation reactions were performed in 200 mL glass bubblers purchased from Brooks Rand LLC (Seattle, WA). The traps used for retaining mercury species were deactivated inlet liners (5.8 mm o.d. × 9.2 mm length × 4.0 mm i.d.) for PerkinElmer AutoSystem XL GC. The liners were packed with 0.06–0.08 g of Tenax (60/80 mesh, SUPELCO) and plugged with deactivated glass wool on both ends to hold the Tenax grains in place. The temperatures of injection port and detector of the GC were set at 200 and 250 °C, respectively. A fused-silica capillary column (Rtx®-1, 0.53 mm × 15 m, 1.5-μm film, Restek Corporation, Bellefonte, PA) was used for mercury species separation, and a Rtx®-50 capillary column (0.53 mm × 15 m, film 1.0 μm) was used for purposes of verification. The column temperature was programmed as follows: 50 °C for 1 min, ramped from 50 to 150 °C at a rate of 20 °C/min, maintained at 150 °C for 1 min, ramped to 250 °C at a rate of 20 °C/min, and then maintained at 250 °C for 3 min. A split/splitless injection port was used in splitless mode. The carrier gas was argon at 4 mL/min. Organomercury species eluting from the GC capillary column were converted to elemental Hg via a pyrolysis oven (800 °C). A piece of PFA tubing (0.635 cm i.d. × 150 cm length, NALGENE NUNC, Rochester, NY) operated at room temperature was used to transfer elemental Hg to ICPMS. The nebulizer gas flow for ICPMS was 1.05 L/min and the ICP RF power was1300 W ^{202}Hg and ^{199}Hg were monitored with a dwell time of 25 ms and 5 sweeps/reading.

GC-AFS system used was a capillary GC (Model GC94, Ai Cambridge, UK) coupled to mercury AFS (PSA Merlin) via a pyrolysis oven maintained at 800 °C. Details of the system can be found elsewhere (Mao et al., 2008). Temperature program used was the same as the one described above. Carrier and makeup gases were argon, at flow rates of 4 and 35 mL/min, respectively.

Identification of organomercury species was also accomplished on an HPLC-AFS system. Details of the instrumentation can be found elsewhere (Gao et al., 2008). A C_{18} column (Shimadzu Shimpack CLC-ODS, 6 mm × 150 mm, 5 μm particle size) and a 100 μL sample loop were employed. Isocratic elution was applied with a mobile phase containing 0.1% L-cysteine and 0.03 mol/L of ammonium acetate in water.

GC/MS used for the purpose of verifying the presence of organomercury species was a Hewlett Packard 6890 GC (Agilent) hyphenated to a Hewlett Packard 5973 mass detector. A DB-5MS fused-silica capillary column (0.25 mm × 30 m, 0.25-μm film) was used with the same temperature program as that used for GC-ICPMS analysis. The traps used on GC/MS were inlet liners for HP 6890 GC packed with Tenax and glass wool in the same way as above mentioned. Total mercury was determined by cold vapor atomic fluorescence spectrometry (CVAFS) (Merlin 10.035, PS Analytical, UK) following a procedure reported previously (Liu et al., 2008).

2.3. Sample preparation and analysis

Soil samples were collected at seven locations (Table 1 and Figure SI-1), which have been used in previous studies and extensively characterized in terms of environmental conditions, throughout the Florida Everglades. A significant gradient exists for geochemical parameters such as sulfur and organic carbon among these sites, with an increasing trend from south to north (S1 to S7) (Liu et al., 2008). Samples were collected in polyethylene specimen cups and then doubly bagged with polyethylene Zip-lock bags. The samples were kept on ice in a cooler during transport and were kept in a refrigerator (4 °C) upon arrival in the laboratory.

When GC-ICPMS was used as the detection method, sample preparation and analysis were conducted using the aqueous phenylation followed by purge-and-trap preconcentration procedures reported previously (Mao et al., 2008). Briefly, soil samples were digested with acidic $KBr/CuSO_4$ and extracted with CH_2Cl_2. Organomercury species present in CH_2Cl_2 was back extracted into 1% HCl via volatilization of the CH_2Cl_2, followed by aqueous phase phenylation and purge-and-trap. The traps retaining the phenylation products were inserted into the GC injection port and analyzed by GC-ICPMS. The transient signals recorded by ICPMS were processed by Origin 6.0 (Microcal Software Inc., MA). Quantitation and $^{202}Hg/^{199}Hg$ ratios were calculated based on the peak areas of the corresponding species. Strict quality control and quality assurance procedures were followed during sample analysis as described previously (Liu et al., 2008). This GC-ICPMS technique has a limit of detection (LOD) of 0.001 ng/g for EtHg and acceptable recoveries (75–135%) were obtained for all matrix spikes or certified reference materials (IAEA-405) during analysis.

With respect to HPLC-AFS analysis, 20 g of soil sample were digested with 16 mL of acidic $KBr/CuSO_4$ (3:1, v/v) and extracted with 20 mL of CH_2Cl_2. Organomercury species in the CH_2Cl_2 phase was back extracted into 1.6 mL of NaS_2O_3 (10 mM), which was then analyzed by HPLC-AFS (He et al., 2006). The LOD of this method is 0.014 ng/g for EtHg.

Table 1
$CH_3CH_2Hg^+$ artifact test during sample homogenization, storage, extraction and analysis processes.

Sample ID	Site coordinates		Total Hg (ng/g)	CH_3Hg^+ (ng/g)	$CH_3CH_2Hg^+$ (ng/g)	$^{199}Hg^{2+}$ spike (ng/g)	$^{202}Hg/^{199}Hg$ of $CH_3CH_2Hg^+$	
	Latitude	Longitude					Original	Spiked
S-1	25.35161	−80.65897	27.9 ± 4.20	0.24 ± 0.01	0.36 ± 0.03	40.0	1.76 ± 0.01	1.76 ± 0.02
S-2	25.56223	−80.78036	192 ± 3.60	1.81 ± 0.11	1.76 ± 0.03	333	1.74 ± 0.01	1.76 ± 0.02
S-3	25.75463	−80.74082	91.3 ± 5.23	0.69 ± 0.09	0.87 ± 0.01	100	1.75 ± 0.03	1.76 ± 0.03
S-4	25.79113	−80.75604	204 ± 14.0	0.54 ± 0.04	1.33 ± 0.25	200	1.76 ± 0.01	1.72 ± 0.04
S-5	25.85007	−80.61682	112 ± 33.3	0.41 ± 0.09	0.95 ± 0.09	200	1.73 ± 0.01	1.74 ± 0.01
S-6	26.29352	−80.45990	238 ± 8.35	2.43 ± 0.18	0.23 ± 0.00	200	1.74 ± 0.01	1.73 ± 0.03
S-7	26.48657	−80.32231	132 ± 6.08	1.68 ± 0.10	0.45 ± 0.03	250	1.74 ± 0.01	1.72 ± 0.04

Note: (1) For all the samples, $n = 3$; (2) All the concentrations were calculated on dry weight basis.

CH_3SHg^+ was synthesized by mixing aqueous solution of CH_3SNa with aqueous solutions of Hg^{2+} (Wilken et al., 2003). Briefly, 50 μL of CH_3SNa (30 mM in degassed deionized water) were added into 10 mL of Hg^{2+} (0.15 mM in degased deionized water). After shaking for 10 min, the product obtained was immediately analyzed by HPLC-AFS after appropriate dilution. With respect to GC/MS analysis, CH_3SHg^+ synthesized (10 mL) was phenylated by adding 1 mL of $NaB(C_6H_5)_4$ (1%) and 2 mL citric buffer (pH = 5.0), followed by extraction of the product, $CH_3SHgC_6H_5$, into 2 mL of hexane.

In order to obtain reliable mass spectra of the organomercury species (CH_3Hg^+ and $CH_3CH_2Hg^+$) present in soil samples, a multi-step sample extraction, preconcentration, and cleanup method was developed and employed prior to GC/MS analysis. In addition to the acidic $KBr/CuSO_4$ digestion/CH_2Cl_2 extraction steps, 10 mM NaS_2O_3 was used for cleanup purpose (Cai et al., 1997) and solid phase extraction (SPE) with sulfhydryl cotton fibers (SCF) was carried out to enrich the organomercury species (Cai et al., 1996). SCF were synthesized following the procedures used by Lee and Mowrer (Lee and Mowrer, 1989). The SCF column used in this experiment was a piece of PFA tubing (0.635 cm i.d., 10 cm length) packed with 0.5 g of SCF. Details of the operation procedures can be found in Supporting Information (Figure SI-2). The LOD of this multi-step preconcentration GC/MS method for EtHg can reach 0.02 ng/g.

3. Results and discussion

3.1. Identification of $CH_3CH_2Hg^+$ in soil of the Florida Everglades

Phenylated mercury species were preliminarily identified according to their retention times on GC columns. Fig. 1A illustrates GC-ICPMS chromatograms of mercury standards, a real soil sample collected from the Florida Everglades and a method blank obtained with an Rtx®-1 column. Similar to the standard, the sample chromatogram shows two major peaks. The peak 2 on the chromatogram derived from the real sample showed exactly the same retention time as $CH_3CH_2Hg^+$ in the standard, suggesting the possible existence of $CH_3CH_2Hg^+$ in the sample. Meanwhile, the absence of peak 2 from the chromatogram of the method blank indicates that it was not from the reagents used in sample preparation and analysis. Similar results were obtained using an Rtx®-50 column (Fig. 1B). Organomercury species derived from the Everglades soil were further identified by HPLC-AFS analysis. On the chromatogram of the real sample (Fig. 2A), a peak bearing the similar retention time as that of $CH_3CH_2Hg^+$ standard (peak 3 in Fig. 2B) can be reasonably ascribed to $CH_3CH_2Hg^+$.

It was reported that another organomercury species, CH_3SHg^+, was eluted from the column at the same retention time as $CH_3CH_2Hg^+$ standard when an HPLC-ICPMS system was used (Wilken et al., 2003). On the chromatograms obtained with our HPLC-AFS system, CH_3SHg^+ (peak 4 in Fig. 2C) appeared at the same retention time with $CH_3CH_2Hg^+$ standard (peak 3 in Fig. 2B), in agreement with the report by Wilken et al. (Wilken et al., 2003), although the mobile phases used in these two studies were different. The synthetic yield of CH_3SHg^+ was very low, as indicated by the presence of a huge Hg^{2+} peak on the HPLC chromatogram (peak 1 in Fig. 2C). Bearing a similar retention time with $CH_3CH_2Hg^+$ on HPLC column, CH_3SHg^+, if present in the samples, would interfere with HPLC analysis of $CH_3CH_2Hg^+$.

In order to exam whether CH_3SHg^+ interferes with $CH_3CH_2Hg^+$ analysis by phenylation-GC-ICPMS or GC-AFS methods, the synthesized CH_3SHg^+ was phenylated using $NaB(C_6H_5)_4$, followed by extraction of the products into hexanes. The identity of $CH_3SHgC_6H_5$ was confirmed by GC/MS spectra (Figure SI-3). On the total ion count (TIC) chromatogram of GC/MS, $CH_3SHgC_6H_5$ showed

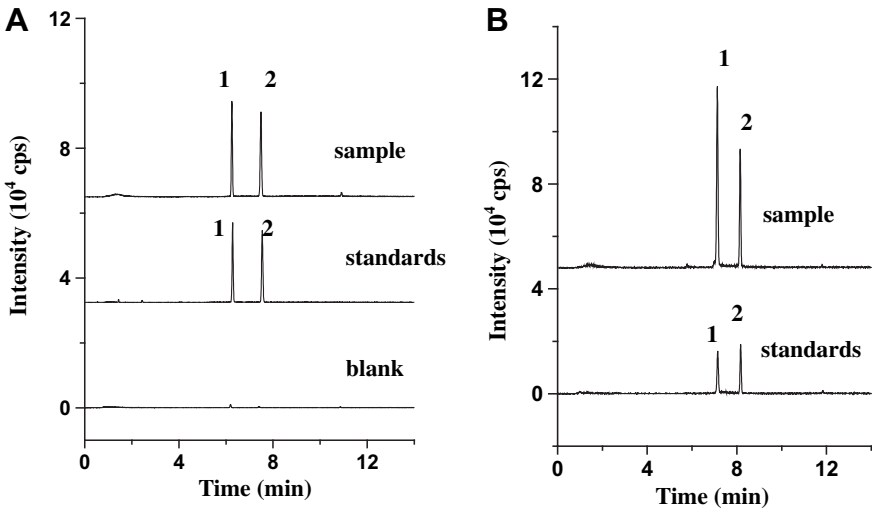

Fig. 1. GC-ICPMS chromatograms of organomercury standards and the Everglades soil sample after aqueous phenylation-purge-and-trap with the use of an Rtx®-1 (A) and an Rtx®-50 (B) capillary column. Peaks identification: 1, $CH_3HgC_6H_5$; 2, $CH_3CH_2HgC_6H_5$.

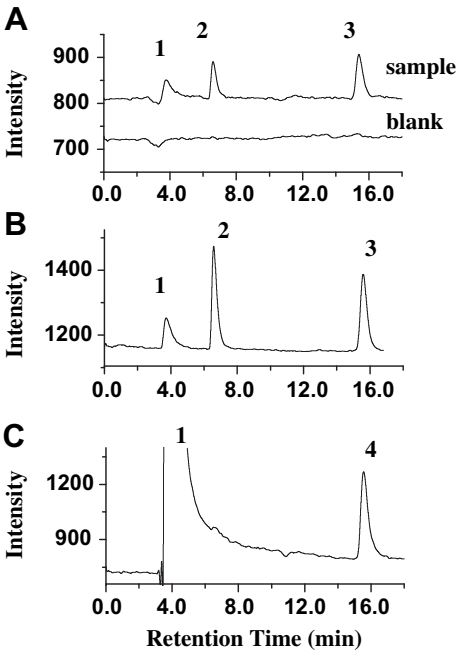

Fig. 2. HPLC-AFS chromatograms of the Everglades soil sample and method blank (A), CH_3Hg^+ and $CH_3CH_2Hg^+$ standards (B), and CH_3SHg^+ synthesized (C). Peaks identification: 1, Hg^{2+}; 2, CH_3Hg^+; 3, $CH_3CH_2Hg^+$; 4, CH_3SHg^+.

a retention time of 16.8 min, longer than the retention time of $CH_3CH_2HgC_6H_5$ by 2 min (Figure SI-3). The synthesized $CH_3SHgC_6H_5$ was further analyzed by both GC-AFS and GC-ICPMS. Despite the difference in design of the injection ports and interfaces of the two systems (Mao et al., 2008), $CH_3SHgC_6H_5$ (peak 4 in Fig. 3) showed a longer retention time than $CH_3CH_2HgC_6H_5$ (peak 3 in Fig. 3) on both systems. These results demonstrate that CH_3SHg^+ should not interfere with the analysis of $CH_3CH_2Hg^+$ when the phenylation-GC-ICPMS or GC-AFS method is used. In addition, this $CH_3SHgC_6H_5$ peak has never been observed in the analysis of real samples.

To confirm the detection of $CH_3CH_2Hg^+$ derived from real samples, a GC/MS analysis was performed. Because of the poor detection limit of GC/MS, organomercury species, which are generally present at ultra trace levels in the environment, should be highly enriched prior to GC/MS analysis. It was estimated that an approximate 1000-fold enrichment is required to obtain a reliable MS signal from soil samples of the relatively pristine Florida Everglades ecosystem. Additionally, matrix interference is a problem as MS acts as a universal detector, especially in cases of analyses requiring high sample preconcentration factors. Therefore, extensive cleanup steps must be utilized to minimize matrix interference. In this study, SPE was selected as the enrichment technique, where SCF was used as the adsorbent because of its high enrichment capacity and affinity to organomercury species (Cai et al., 1996; Celo et al., 2004; Lee and Mowrer, 1989).

After the multi-step extraction/cleanup procedures (Figure SI-2), phenylated organomercury species retained on the Tenax traps were analyzed by GC/MS under scan mode. As shown in the TIC chromatogram (Fig. 4A), the matrix was still complex. However, the mass spectra of both $CH_3HgC_6H_5$ and $CH_3CH_2HgC_6H_5$ were clearly identified at the retention times of the corresponding mercury standards (Fig. 4C–F). The similar isotopic patterns and spectra of the sample and standards strongly suggest that the two mercury species detected in the soil of the Florida Everglades were CH_3Hg^+ and $CH_3CH_2Hg^+$. This multi-step extraction/cleanup and GC/MS confirmation experiment was successfully repeated using another sample from the Everglades and a different GC temperature program (data not shown). The method blank was tested using the same procedures (Fig. 4B). Characteristic spectra and isotopic patterns of $CH_3HgC_6H_5$ and $CH_3CH_2HgC_6H_5$ were absent from the chromatogram of the method blank (Figure SI-4), excluding the possibility that the detected CH_3Hg^+ and $CH_3CH_2Hg^+$ originated from either chemical reagents or analytical processes. The TIC chromatogram of the method blank was similar to that of the sample, indicating that the matrix interference on GC/MS should be primarily attributed to the chemical reagents used during the extraction/cleanup and analytical processes. The matrix interference from soil sample was successfully eliminated by the sample pretreatment and cleanup steps. To the best of our knowledge, this is the first time that GC/MS spectra of $CH_3CH_2Hg^+$ present in soil samples from a natural (i.e., not contaminated) environment were obtained.

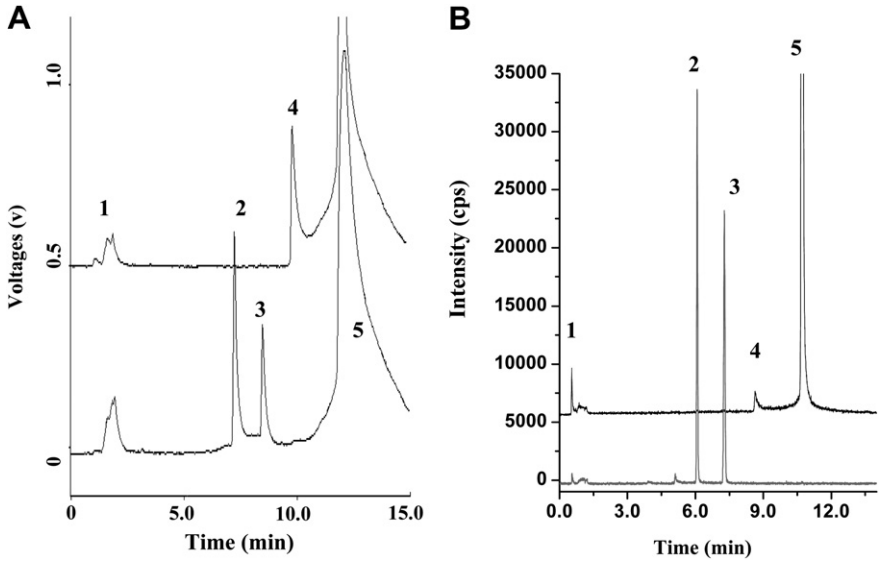

Fig. 3. Chromatograms of phenylated CH_3SHg^+ (chromatograms on the top) and mixture of CH_3Hg^+ and $CH_3CH_2Hg^+$ standards (chromatograms on the bottom) obtained with GC-AFS (A) and GC-ICPMS (B). Peaks identification: 1, Hg^0; 2, $CH_3HgC_6H_5$; 3, $CH_3CH_2HgC_6H_5$; 4, $CH_3SHgC_6H_5$; 5, $Hg(C_6H_5)_2$.

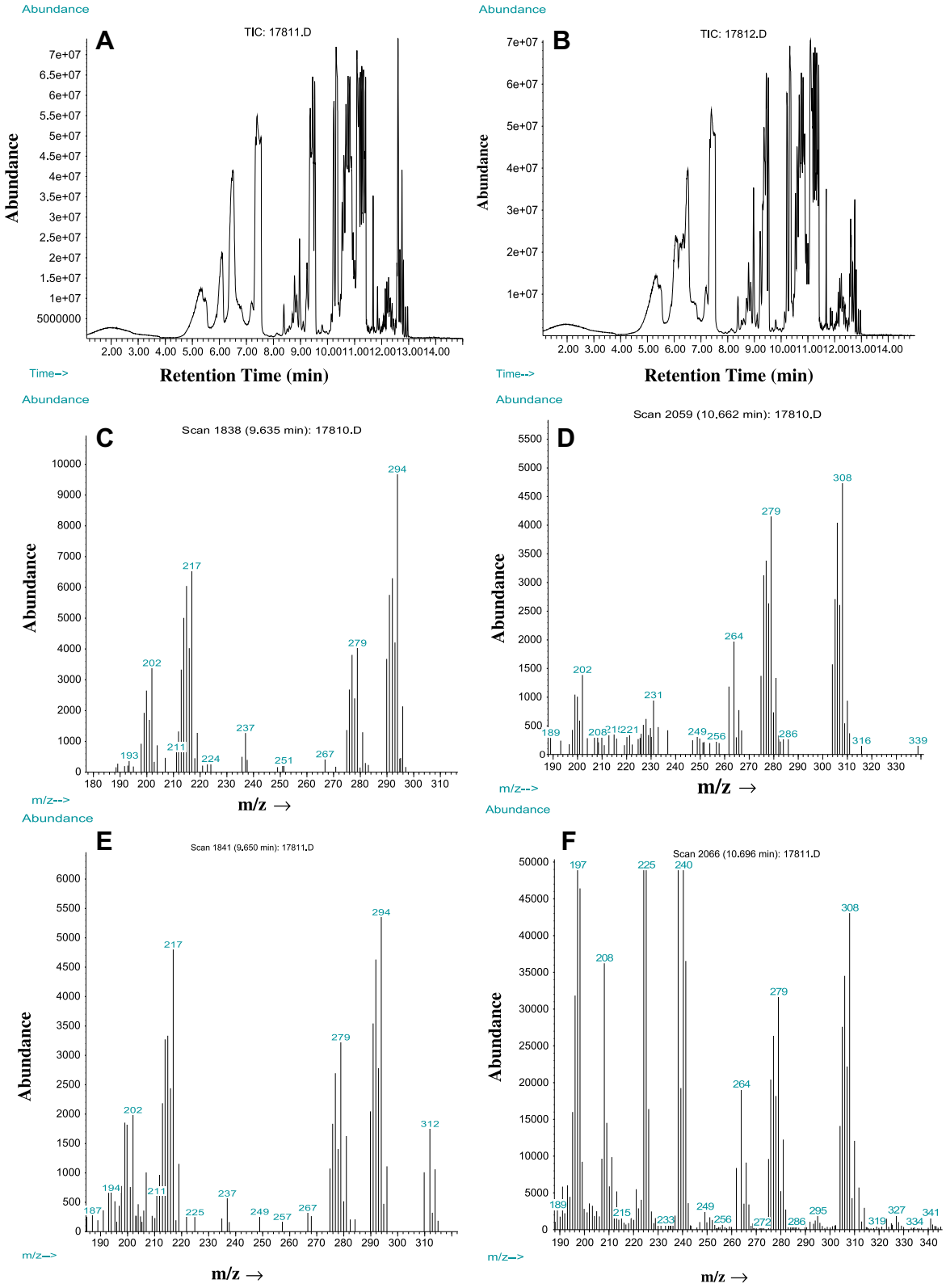

Fig. 4. TIC chromatograms of a real soil sample of Florida Everglades (A) and of a method blank (B); spectra of $CH_3HgC_6H_5$ (C) and $CH_3CH_2HgC_6H_5$ (D) of the corresponding standards, and spectra of $CH_3HgC_6H_5$ (E) and $CH_3CH_2HgC_6H_5$ (F) of a real sample obtained with phenylation-purge-and-trap-GC/MS. MS molecular/fragmental ions identification: m/z = 308, $CH_3CH_2HgC_6H_5^{+\cdot}$; 279, $C_6H_5Hg^+$; 231, $CH_3CH_2Hg^+$; 294, $CH_3HgC_6H_5^{+\cdot}$; and 217, CH_3Hg^+.

3.2. Analytical artifact test

Formation of CH_3Hg^+ artifact during different sample preparation and analysis processes has been a concern for mercury speciation in real environmental samples (Falter, 1999a,b; Hintelmann, 1999; Hintelmann et al., 1997). In this study, the possibility of artifact formation of $CH_3CH_2Hg^+$ during the sample extraction and analysis was examined using a stable isotope tracer technique.

Fresh soil samples were collected from different areas of the Florida Everglades (Table 1). Most of the sampling sites were covered with water of different depths (2–50 cm) and thick sawgrass. Only station S-1 was under dry conditions with less sawgrass. Total Hg concentrations were in the range of 27.9–239 ng/g, while CH_3Hg^+ was present at approximately 0.24–2.43 ng/g levels. $CH_3CH_2Hg^+$ was detected in all samples at levels similar to those of CH_3Hg^+. Aliquots of the fresh samples were spiked with enriched ^{199}Hg immediately upon arrival in the lab and then homogenized. After refrigerated storage (4 °C) for 24 h, the original and spiked samples were digested and extracted using the protocol for GC-ICPMS analysis. After phenylation and purge-and-trap preconcentration, the $^{202}Hg/^{199}Hg$ ratios of $CH_3CH_2Hg^+$ were measured with GC-ICPMS. The logic is that if artifact formation occurred during the sample preparation/analysis processes, the $^{202}Hg/^{199}Hg$ ratios of these organomercury species would decrease. As shown in Table 1, $^{202}Hg/^{199}Hg$ ratio of $CH_3CH_2Hg^+$ did not change significantly (one-tail t test, $P > 0.1$) between the original samples and those enriched with ^{199}Hg, indicating that the detected $CH_3CH_2Hg^+$ originated from the soil samples.

Based on the evidence described above, it can be concluded that the presence of $CH_3CH_2Hg^+$ in the soil of the Florida Everglades was confirmed. Different from $CH_3CH_2Hg^+$ observed in the polluted areas (Hintelmann et al., 1995; Jerneloev and Wennergren, 1980), no obvious natural or anthropogenic source appears in adequate amounts to account for the presence of $CH_3CH_2Hg^+$ in the Florida Everglades (Cai et al., 1997). The prevalence of $CH_3CH_2Hg^+$ in the Florida Everglades indicates that this organomercury species most probably occurs widely in the environment, although not commonly reported in the literature. Again the lack of information on $CH_3CH_2Hg^+$ occurrence in the environment is likely due to the absence of adequate analytical methods. In the Everglades soil analyzed in this study, $CH_3CH_2Hg^+$ and CH_3Hg^+ accounted for 0.10–1.30 and 0.26–1.27% of the total mercury concentrations respectively. The similarity between levels of $CH_3CH_2Hg^+$ and CH_3Hg^+ reveals that ethylation of Hg could be an important transformation pathway of Hg. Unlike CH_3Hg^+, $CH_3CH_2Hg^+$ was not detectable in other matrices of the Florida Everglades, such as water and periphyton using the sensitive aqueous phenylation-purge-and-trap-GC-ICPMS, indicating that $CH_3CH_2Hg^+$ could present in these matrices at much lower levels than CH_3Hg^+. The obvious differences in their occurrence in the Everglades warrant further studies in terms of the environmental fate and transport of $CH_3CH_2Hg^+$.

4. Conclusions

Lines of evidence, including direct verification of molecular identity of $CH_3CH_2Hg^+$ by GC–MS and elimination of analytical artifact by isotope-tracer experiments, confirm that $CH_3CH_2Hg^+$ indeed occurs in Everglades soil. $CH_3CH_2Hg^+$ is present, at ng/g levels that are similar to those of CH_3Hg^+, in all soil samples collected at different locations throughout the Everglades. Since there are no known sources that discharge $CH_3CH_2Hg^+$ to the Everglades, the occurrence of $CH_3CH_2Hg^+$ in Everglades soil is presumably a consequence of ethylation processes naturally occurring in this wetland. The prevalence of $CH_3CH_2Hg^+$ in Everglades soil warrants further investigation on Hg ethylation process and its role in the biogeochemical cycling of Hg.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envpol.2010.07.031.

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