TO: A. Weissbach, J. Killian (Wisconsin DNR)
FROM: J. Aldstadt (UW-Milwaukee / Chemistry)
DATE: December 10, 2003
SUBJECT: Survey of Organoarsenic Speciation at Kewaunee Marsh

Summary

Sampling was conducted on two occasions at the Kewaumee Marsh in July and September 2003. Grab samples were studied using solid-phase microextraction gas chromatography and inductively-coupled mass spectrometry. Aqueous surface and monitoring well samples showed appreciable levels of monomethyl arsonic acid (MMAA), low ppb levels of dimethyl arsinic acid, and the absence of organoarsines. The concentration data for MMAA is semi-quantitative in that an unknown interference complicated the interpretation of the MMAA mass spectra. Solid samples (peat) showed only trace levels of MMAA, with the ppm-levels apparently dominated by the presence of inorganic arsenicals. It thus appears that MMAA is the only organoarsenical found at appreciable levels and it does not appear to be associating significantly with solid material to possibly mitigate its transport toward the Kewaunee River.

Introduction.

The objective of this work was to conduct an initial survey of the Kewaunee Marsh to define the speciation of organoarsenicals. We studied the possible presence of volatile and non-volatile organoarsenicals, as well as the association of arsenicals with particulate matter. Volatile organoarsenicals are the methylated derivatives of trivalent arsenious acid, such as monomethyl arsine (MMA), dimethyl arsine (DMA), and trimethyl arsine (TMA).[1] These compounds have been shown to

[1] Contrary to popular usage, we use the abbreviation "DMAA" for dimethylarsinic acid (in the literature, usually just "DMA") and "MMAA" for monomethyl arsonic acid (in the literature, "MMA") to distinguish these non-volatile organoarsenicals from their volatile counterparts (MMA – monomethylarsine and DMA – dimethylarsine). The reason for this practice is that we measure both organoarsines as well as non-volatile organoarsenicals; methods for the arsines are not commonly employed by other investigators.

be formed by microbial action. As a class, the organoarsines are highly toxic; however, they are also unstable in aerobic environments. For non-volatile organoarsenicals, the alkylated (usually methylated) derivatives of pentavalent arsenic acid are commonly found in the environment. These include monomethyl arsonic acid (MMAA) and dimethylarsinic acid (DMAA).[2] These species, until recently, were thought to be relatively non-toxic to humans – however, recent toxicological reports indicate that MMAA and DMAA may be toxic once ingested.

To determine the possible presence of these types of compounds at the Kewaunee Marsh, samples were collected on July 8th and September 30th at the locations shown in Figure 1. The Sampling Plan that was followed is shown in Appendix A. We applied methods developed in our laboratory based on solid-phase microextraction (SPME) gas chromatography (GC) with mass spectrometric (MS) and pulsed flame photometric (PFP) detection for organoarsenic determination (Appendix B), and a method based on inductively-coupled plasma mass spectrometry (ICP-MS) for inorganic (total) arsenic determination (Appendix B).[3]

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[2] (a) W.R. Cullen and K.J. Reimer, "Arsenic Speciation in the Environment" *Chemical Reviews* **1989**, *89*, 713.

(b) J. Nriagu, *Arsenic in the Environment: Cycling and Characterization*, Advances in Environmental Science and Technology, No. 26, John Wiley & Sons, New York, 1994.

[3] (a) B. Szostek, J.H. Aldstadt. "Determination of Organoarsenicals in the Environment by Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry" *Journal of Chromatography* **1998**, *807A*, 253.

(b) D.R. Killelea, J.H. Aldstadt. "A Solid-Phase Microextraction Method for Gas Chromatography with Mass Spectrometric and Pulsed Flame Photometric Detection: Studies of Organoarsenical Speciation" *Journal of Chromatography* **2001**, *918A*, 169.

(c) D.R. Killelea, J.H. Aldstadt. "Determination of Arsines in Freshwater Sediment Near a Former Herbicide Factory by Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry" *Chemosphere* **2002**, *48*, 1003.

(d) A.R. Roerdkink, J.H. Aldstadt. "A Solid-Phase Extraction Multi-Detector Gas Chromatographic Method for the Sensitive Determination of Feed Additive Arsenicals in Natural Waters", in preparation.

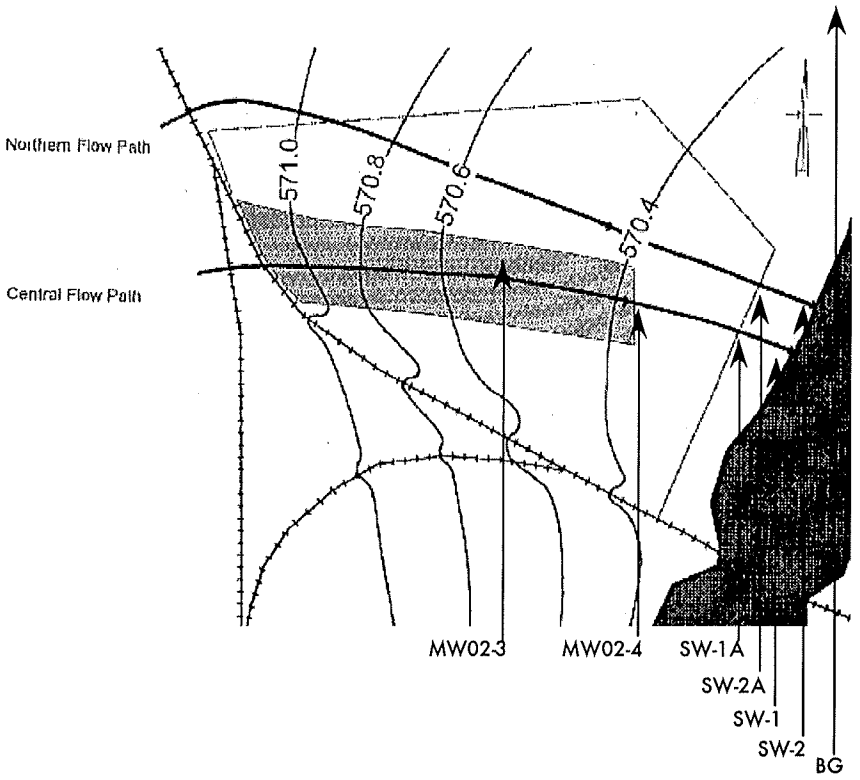


Figure 1. Locations of sampling sites at the Kewaunee Marsh. "MW" are monitoring wells; "SW" are surface waters; "BG" is the location where a background surface water sample was collected.

Results & Discussion

Sampling Trip #1. Table I shows the sample results that were obtained. Work to determine the presence of volatile organoarsenicals was undertaken upon return to the laboratory (< 7 day holding time). Sample containers were stored at 4°C and the caps were punctured immediately prior to SPME sampling. Volatile organoarsenicals were not detected (limit of detection < 1 µg/L, parts-per-billion) in these samples. The only volatiles detected were found in samples taken in the slough samples *and* in the background sample (i.e., adjacent to the river) – these appeared to be petroleum-derived compounds (e.g., toluene), perhaps the result of motor-boat pollution. These compounds did not produce a response on the GC-PFPD (i.e., arsenic-selective detector), so they were not studied further.

Two non-volatile organoarsenicals were found – primarily in the slough samples (SW-1, SW-2): monomethylarsonic acid (MMAA) was identified at appreciable concentrations (approx. 50-300 ppb) and dimethylarsinic acid (DMAA) was identified at much lower concentration (< 50 ppb) levels. Both compounds were

also observed at levels above background in the "intermediate depth" sample from monitoring well #2 (Table I, Figure 2).

Quantitation was performed using a linear calibration model using phenylarsonic acid (PAA) as internal standard. The first-order calibration model for DMAA (0-100 ppb, four levels) was $y = 2.36 \times 10^{-3} x + 4.80 \times 10^{-2}$ with a coefficient of determination (R^2) of 0.999 and standard error of the estimate (in y) 6.0×10^{-3}. The first-order calibration model for MMAA (0-100 ppb, four levels) was $y = 1.55 \times 10^{-3} x + 4.99 \times 10^{-2}$ with a coefficient of determination (R^2) of 0.999 and standard error of the estimate (in y) of 5.9×10^{-4}. The detection limit for both methods (3σ) was approximately 0.50 ppb. Quality control samples (air and liquid travel blanks) did not show the presence of incidental contamination. Examples of GC-MS results for MMAA and DMAA are found in the Appendix.

A complication to our quantitation approach was observed. A "matrix effect" was evident with the slough samples – that is, those apparently containing the highest concentrations of organoarsenical. We observed that upon addition of the derivatizing reagent (1,3-propanedithiol, PDT), a white precipitate readily formed. This caused the deterioration of the SPME fiber coating upon repeated use, an effect that we had not observed previously. The variability in measuring organoarsenicals in the slough samples was consequently very high (>>25% RSD) compared to the typical variability we observe with other surface water samples using the SPME GC method (<10% RSD). We examined several possibilities (e.g., Zn ion forming ZnS precipitate), but could not repeat the phenomenon.

Because SPME is an *equilibrium* extraction technique, the extent to which the formation of the white precipitate altered the quantitation of organoarsenicals is not clear and needs further investigation. Specifically, in the SPME technique the analyte is distributed between the aqueous phase and the SPME fiber phase (modified polydimethylsiloxane). The equilibrium distribution can be described by a partition coefficient (i.e., a temperature-dependent equilibrium coefficient):

$$(R\text{-}As)_{aq} \rightarrow (R\text{-}As)_{PDMS}$$

$$\text{where } K_{eq} = (R\text{-}As)_{PDMS} / (R\text{-}As)_{aq}$$

Thus quantitation using aqueous standards will create a model that is different from that obtained if authentic (uncontaminated) matrix samples are used. If the matrix changes, however, the phase distribution will change as well. Although qualitatively the MMAA and DMAA were clearly present, the reported MMAA and DMAA quantitative information for the slough samples is less certain. A reasonable interpretation of our results could be: "Like at the monitoring well, the slough concentrations are actually on the order of just 20 ppb or so – the matrix effect in the slough enhanced partitioning of these compounds into the fiber phase". Note that the

use of the method of standard addition (MSA) as an alternative to the calibration procedure did not correct for the observed matrix effects in the slough samples.

Sample Location	Description	[MMAA], ppb	95% CI*	[DMAA], ppb	95% CI*	
SW3 (vicinity)	background	6.07	7.47	ND		
SW-2F	slough, near fence	158	33.5	3.74	1.54	**
SW-2	slough, middle	343	367	19.9	3.03	**
SW-2R	slough, near river	168	57.8	27.4	71.7	**
MW02-3	well #3, shallow	2.79	3.05	ND		
MW02-4	well #4, shallow	4.65	4.10	ND		
MW02-4i	well #4, intermed.	27.9	14.0	ND		
MW02-4d	well #4, deep	8.05	6.80	ND		

* 95% confidence intervals calculated using Student's t-value for a two-tailed distribution, n=3

** unusually high variability observed in these samples – a white precipitate formed upon addition of dithiol

Table I. Summary of results obtained during Sampling Trip #1 at the Kewaunee Marsh.

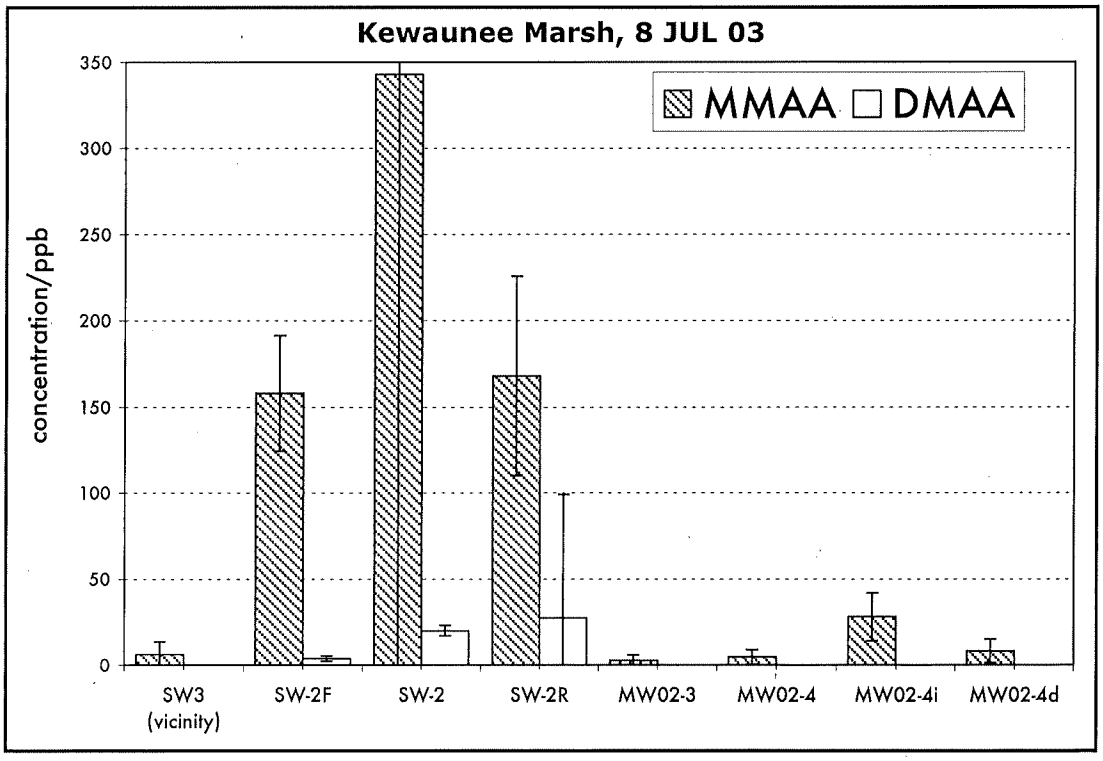


Figure 2. Graphical representation of results obtained for MMAA and DMAA in the Kewaunee Marsh samples. Confidence intervals are calculated using Student's t-value (two-tailed distribution, n=3).

Sampling Trip #2. Based on the results of Trip #1, for the second sampling trip we sampled at two types of locations: (a) monitoring well samples just west of the slough to determine the vertical distribution of organoarsenicals; and (b) solid (i.e., peat) samples along the slough to determine if organoarsenicals are associated with this material. Table II shows the sample results that were obtained. For organoarsenicals, traces of MMAA were observed in the monitoring well at two depths. For the "intermediate" sample, we again observed the formation of a white precipitate and consequent deterioration of the SPME fiber. DMAA was not observed, i.e., it was below the limit of detection for the method. For the "grab" samples of peat at S2, extraction with reagent water yielded a slightly higher concentration of MMAA – though still very low (< 20 ppb) in both the surface and deeper (~0.5 m) peat samples. We then subjected the surface peat sample to "mild" (10 mM HCl) and "harsh" (6 M HNO_3) extractions. These extracts were analyzed by ICP-MS, so "total arsenic" concentrations could be measured. The levels observed were high, as shown in previous investigations of this region of the marsh. It thus appears that inorganic arsenic (arsenate, arsenite) are the major species that are adsorbed onto the solid material.

Sample Location	Description	[MMAA], ppb	95% CI*	
MW02-5	well #5, shallow	6.00	1.08	**
MW02-5i	well #5, intermed.	6.08	31.1	**
S2 Surface - raw	surface peat	18.3	37.6	**
S2 Deep - raw	surface peat	15.1	46.2	**

Sample Location	Description	[total As], ppb	95% CI*	
MW02-5	well #5, shallow	606	125	
MW02-5i	well #5, intermed.	3.10	6.82	
S2 Surface - 10.0 mM HCl	surface peat, ext'd	1.26E+03	0.898	
S2 Surface - 6 M HNO3	surface peat, ext'd	8.89E+03	0.989	

* 95% confidence intervals calculated using Student's t-value for a two-tailed distribution, n=2
** DMAA was not detected in these samples

Table II. Summary of results obtained during Sampling Trip #2 at the Kewaunee Marsh.

Suggestions for Further Work

The questions raised by the analytical results from this study include:

1. Why do certain samples cause deterioration of the SPME fiber?
2. What is the chemical speciation of arsenic in other areas of the marsh (horizontally and vertically, aqueous and solid material)?
3. How does the dynamic nature of the marsh environment affect the abundance and distribution of the arsenicals? To what extent are chemical transformations (e.g., redox reactions) occurring?
4. How far have mobile species such as arsenite and MMAA been transported? Is the arsenate completely adsorbed to iron oxide minerals?
5. What other arsenic species are associated with particulate material? How are different arsenic species distributed as a function of particle size? What are the ramifications of this distribution for sample pre-treatment (e.g., filter porosity) as well as for transport and fate?

Appendix A. Sampling Plan.

Chemical Speciation of Organoarsenicals in Kewaunee Marsh: Sampling Plan

Prepared by J. Aldstadt, Chemistry Dept., Univ. Wisconsin-Milwaukee

Submitted June 13, 2003

1.0 OBJECTIVE

To conduct an initial survey of the Kewaunee Marsh to define the chemical speciation of organoarsenic.

2.0 APPROACH

2.1 Sample Collection

When dealing with the speciation of arsenicals in natural systems, many factors can affect the data quality. These include: sample container type, filtration method, acidification method, presence of light, presence of dissolved oxygen, sample storage temperature, and sample holding time. Based on our experience, the following conditions are preferred:

- Samples are stored in opaque high-density polyethylene containers
- To preserve the physical integrity of the sample, filtration (e.g., 0.45 μm) is not performed
- Preservation is done by using a non-oxidizing acid (e.g., 0.1 M HCl)
- Samples are not de-gassed because volatile organoarsenicals will be purged
- Samples are transported off-site at 4 °C until determination
- The holding time for volatile arsenicals should not exceed 7 days; holding time for ionic species should not exceed 30 days

2.2 Organoarsenic Determination

We will study various sample types, e.g., surface & pore waters and/or extracts of solids (peat & sediment). The methods we will use are:

Class	*Examples*	*Methods*
Non-volatile organics	Alkylated oxyacids, e.g., $CH_3AsO(OH)_2$, $(CH_3)_2AsOOH$	Solid-Phase Microextraction (SPME); GC-MS, -PFPD
Volatile organics	Alkylated arsines, e.g., CH_3AsH_2, $(CH_3)_2AsH$	Purge & Trap; SPME-GC-MS, GC-PFPD

2.3 Quality Control

Travel (liquid) blank: a sample container will be filled with 0.1 M HCl and sealed; this sample will not be opened until it is returned to the lab.

Travel (air) blank: a sample container will be filled with 0.1 M HCl and sealed; this sample will be uncapped during the on-site work, then re-capped at the end of the day and returned to the lab.

Matrix (recovery) spike: A phenylarsonic acid (PAA) standard (50.0 µg/L) will be added to each sample.

3.0 LOGISTICS

Work will be conducted on Tuesday July 8th (tentatively from 10 am to 3 pm). Joe Aldstadt and two graduate students (Aaron Roerdink and Jessica Ammerman) will perform sample collection at six locations identified by the DNR.

The following will be brought on-site by UWM. Additional personal protective gear will be provided by the sponsor.

Equipment

- HDPE bottles, 250 mL, wide-mouth (18)
- Container labels (24)
- Auto-Pipettes (various)
- Battery-operated balance (volume by mass)
- Syringes (12)
- Zip-Lok bags (box)
- Kimwipes (box)
- Parafilm & scissors (ea)
- Silicone rubber gloves (9 pr)
- Safety goggles (3)
- Ice chest (1)

Reagents

Hydrochloric acid, Optima-grade, 0.10 M (1 L)
Quality control standards (various)

NOTE 1: Sample containers will be soaked in 5% (v/v) nitric acid (AR-grade) and rinsed with high-purity reagent water (18 MW-cm) beforehand.

NOTE 2: To minimize contamination, samples will be collected using the "clean-hands/dirty-hands" protocol.

NOTE 3: Any waste generated during the test (e.g., acid washes) will be transported back to UWM for disposal in accordance with ES&H policies.

Appendix B. Experimental Methods.

Reagents

All reagents used were analytical reagent grade (AR) or better. Reagent water (18 MΩ-cm) was prepared by passing house deionized water through a Barnstead NanoPure™ filtration system (Dubuque, IA, USA) equipped with an ultraviolet lamp (deuterium, 14 watts). Stock standards (100mg/L) were stored in opaque high-density polyethylene (HDPE) bottles in the dark at 4 C. Standards less than 1mg/L were made on the day of use. All glassware and plasticware were washed and soaked for at least 36 hours in 5%(v/v) nitric acid (AR grade, Fisher Scientific, Pittsburgh, PA, USA) followed by copious rinsing with reagent water before use.

The derivatizing reagent used to volatilize the organoarsenicals was 1,3-propanedithiol (PDT) (Aldrich, Milwaukee, WI, USA). Standard solutions of MMAA (synthesized in-house), DMAA (98%, Sigma, St. Louis, MO, USA), and PAA (98%, Aldrich) were prepared by dilution of the appropriate amount of solid to achieve a solution with a concentration of 1000 mg/L of As. A 10.0 mM hydrochloric acid (TraceMetal grade, Fisher) was used to dilute standards to the appropriate concentration and acidify the samples.

Sample Collection

Marsh water samples were collected from the Kewaunee Marsh near Kewaunee, WI. Opaque HDPE sample bottles (500 mL) were filled with the sample from either surface or well water sources. The samples were preserved by spiking with HCl (TraceMetal™ Grade, Fisher) to a final concentration of 0.01 M. Also, the samples were spiked with phenylarsonic acid (99% in 0.010 M HCl, Aldrich) internal standard at 50 ppb. Samples were transported and stored at 4 C.

Gas Chromatography

Organic arsenic compounds were determined by gas-liquid chromatography (GC) using a Varian (Walnut Creek, CA, USA) GC-MS system which consisted of the following components: Model 3800 capillary gas-liquid chromatograph; Varian Model 1079 split/splitless injector; Varian SPME apparatus; 5% phenyl-polydimethylsiloxane (PDMS) low-bleed analytical column (Model CP-SIL 8CB Low Bleed, Varian) that was 30 m x 0.25 mm i.d. with 0.25 μm film thickness; Varian pulsed flame photometric detector (PFPD) in arsenic mode using a high-pass (695 nm) optical filter (Schott RG695, BES optics, Warwick, RI) and Model R5070 photomultiplier tube (Hamamatsu, Bridgewater, NJ, USA) set to 610 V with a 200 mV trigger level; Varian electron impact ionization source (70 eV); and a Varian Model

Saturn 2000 quadrupole ion trap mass spectrometer (IT-MS) (10-650 m/z range, unit resolution). The automatic gain control of the MS system was used throughout the study. The mobile phase was ultra-high purity (99.999% (v/v)) helium (Praxair, Milwaukee, WI, USA) at a constant linear velocity by electronic flow control.

GC instrument control and data acquisition were performed on a Pentium II personal computer (Optiplex GX1, Dell, Dallas, TX, USA) using Saturn Software version 5.4 and Varian PFPD analysis software version 1.0. Response was reported as peak area for MS detection and peak height for PFPD detection. Automated library searching was accomplished using the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) Mass Spectral Database version 3.0.

Determination of Volatile Organoarsenicals by GC

Samples containing volatile organoarsenicals were sampled from the headspace of the container by solid-phase microextraction (SPME). The cap of the sample container was punctured with a steel awl and immediately covered with a piece of aluminum foil. A 75 µm carboxen-polydimethylsiloxane (Carb-PDMS) (Supelco, Bellefonte, PA, USA) was allowed to equilibrate with the sample for 60.0 min. The analytes that partitioned into the fiber were then desorbed in the GC injection port (splitless) at 250°C for 5 min. The initial column temperature was 30°C and held for 5.0 min. The column oven was then programmed at 20°C per min to 165°C, then 8°C per min to 213°C, then 45°C per min to 303°C. The oven was held at 303°C for 6.5 min; this program gave a total run time of 26.25 min.

Determination of Non-Volatile Organoarsenicals by GC

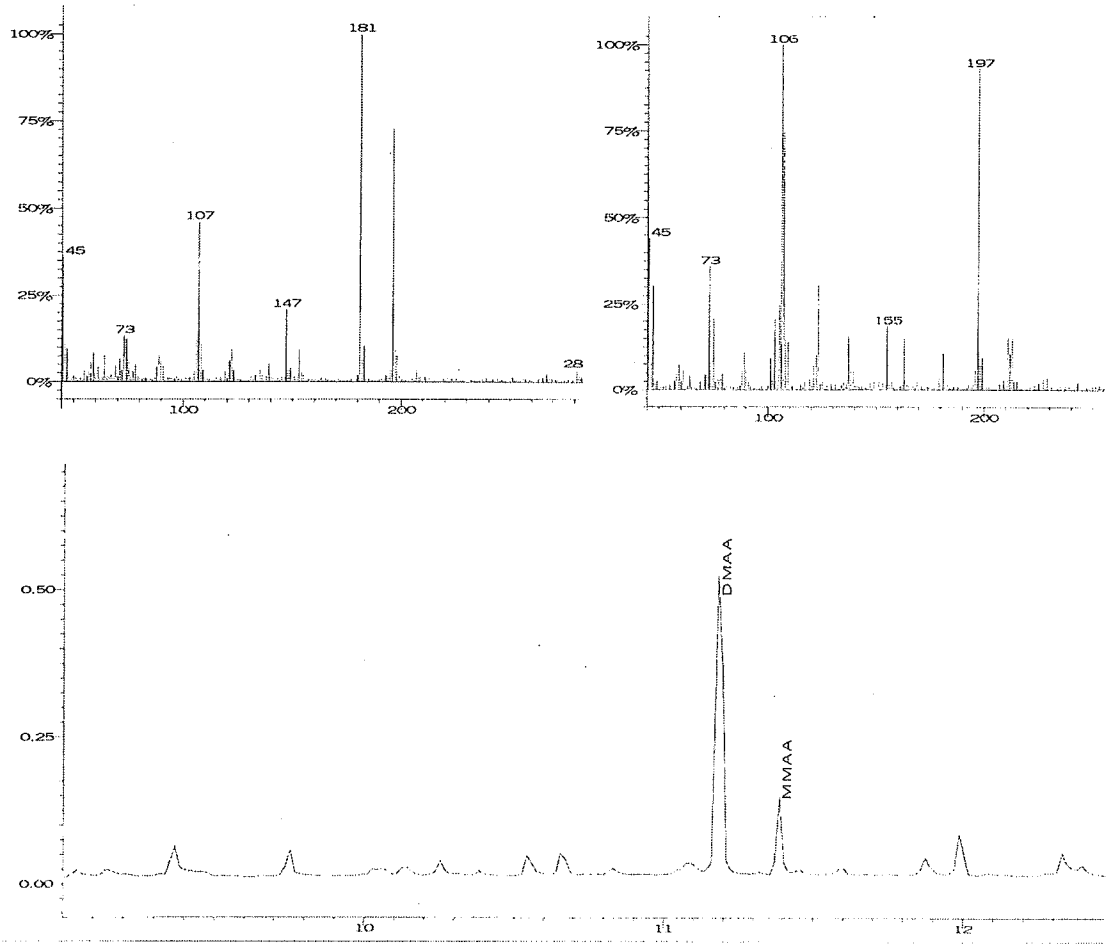
For the determination of non-volatile organoarsencials in standards and spiked surface water samples, the sample was acidified to pH 2 with 6.0 M HCl (TraceMetal™ grade, Fisher), as required. Next, a 2.5 mL portion of the sample was pipetted into a 4.0 mL vial (clear vial, screw top, white silicone/PTFE septa, Supelco) along with a magnetic stir bar (10 mm length, 3 mm diameter, Fisher) to provide agitation. The sample was then heated at 70°C and reacted with 0.5 µL (Hamilton 10 µL syringe, Fisher) of neat PDT for 5 min. The organoarsenicals were extracted from the sample matrix by SPME. A 65 µm polydimethylsiloxane-divinylbenzene (PDMS-DVB) (Supelco) was allowed to equilibrate with the sample for 15.0 min. The analytes that adsorbed onto the fiber were then desorbed into the GC injection port (splitless) at 250°C for 5 min. The initial column temperature was 45°C and held for 5.0 min. The column oven was then programmed at 20°C per min for to 165°C, then 8°C per min to 213°C, then 45°C per min to 303°C. The oven was then held at 303°C for 6.5 min; this program gave a total run time of 25.50 min.

Determination of Total Arsenic by ICP-MS

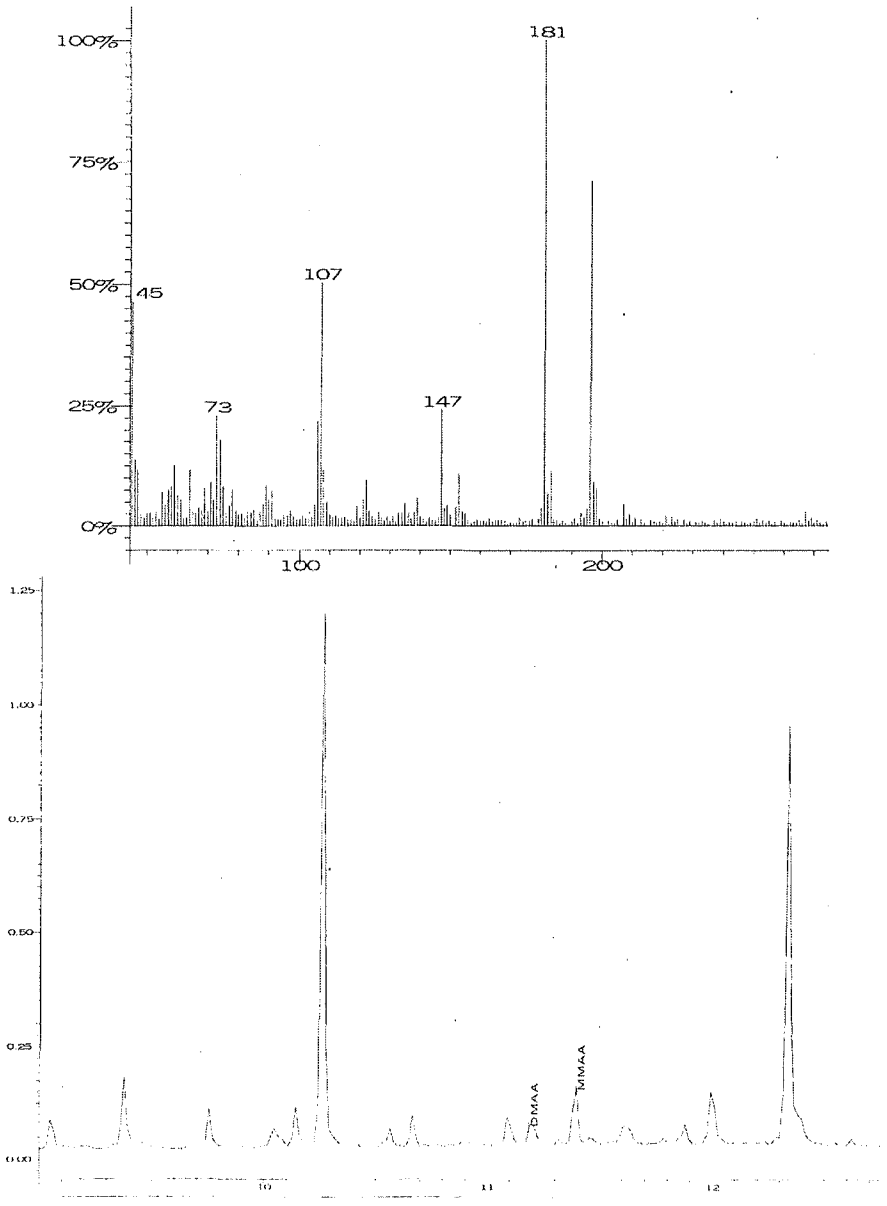
The inductively coupled plasma-mass spectrometer (ICP-MS) determination was completed using a Platform ICP-MS (Micromass, MA, USA) and Masslynx (version 4.0) software. Samples were selected by an autosampler (Model AXS-500, Cetac Technologies, Omaha, Nebraska, USA) and aspirated into the spray chamber by a peristaltic pump (Model Miniplus 3, Gilson, Villiers le Bel, France). The algorithm for correction of the polyatomic ion interference ($^{40}Ar^{35}Cl+$) to the m/z 75 response (^{75}As - (3.127 * $^{77}ArCl$) + (^{82}Se * 2.4585)) was used. ICP-MS system control and acquisition were performed on a Pentium II personal computer (Professional Workstation AP200, Compaq, Houston, TX, USA) using Masslynx (version 4.0) software (Micromass). All data was transferred to Microsoft Excel 2000 (Microsoft) for data analysis.

A 1.0 g sample of peat was extracted with 10 mL of 0.01 M HCl or 10 mL of 6 M HNO_3 and was tumbled overnight. The solution was then passed through a 0.45 µm cellulose filter to remove suspended solids (e.g., silicates). The sample was then acidified to 5% HNO_3 (with inclusion of ^{115}In as internal standard) prior to ICP-MS analysis.

Appendix C. Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry (attachments).



#1. GC-MS of standards; (a) mass spectrum of MMAA-PDT (top left) and DMAA-PDT (top right) and (b) gas-liquid chromatogram (bottom).



#2. GC-MS of SW2-F (northern slough, near fence line); (a) mass spectrum of MMAA-PDT (top) and (b) gas-liquid chromatogram (bottom). Note that the large peak at ~12.5 min is the internal standard (PAA).