ELSEVIER

The Science of the Total Environment 180 (1996) 165–182

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

Arsenic in subArctic lakes influenced by gold mine effluent: the occurrence of organoarsenicals and 'hidden' arsenic

Doug A. Bright*[1], Matthew Dodd[1], Kenneth J. Reimer[2]

Environmental Sciences Group, Royal Roads Military College, FMO Victoria, British Columbia, V0S 1B0, Canada

Received 30 March 1995; accepted 7 August 1995

Abstract

A series of subArctic lakes near Yellowknife, Northwest Territories, Canada, are contaminated with arsenic released from a gold mine. The high environmental arsenic levels afforded an opportunity to identify factors that control methylarsenical production in freshwater sediments. A large variety of methylated arsenicals were observed in most pore water and water column samples from lakes contaminated by aqueous or atmospheric arsenic inputs, albeit at low concentrations relative to inorganic arsenic (generally less than 10% of the total dissolved arsenic concentration). Biologically-mediated methylation/demethylation reactions, therefore, may influence the local arsenic cycle. Water column samples exhibited a consistent methylarsenic composition in which the dominant form was dimethylarsonic acid, followed by monomethylarsinic acid. A much broader range of mono-, di- and trimethylated arsenicals was found in sediment pore water, and the composition of methylarsenic species was highly variable both within and between cores. We provide indirect evidence that many samples contained mono-, di-, and trimethylated arsenic(III)thiols, of the form $(CH_3)_nAs^{III}(SR)_{3-n}$ ($n = 1, 2, 3$), the existence of which has been predicted but not previously demonstrated in the environment. The total dissolved methylarsenic concentration in cores consistently exhibited a subsurface maxima between 5 and 15 cm depth, which suggests — in conjunction with the presence of other metabolites in pore water — that methylation may be enhanced by sulfate-reducing bacteria. The role of sulfate-reducers in the production of methylarsenicals observed in sediment pore water is also supported by co-variations with depth between the concentration of methylarsenic species and iron dissolution or other chemical distributions attributed to metabolism by heterotrophic bacteria across different redox zones. Many samples contained appreciable concentrations of arsenicals the specific identity of which are presently unknown, and some of which are 'hidden' to conventional hydride-generation analyses. At least one unidentified arsenical found in lake water and sediment pore water appears to have been produced directly or indirectly from atmospheric emissions from the gold mine. The occurrence of methylated arsenicals and hidden arsenic at concentrations that comprise a substantial portion of the total arsenic budget in some aquatic systems suggests that current models of lacustrine arsenic cycling that incorporate only inorganic arsenicals need to be revisited.

Keywords: Arsenic; Organoarsenicals; Methylation; Freshwater; Porewater; Diagenesis; Sediments

* Corresponding author. Tel.: +1 604 3912584; Fax: +1 604 3912522; e-mail: DBRIGHT@post.Royalroads.ca.
[1] Applied Research Division, Royal Roads University, 2005 Sooke Road, Victoria, B.C., V9B 1W2, Canada.
[2] Environmental Sciences Group, c/o Chemistry and Chemical Engineering, Royal Military College, Kingston, Ontario, K7K 5LO, Canada.

SSDI 0048-9697(95)04940-3

1. Introduction

The toxicity of arsenic to aquatic biota is controlled by the type of arsenic species present, as well as the partitioning of arsenicals between environmental compartments. Cullen and Reimer [1] reviewed the environmental chemistry of arsenic, and Maeda [2] recently provided a review of arsenic speciation in freshwater environments. Arsenic occurs in aquatic environments either in inorganic form (as arsenite: As(III), and arsenate: As(V)) or as methylated species (monomethylarsonic acid: MMAA, dimethylarsinic acid: DMAA, trimethylarsine oxide: TMAO; the reduced, highly-volatile analogues monomethylarsine: MMA, dimethylarsine: DMA, and trimethylarsine: TMA; and others). More complex organoarsenicals such as arsenobetaine, arsenocholine, arsenosugars, and arsenolipids have been found in multicellular organisms or in seawater [1,3]. The methylation and oxidation (+3 to +5) of arsenic compounds reduces the toxicity and is hypothesized to be a protective mechanism in some organisms [1]. In general, the toxicity of arsenicals decreases as follows: MMA, DMA, TMA (III) > arsenite (III) > arsenate (V) > MMAA, DMAA, TMAO (V) > arsenobetaine, arsenocholine, arsenosugars, arsenolipids [1].

The presence of MMAA, DMAA, and TMAO in marine sediments or sea water has been taken as evidence that biological methylation/demethylation is an important influence on the environmental fate and effects of arsenic [4–7] in marine systems. The widespread occurrence of methylated arsenicals in lacustrine systems, however, was only recently demonstrated [8], and we are aware of only one previous detailed study of methylarsenic species in lake sediments; Takamatsu et al. [9] detected MMAA and DMAA in the pore water of lake sediment at concentrations of approximately 0.1% of the total dissolved arsenic level.

Several researchers have recently demonstrated in non-biotic environmental compartments the presence of additional complex arsenicals which are hidden to commonly used hydride generation techniques [10–12]. These arsenic species have been collectively referred to as 'hidden' [10] or 'refractory' [11]. Hidden arsenic comprises that portion of all arsenicals that does not generate a volatile arsine on reaction with borohydride, unless first decomposed to simpler forms [11,12]. Reimer et al. [13] have also detected organoantimony compounds in aquatic plant samples which are hidden to hydride-generation, atomic absorption spectroscopy analysis unless first subjected to microwave digestion with added potassium dichromate. Hidden arsenicals in water or sediment potentially include arsenocholine, arsenobetaine, arsenolipids, and arsenosugars; their metabolites (or precursors); possible sulfur- or thiol-containing arsenicals; and other presently undescribed compounds. In practice, the identity of hidden arsenic in environmental samples has rarely been determined, although hidden arsenic may account for in excess of 50% of the local arsenic budget in marine sediment interstitial water [12].

The biomethylation of mercury in freshwater sediment is directly related to bioavailability and toxicity. The importance of arsenic methylation and demethylation, however, is not known and the factors that control the concentrations of organoarsenicals in water and sediment have not been elucidated. In sediments, bacterial methylation and demethylation as well as microbially-mediated oxidation-reduction reactions might alter the lipophilicity and bioavailability of pore water arsenicals, directly alter arsenic toxicity, and/or alter the rates of arsenic remobilization from and demobilization into the sediment.

A series of small, shallow lakes in the Canadian subarctic, near Yellowknife, Northwest Territories exhibit elevated concentrations of arsenic owing to the input of gold mine effluent over several decades [14]. This study examines the distribution of methylarsenic species and hidden arsenic in the pore water of sediment cores and the water column of the Meg, Keg, Peg Lake, Great Slave Lake drainage system. We previously demonstrated [15,16] that bacteria from sediments in one of these lakes, isolated into arsenic-enriched aerobic and anaerobic cultures, were capable of methylating and demethylating arsenic. Aerobic, iron-reducing, manganese-reducing, and sulfate-reducing bacteria were all able to methylate inorganic arsenic. The same metabolic groups are also involved in the diagenetic behaviour of a wide

variety of substances. This study also examines the relationship between organoarsenicals and concentrations of inorganic arsenic, iron, manganese, sulphate, phosphate, and ammonium ion in pore water, as well as levels of arsenic and several other elements in the particle phase.

2. Materials and methods

2.1. Study area

Meg, Keg and Peg Lakes comprise a series of shallow (<2 m maximum depth), organic-rich lakes which eventually drain into southwest Yellowknife Bay, Great Slave Lake (Fig. 1). Since the 1930s, the watershed has received contaminant inputs (including SO_2^- and AsO_3) from the atmosphere as a result of stack emissions from two separate gold mines, as well as water-borne inputs of decant water and mine-tailings from one of the gold mines. The recent history of the contaminant inputs and watershed transport is provided by Bright et al. [14]. Prior to 1983, treatment of the tailings involved settlement of the solids and decanting of the overlying water. The present treatment of mine-tailings/effluent involves the addition of peroxide to oxidize cyanide to cyanate, the addition of ferric sulfate and lime to precipitate arsenic as ferric arsenate ($FeAsO_4$) and the addition of polyacrylamide flocculant to enhance sedimentation. Treated effluent is discharged into a drainage ditch leading into Meg Lake, which drains into Great Slave Lake via Peg Lake and Keg Lake.

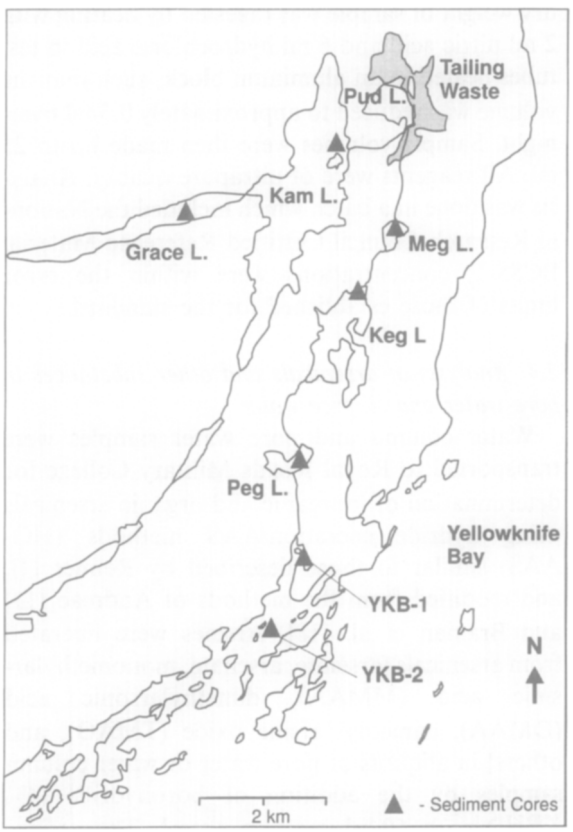

Fig. 1. Study site near Yellowknife, Northwest Territories, Canada.

Kam Lake is not in the Meg, Keg, Peg Lake drainage system, but was included in the study because of the documented contamination by arsenic, copper, and zinc. Kam Lake received several accidental discharges of effluent and tailings from the mine-tailings pond in the early 1970s and has also been subjected to nutrient enrichment through raw sewage discharge. Grace Lake, which is further from the gold mine and flows into Kam Lake via a small stream, was included as a reference lake for both Kam Lake and the Meg, Keg, Peg Lake systems.

2.2. Sample collection and processing

Sediment cores and water samples were obtained in August 1991, from Kam Lake; Grace Lake; Meg, Peg, and Keg Lakes; and Yellowknife Bay, Great Slave Lake within 0.5 km of the Meg, Keg, Peg system discharge point. A 9.0-cm diameter gravity corer, deployed from a small boat, consisted of either a 122-cm or 91-cm long polyacrylic tube mounted on an aluminum-alloy head with detachable lead weights and fitted with a stainless steel cutter/catcher. The cores were collected, immediately transported back to the laboratory, and stored before processing for a maximum period of 18 h in a walk-in cooler maintained at approximately 4°C. All cores were sectioned and squeezed through a 0.22-μm Millipore filter under a nitrogen atmosphere using previously established techniques [4]. A description of the procedure follows.

Each core was extruded from the core tube in a

nitrogen-filled modified glove bag, and sectioned into 5-cm sections with a Teflon-coated knife into pore water squeezer cells; these consisted of a base plate, a donut and a top — all made of polypropylene. The base plate was fitted with a drainage spout and a 0.22-μm Millipore filter overlying a precleaned polyethylene filter support. The donut (9.0 cm inside diameter × 5 cm high) was placed over the base plate/filter and filled with the sectioned sediment, which was then covered with precleaned latex rubber dental dam. The top of the squeezer, which has a pressure quick-coupler for applying nitrogen pressure, fit snugly over the dental dam and top of the donut assembly. The filled squeezers were removed from the glove bags and clamped into squeezer racks. Nitrogen pressure (345 kPa) was applied to the dental dam in order to express the pore water out of the sediment. The first 5 ml of sample was discarded, and approximately 80–180 ml of pore water was collected under a nitrogen purge line into a 250-ml acid washed polyethylene bottle over a period of 30–120 min, depending on the particle size and porosity of the sediment.

The pore water was split under N_2 gas into sterile, contaminant-free 50-ml Evergreen™ containers. A 3-ml aliquot of pore water was acidified by adding 30 μl of 4 M HCl and stored at room temperature for measurement of iron and manganese. Aliquots (6 ml) of pore water were collected for analysis of nutrients and selected ions (ammonium, phosphate, sulfate). Pore water samples (15–50 ml) were placed into Evergreen containers for analysis of arsenic and organoarsenicals. All samples except those acidified for iron and manganese analysis were immediately frozen over dry ice and transferred to a chest freezer (−20°C).

Surface water samples at each coring location were obtained by immersing the mouth of an acid-washed 4-l polyethylene container approximately 30 cm below the surface. The samples were then transported to the laboratory, filtered using Whatman 934-AF filters (1.5 μm nominal pore size) and immediately frozen.

2.3. Sediment analysis

Elemental analyses of the sediment solid phase were carried out at the Analytical Services Unit at Queen's University, Kingston, using neutron activation analysis (NAA) (for As, Al, Ca, Cl, Mn, Na, Br, Fe, K, Sb and Au) or flame atomic absorption spectroscopy (AAS) (for Cu and Zn). For NAA, previously frozen, dried and ground sediments were irradiated using the SLOWPOKE reactor facility at Royal Military College, Kingston with a flux of 5×10^{11} n cm^{-2} s^{-1}. Counting was done using a GMC HpGe detector coupled to a Nuclear Data mMCA. The sample (0.35–1.2 g) was weighed into 1.5-ml polyethylene vials and heat sealed. Short-lived isotopes, including Al, Ca, Cl, Mn and Na, were analyzed by irradiating for 1 min followed by a 15-min delay time and 10-min counting time. Long-lived isotopes (As, Br, Fe, K, La, Na, Sb, Au) were assayed by irradiating for 2 h followed by a 80- to 120-h delay time and 1.5-h counting time. For AAS, previously frozen sediments were air dried and ground to a fine powder. Approximately 0.5 g dry weight of sample was digested by heating with 2 ml nitric acid and 6 ml hydrochloric acid in test tubes heated in an aluminum block, such that the volume was reduced to approximately 0.5 ml overnight. Sample volumes were then made up to 25 ml. All reagents were of ultrapure quality. Analysis was done in a batch which included the National Research Council Certified Reference Material BCSS-1; concentrations were within the error limits of those established for the standard.

2.4. Analysis of arsenicals and other substances in pore water and surface water

Water column and pore water samples were transported to Royal Roads Military College for determination of inorganic and organic arsenicals using hydride-generation/AAS methods (HG-AAS) similar to those described by Reimer [4], and modified from the methods of Andreae [17] and Braman et al. [18]. Arsines were liberated from arsenicals [arsenite, arsenate, monomethylarsinic acid (MMAA), dimethylarsonic acid (DMAA), trimethyl arsine oxide (TMAO), and others] in aliquots of pore water or water column samples by the addition of borohydride (4% KBH_4) to a 60-ml reaction vessel. The arsines were purged from the reaction vessel using a helium carrier gas through a −78°C trap to remove

water and then cryofocussed in a U-shaped Teflon tube immersed in liquid nitrogen. After a 5-min trapping period, the liquid nitrogen bath was replaced with a 50°C water bath, and the trapped arsines were swept through a 50–60 cm × 1.2 mm i.d. Teflon column filled with Poropak PS 60/80. This was cycled from 50 to 150°C in a GC oven at a rate of 30°C min^{-1}. Arsines emerged from the column primarily in relation to their boiling points, although other interactions with the column cannot be discounted. The resulting arsines were monitored with an Instrument Laboratories 351 Atomic Absorption Spectrophotometer fitted with a hydrogen air flame contained within a quartz cuvette.

Samples were reacted with borohydride at two separate pHs in order to separate As(III) species (including arsenite) that are amenable to hydride-generation at near-neutral pH conditions from As(V) species such as arsenate, MMAA, DMAA, and TMAO which only react with borohydride under strongly acidic conditions. Each sample was initially buffered to pH 5.8 using Tris–HCl buffer for the determination of As(III) species. Three microlitres of 4 N HCl were then added to the reaction vessel to increase the acidity to pH 1 and the hydride-generation, cryofocussing, boiling point separation and AAS analysis of arsenicals were repeated. We found it necessary to optimize the analytical system in order to obtain reliable and routine detection of trimethylated arsenicals. This included the elimination of any materials (for example, glass) from the analytical system that might otherwise adsorb analytes, ensuring that all fittings were gas-tight, and conditioning the Porapak 68/80 column daily. Conditioning of the column involved thorough silanization, followed by saturation of microscopic pores in the packing material by hydride-generation/AAS analysis of aliquots containing a MMAA standard at high concentrations.

It was necessary to frequently run analytical blanks in order to prevent analyte carry over from one sample to the next. The performance of the system was periodically checked with aliquots of NASS-1, a Marine Analytical Chemistry Standard obtained from National Research Council Canada; no freshwater reference material certified for arsenic was available. Total arsenic concentrations were between 1.5 and 1.7 μg As l^{-1} which were within the certified values of 1.65 ± 0.19. About 10% of the samples and standards were run in replicate: reproducibility was within ±5% for arsenite or arsenate, and ±15% for methylarsenic species. Absolute detection limits were 0.12 ng (of arsenic) for arsenite and 0.25 ng for arsenate and the methyl arsenicals.

The concentration of total dissolved arsenic in pore water was measured in a subset of pore water samples before and after ultraviolet (U.V.) irradiation or microwave digestion in order to estimate the amount of arsenic present which is otherwise hidden to hydride-generation techniques. Samples of pore water from the core Yellowknife Bay-2 were irradiated in a U.V. chamber which was a modification of a design described previously [10]. The chamber contained a 450-W medium pressure lamp and four cooling fans instead of a 1000-W lamp and a single fan. Samples (10–20 ml) were placed into 24 × 2.5 cm quartz tubes. The tubes were sealed after the addition of 1.0 ml of 0.1 M borax, 0.1 ml of hydrochloric acid (Analar 35%) and 20 μl of hydrogen peroxide (30% BDH Chemicals). The samples were arranged in a carousel around a lamp and irradiated for 12 h. After irradiation, the tubes were allowed to cool. The tubes were then opened and the solutions analyzed by hydride generation.

Decomposition of hidden arsenic by microwave digestion was conducted according to the methods of Le et al. [23]. Briefly, pore water samples were injected into a flow injection, hydride-generation, AAS system with or without digestion using potassium persulphate, sodium hydroxide and microwave energy.

Iron and manganese in pore water were measured by flame AAS. Nutrient or metabolite (PO_4^{3-} as P, SO_4^{2-}, and NH_4^+ as N) concentrations were determined by ion-exchange chromatography at Queen's University, Analytical Services Unit, Kingston.

3. Results and discussion

3.1. The occurrence of methylarsenic species

Several of the pore water samples isolated from

lake sediment near Yellowknife contained a large number of methylarsenic species: more than has been documented in the literature or observed previously in our laboratory. Fig. 2 illustrates a series of HG-AAS traces typical of many of the pore water and water column samples. When hydride-generation, which converts aqueous arsenicals to volatile arsines, was conducted at pH 6, arsine (from arsenite) was routinely detected along with up to four other arsenicals (Table 1). At pH 1, the arsine peak corresponding to arsenate was routinely observed, along with up to four other arsines that were identical in retention time (and, presumably, boiling point) to those observed after hydride generation at pH 6 (Fig. 2). The identity of four of the five peaks after hydride-generation at either pH is known: the volatile arsines emerge from the Poropak column, in order of increasing boiling point, and include arsine, monomethylarsine (MMA), dimethylarsine (DMA) and trimethylarsine (TMA).

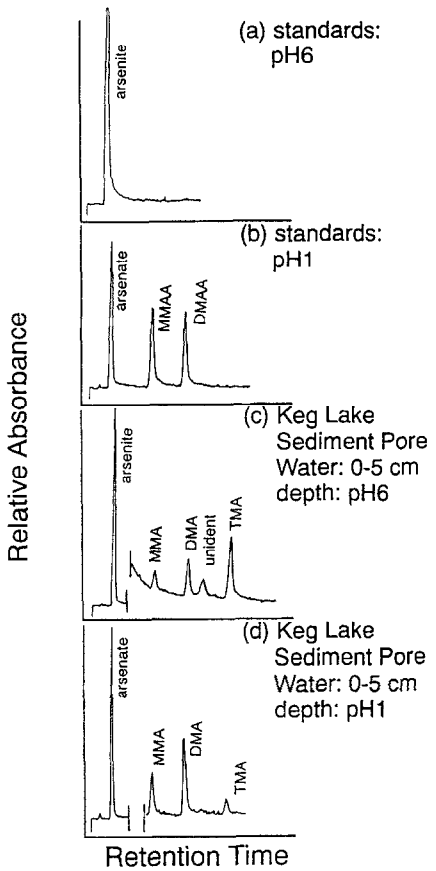

Fig. 2. Hydride generation, gas chromatography, atomic absorption spectroscopic analysis of inorganic and methylated arsenicals in standard solutions of porewater isolated from lake sediment collected near Yellowknife, NWT. At a reaction pH of 1, standards of arsenate, monomethylarsenic acid, dimethylarsonic acid and trimethylarsine oxide in aqueous solution yield arsine, monomethylarsine (MMA), dimethylarsine (DMA), and trimethylarsine (TMA) respectively following hydride-generation. The detection/quantification of arsine after hydride-generation at pH 6 is attributed to arsenite, whereas the production of MMA, DMA and TMA at pH 6 may be attributable to the presence of methylated arsinothiols in the original sample (see text). At reaction pHs of both 6 and 1, the arsenical eluting between DMA and TMA is hitherto undescribed, and the identity has not been determined. Fig. 2c,d: note that inorganic arsenicals (arsenate, arsenite) were analyzed separately from methylated species, where much larger aliquots of water were injected into the reaction vessel.

Detection of MMA, DMA and TMA following hydride-generation has routinely been attributed to the presence of the As(V) species MMAA, DMAA and TMAO, respectively, in the original sample [5–9]; we concur with this assessment when hydride-generation has been conducted under strongly acidic conditions. The concentrations of MMAA, DMAA, and TMAO listed in Table 1 are calculated by quantification of the corresponding arsines when produced by reaction of the aqueous samples at pH 1. However, pure standards of MMAA and DMAA freshly made up in sterilized, de-ionized water do not react at pH 6 with borohydride to produce a volatile arsine. Monomethyl-, dimethyl-, and trimethylarsinothiols, such as dimethylarsinoglutathione and monomethylarsinocysteine, synthesized and supplied by Cullen et al. [19], did produce the equivalent methylarsines when reacted at pH 6.

Cullen et al. [19,21] have shown that As(V) methylarsenicals are readily reduced by a variety of thiols and dithiols including cysteine, glutathione, dithiothreitol, 2,3-mercaptopropanol and lipoic acid. In addition, several thiols, including glutathione, cysteine, 3-mercaptopropionate and methanethiol, have been identified in the pore water of reduced marine sediments ([25] and refs. therein), and various thiols are probably present in freshwater sediment. The probable identity of the pH 6 reactive methylarsenicals, therefore, is arsenothiols of the form $(CH_3)_nAs^{III}(SR)_{3-n}$ (n = 1, 2, 3) [15].

Table 1
Distribution of arsenic species in water, sediment, or porewater in lakes near Yellowknife, NWT, influenced by gold mining activity

Sediment depth (cm)	Solid-phase As (ppm)	ΣDAs	(pH 1) Arsenate	(pH 6) Arsenite	ΣMe sp.	pH 1				pH 6			
						MMAA	DMAA	TMAA	Unident.	MMA	DMA	TMA	Unident.
Grace Lake													
Water column	—	11	11	0.19	0.44	0.06	0.22	ND	ND	0.04	ND	ND	0.12
0–5	22	15	6.4	8.6	0	ND	ND	ND	ND	ND	ND	—	—
5–10	56	43	41	1.3	0.05	ND	ND	0.05	ND	ND	ND	ND	ND
10–15	15	17	13	4.5	0.07	ND	ND	ND	ND	ND	ND	ND	ND
15–18	23	70	63	6.6	0.12	0.10	0.02	ND	ND	ND	ND	ND	ND
Effluent	—	36	35	ND	1.6	ND	ND	ND	ND	ND	ND	ND	1.63
Meg Lake													
Water column	—	65	58	5.9	0.17	0.06	0.10	ND	ND	ND	ND	ND	0.59
0–5	1160	1370	660	710	2.2	0.95	0.11	0.18	0.13	0.06	0.02	0.72	0.07
5–10	1475	1110	230	870	4.7	0.64	0.06	ND	ND	2.7	0.02	1.3	0.02
10–15	1080	464	150	310	4.1	2.0	0.07	0.02	ND	1.1	0.072	0.56	0.29
15–20	209	139	100	36	2.9	1.9	0.18	0.02	0.19	0.22	ND	0.02	0.40
Keg Lake													
Water column	—	64	58	5.9	0	ND	ND	ND	ND	ND	ND	ND	ND
0–5	1043	1200	270	930	4.4	0.80	1.2	0.26	ND	0.22	0.52	1.1	0.43
5–10	1905	1050	750	300	1.6	0.53	0.16	0.44	ND	0.02	0.22	0.05	0.16
10–15	1455	643	370	270	2.5	0.62	0.45	0.76	ND	0.10	0.28	0.16	0.18
15–17	480	1590	1050	540	2.2	0.15	0.18	0.32	ND	0.39	0.49	0.49	0.15
Peg Lake													
Water column	—	422	400	22	0.05	0.05	ND	ND	ND	ND	ND	ND	ND
0–5	3090	5170	2150	3020	3.92	0.44	0.68	ND	ND	ND	ND	1.1	1.7
5–10	1405	3860	2450	1400	4.82	0.47	0.38	0.72	ND	0.49	0.56	0.78	1.42
10–16	660	2870	2040	830	4.44	1.03	0.6	1.08	ND	0.15	0.09	0.21	1.28
Yellowknife Bay-1													
Water column	183	180	3.0	0	ND	ND	ND	ND	ND	ND	ND	ND	
0–5	115	48	26	21	0.56	0.28	0.28	ND	ND	ND	ND	ND	ND
5–10	61	95	11	83	0.75	0.20	0.3	ND	ND	ND	0.25	ND	ND
10–15	46	21	11	9.0	0.60	0.14	0.34	ND	ND	ND	0.12	ND	ND
15–18	34	46	12	33	1.163	0.13	0.67	ND	ND	ND	0.36	ND	ND
Yellowknife Bay-2													
Water column	2.1	1.2	0.70	0.23	0.08	0.15	ND	ND	ND	ND	ND	ND	
0–5	174	68	52	15	1.04	ND	1.04	—	—	—	—	—	—
5–10	204	76	54	22	0.15	ND	0.15	—	—	—	—	—	—
10–15	94	15	8.6	6.8	—	ND	ND	—	—	—	—	—	—
15–20	22	12	5.9	6.2	0.37	0.1	0.27	—	—	—	—	—	—
20–30	17	4.1	2.1	2.0	—	ND	ND	—	—	—	—	—	—
30–40	14	4.3	2.5	1.9	—	ND	ND	—	—	—	—	—	—
40–44	16	4.6	2.8	1.8	—	ND	ND	—	—	—	—	—	—
Kam Lake													
Water column	—	530	520	5.4	3.3	0.55	0.72	0.22	0.60	ND	ND	ND	1.24
0–5	2186	1120	280	840	2.7	0.61	0.40	0.02	0.02	0.81	0.12	0.80	0.02
5–10	1465	800	190	610	5.2	1.6	0.36	0.24	0.02	1.6	0.16	1.23	0.02
10–15	276	650	160	490	5.2	3.0	0.58	0.26	0.02	1.0	0.09	0.28	0.02
15–20	169	450	110	340	3.1	1.1	0.29	0.02	0.02	1.1	0.12	0.48	0.02
20–25	53	420	130	290	2.7	1.0	0.76	0.26	0.02	0.21	0.09	0.33	0.02
25–37	39	210	68	140	0.67	ND	0.20	0.33	ND	0.02	0.02	0.10	ND

Concentrations in ppb, unless otherwise indicated; ΣDAs, total dissolved arsenic concentration in water; ΣMe sp., sum of all hydride-reactive methylarsenicals in water; ND, not detected; — , not analyzed, or not relevant.

A fifth arsenic-containing volatile compound was detected in many samples when hydride-generation was carried out at either pH (Fig. 2; Table 1). This as yet to be identified arsenical has a retention time that is intermediate between that of DMA and TMA. The identity of the 'unknown' volatile arsenic compound, or that of the aqueous precursor(s) prior to hydride-generation has not

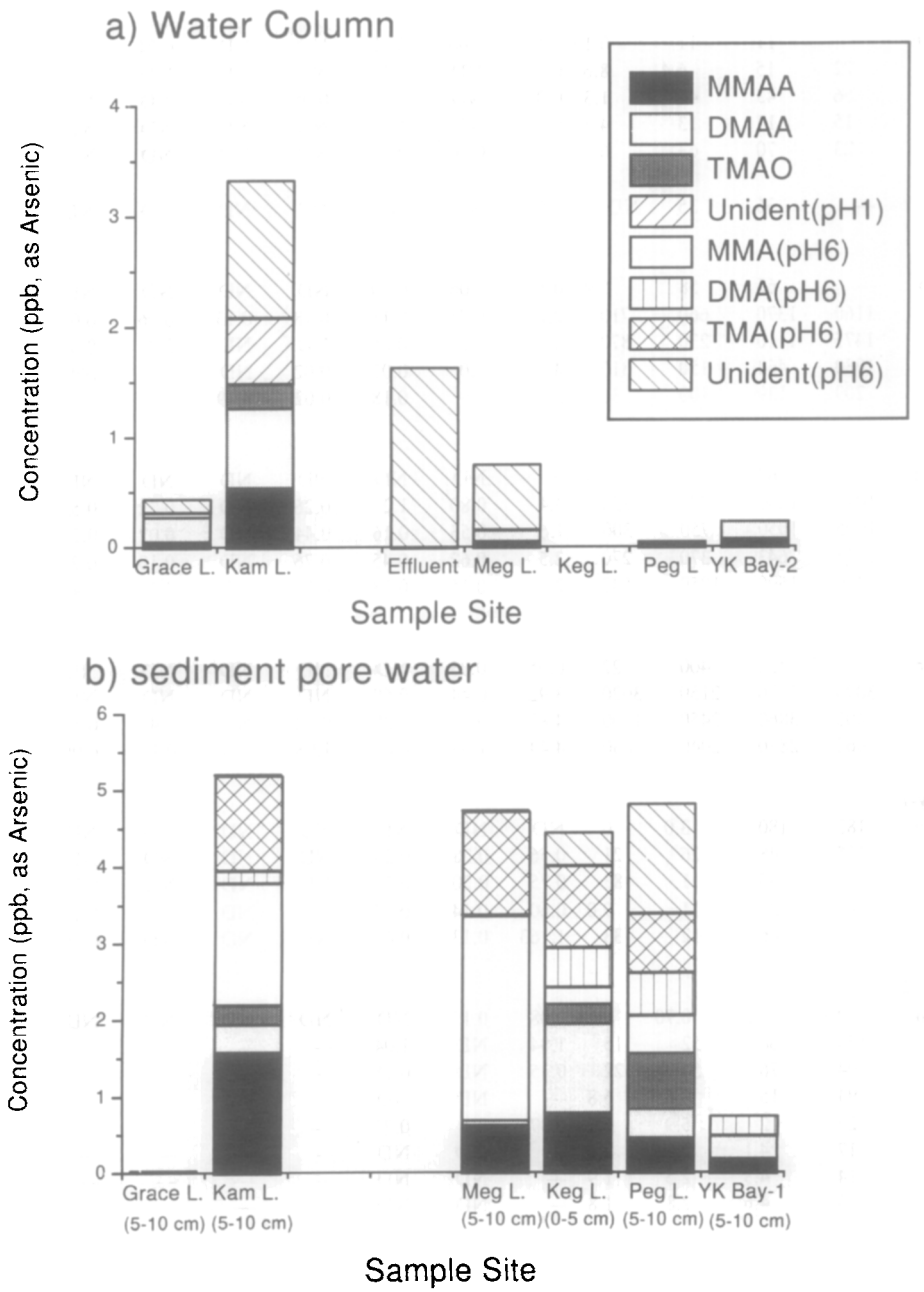

Fig. 3. Composition of methylarsenic species in gold mine effluent and water column samples (Fig. 3a), or sediment pore water at approximately the depth of the ΣMeAs concentration maxima (Fig. 3b) in lakes near Yellowknife, NWT.

been determined, and the analytes will be hereafter referred to as 'unident (pH 1)' and 'unident (pH 6)'. The concentration of the unidentified arsenicals was estimated, as arsenic, assuming that the unidentified species exhibited an AAS detector response similar to that of DMA or TMA.

Methylarsenicals in sediment pore water comprised up to 2.9% of the total dissolved arsenic concentration (TDAs) (methylarsenic concentrations (ΣMeAs) included mono-, di-, and trimethyl species detected after reaction at both pH 6 and pH 1 but excluded unident (pH 1) and unident (pH 6)). The average ΣMeAs concentration in pore water was 0.7% of the TDAs concentration. Similarly, methylarsenicals comprised up to 2.7% of the TDAs level in water column samples except for one sample from Yellowknife Bay, Great Slave Lake (YK Bay-2), where ΣMeAs comprised 10.6% of the TDAs concentration.

3.2. Composition of methylarsenic species in gold mine effluent, lake water or sediment pore water

Anderson and Bruland [8] have described the presence of the methylarsenicals MMAA and DMAA in lake water from a large variety of lacustrine systems: ΣMeAs constituted from 1 to 59% of the TDAs, and DMAA was the dominant methylarsenical in all samples. Takamatsu et al. [9] described methylarsenicals in sediment pore water from a Japanese lake, but like Anderson and Bruland, restricted their analyses of aqueous species to inorganic arsenic, MMAA and DMAA.

Fig. 3 illustrates the relative concentrations of all aqueous arsenicals in gold mine effluent, lake water (Fig. 3a) or sediment pore water samples (Fig. 3b) from the Meg, Keg, Peg Lake, Yellowknife Bay system. The downstream distributions of arsenite, arsenate, and particulate arsenic have been discussed elsewhere [14]. Excluding the unidentified arsenicals, the dominant methylarsenical in the water column of the Meg, Keg, Peg Lake, Yellowknife Bay drainage, as well as Kam and Grace Lakes, was DMAA followed by MMAA. TMAO was also detected in the water column of Kam Lake, at a concentration of 0.22 ppb (Table 1). Methylarsenicals that formed hydrides at pH 6 (precursors for MMA, DMA, TMA at a reaction pH of 6) were not detected in the water column, with one minor exception. The MMA (pH 6) concentration in the water column from Grace Lake was estimated to be 0.04 ppb (Table 1).

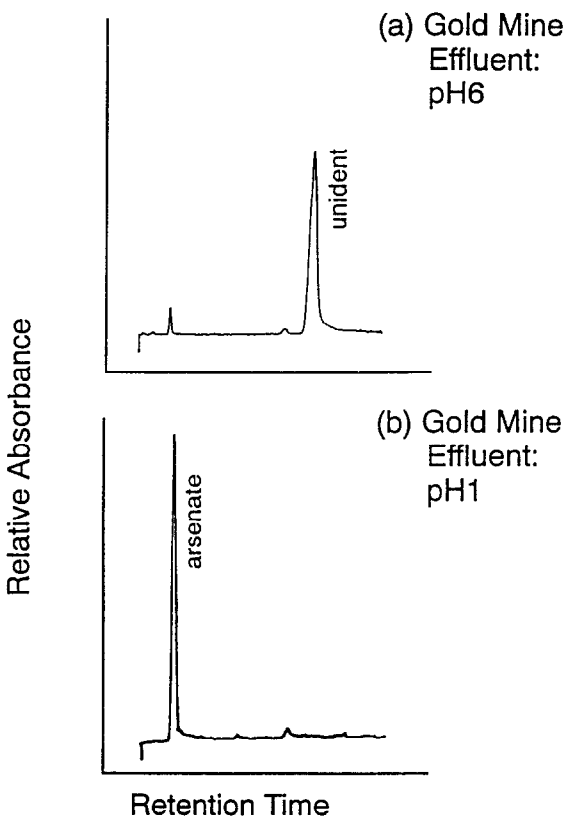

Fig. 4. HG-GC-AAS analytical trace of effluent from a gold mine near Yellowknife, which is discharged to the Meg, Keg, Peg Lake, Yellowknife Bay watershed.

One of two unidentified arsenicals (Table 1; Fig. 2: unident (pH 6)) was observed in water column samples from Grace Lake, Kam Lake, as well as in the gold mine effluent at a concentration (as arsenic) that was higher than that of the other methylarsenicals (Fig. 3). The analyte unident (pH 1) was detected in the water column only in Kam Lake.

The dissolved arsenic composition of the gold mine effluent provides a clue regarding the origin of the unident (pH 6) arsenical. The analytical trace for the gold mine effluent produced from the HG-GC-AAS analysis is provided in Fig. 4.

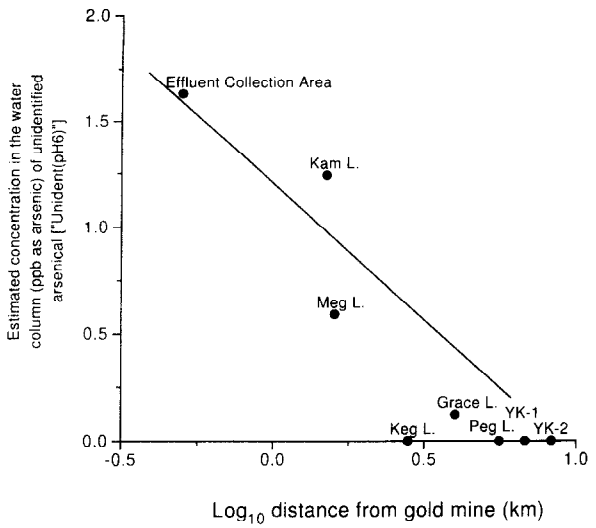

Fig. 5. Correlation between the concentration of an unidentified arsenical [unident (pH 6] in lake water and the $\log_{10}$ of the linear distance from the gold mine stack, used historically in association with the roasting of gold-bearing ore.

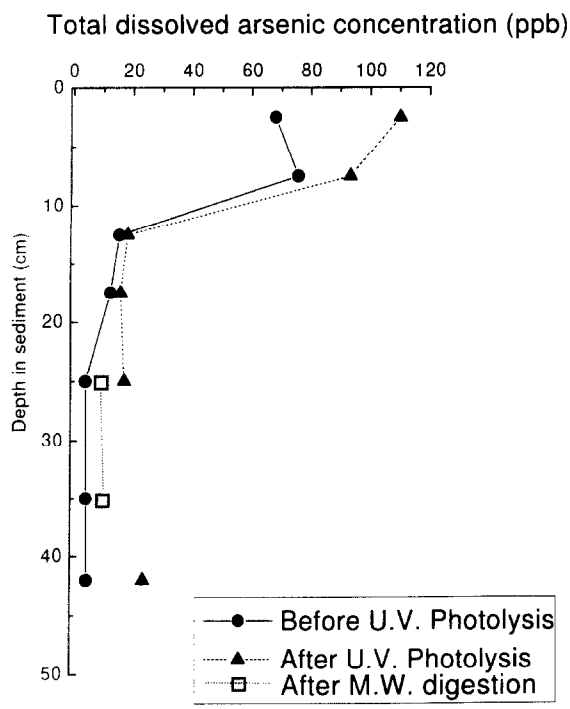

Fig. 6. Hidden arsenic, as determined after U.V. irradiation, in pore water isolated from a sediment core from site Yellowknife Bay-2.

Dissolved arsenic in the gold mine effluent was comprised entirely of arsenate and the unident (pH 6) species; no other arsenicals were detected in spite of their widespread distribution farther downstream. The water column samples and pore water samples collected from sites close to the mine site also contained the unident (pH 6) arsenical. The unident (pH 6) arsenical was found in Grace lake which has only received atmospheric inputs from gold-mining activity. The source of the unident (pH 6) arsenical is probably gold mine stack emissions (at least historically; [20]) in light of the strong correlation between water column concentration and the logarithm of distance of the collection site from the gold mine stack (Fig. 5) (Pearson $r = -0.91$; $P = 0.002$). No statistically-significant relationship was observed between distance from the source and the estimated levels of the unident (pH 6) or (pH 1) arsenicals in sediment pore water (Table 1).

A much broader range of mono-, di- and trimethylated arsenicals was found in sediment pore water than in the overlying water column, and the composition of methylarsenic species was highly variable both within and between cores (Fig. 3; Table 1). Factors that may influence the overall distribution of methylarsenicals in pore water are discussed below.

3.3. *'Hidden' arsenic*

Some of the pore water samples from the Meg, Keg, Peg Lake, Yellowknife Bay system contained other arsenicals, in addition to the aforementioned methylated species, which were only detectable by HG-GC-AAS following strong digestion of the samples. Two sample digestion methods were employed in order to decompose complex arsenicals into simpler forms capable of forming hydrides: U.V. photolysis (as described in [10] and the experimental section above); or continuous-flow HG-AAS in accompaniment with microwave digestion, as described by Le [22] and Le et al. [23]. Pore water samples from the core collected from site Yellowknife Bay-2 were analyzed for dissolved arsenicals before and after U.V. photolysis. The results are shown in Fig. 6. An increase in total dissolved arsenic concentration of 18–420% was observed following irradiation. Howard and

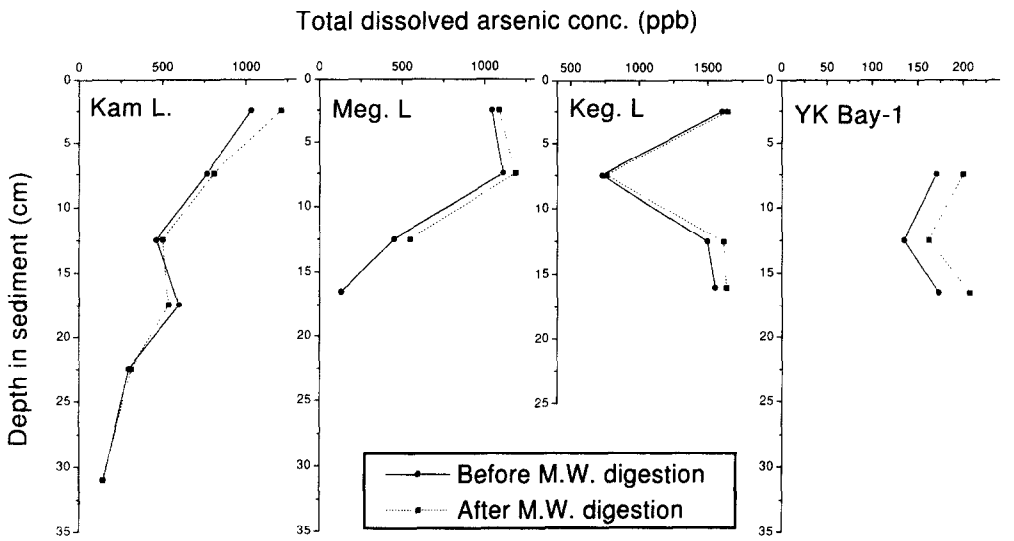

Fig. 7. Hidden arsenic, as determined after microwave digestion, in pore water isolated from sediment cores from sites in Meg Lake, Keg Lake, Kam Lake and Yellowknife Bay-1.

Comber [11] observed on average a 25% increase in the dissolved arsenic levels in seawater after U.V. decomposition. In the Yellowknife Bay core, pore water samples from the top (0–5 cm depth) and bottom of the core (>20 cm depth) exhibited the largest percent increase in arsenic after U.V. photolysis.

Porewater samples from all cores except the Yellowknife Bay-2 site were analyzed for total dissolved arsenic before and after decomposition using the continuous-flow HG-AAS system described by Le et al. [23]. Slight increases in the concentration of dissolved arsenic were consistently observed following microwave digestion (Fig. 7), but in most samples the increase was not significantly greater than the precision of the analytical technique, which was estimated to be 5% [22].

It is likely that neither of the two decomposition techniques provided complete digestion of refractory or hidden arsenicals to simple forms that react to produce volatile hydrides. The relative efficacy of decomposition of the two techniques can be compared based on the results from one pore water sample from the core collected from site Yellowknife Bay-2 (Fig. 6). The TDAs concentration of sediment pore water obtained from a depth of 20–30 cm prior to application of any sample decomposition technique was determined to be 4.1 ppb. The concentration was determined to be 5.2 ppb and 17 ppb following M.W. digestion and U.V. irradiation, respectively. Le [22] provides a detailed discussion of factors that influence the decomposition of organoarsenicals using microwave digestion.

The results herein demonstrate that a considerable portion of the dissolved arsenic in sediment porewater (most of the arsenic in some cases) from southwest Yellowknife Bay occurs in the form of complex arsenicals which do not readily react with borohydride to form volatile hydrides, and which are routinely overlooked in investigations of arsenic cycling. This hidden arsenic might occur in a number of forms; for example, as inorganic arsenicals tightly adsorbed to dissolved or colloidal (<0.22 μm diameter) organic matter such as humic and fulvic substances, or as complex organoarsenicals such as arsenocholine or arsenosugars or other hitherto unrecognized forms. Thanabalasingam and Pickering [24] demonstr-

ated the importance of humic acids in the adsorption of arsenate or arsenite, subject to competition by other anions.

3.4. Factors controlling the methylation of arsenic in lake sediment

Takamatsu et al. [9] hypothesized that the presence of DMAA and MMAA in the pore water of lake sediments is attributable to release from organic detritus at or near the sediment surface, and that the methylarsenicals were originally produced by phytoplankton in the water column. Anderson and Bruland [8] imply that methylated arsenicals in the water column of lakes are attributable to

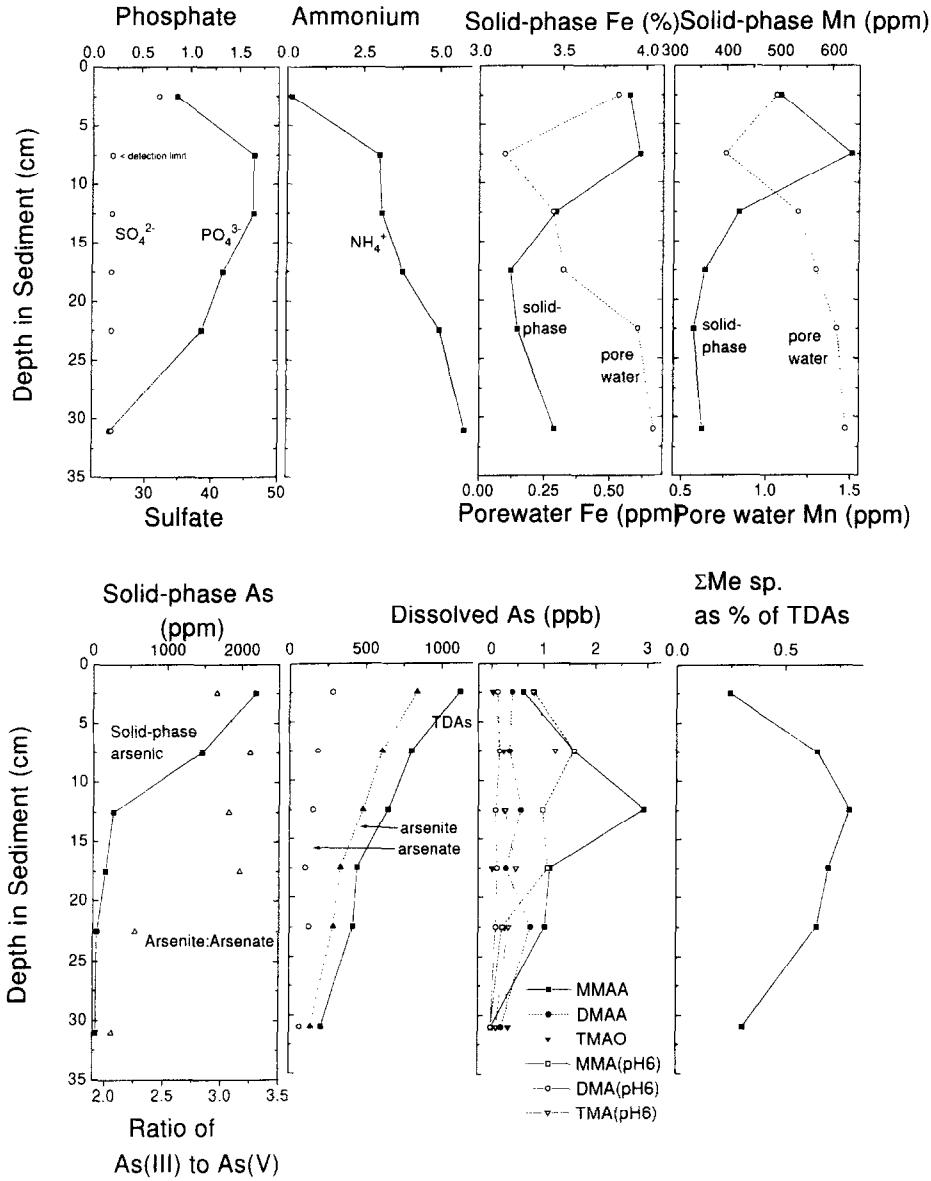

Fig. 8. Vertical profiles in a sediment core collected from Kam Lake, N.W.T., of various metabolites influences by the decomposition of detrital organic matter by heterotrophic bacteria. Vertical profiles of inorganic and methylated arsenicals are shown for comparison.

either direct microalgal production or, indirectly, to the bacterial decomposition of more complex organoarsenicals from algae. If phytoplankton are the major producers of methylated arsenic in freshwater or marine aquatic systems, then the extent of arsenic methylation should be controlled by the primary productivity of a system, and factors that influence the competitive uptake by algae or incorporation into macromolecules of arsenate and phosphate [5,6,26]. Anderson and Bruland

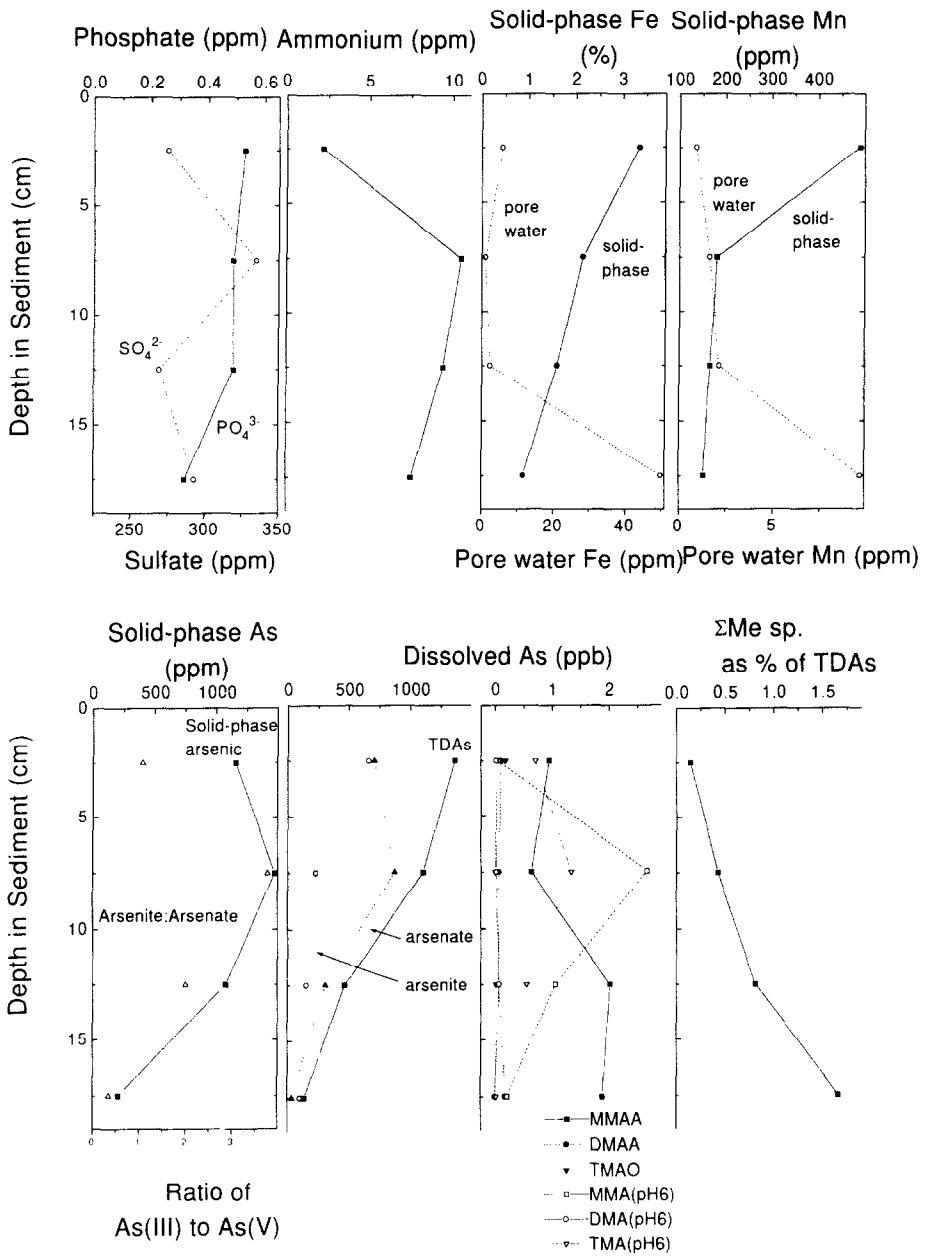

Fig. 9. Vertical profiles in a sediment core collected from Meg Lake, N.W.T., of various metabolites influences by the decomposition of detrital organic matter by heterotrophic bacteria. Vertical profiles of inorganic and methylated arsenicals are shown for comparison.

[8], however, found that methylarsenic concentrations in lakewater were not directly related either to primary productivity or phosphate/arsenate concentration ratios.

The concentration of ΣMeAs species in sediment pore water from cores near Yellowknife exhibited a subsurface rather than surface maximum in all cores except Keg Lake; the data are provided in Table 1. When all porewater data in Table 1 were considered, there was a significant correlation between ΣMeAs and the solid-phase arsenic concentration (Pearson $r = 0.58$; $P = 0.001$). ΣMe-

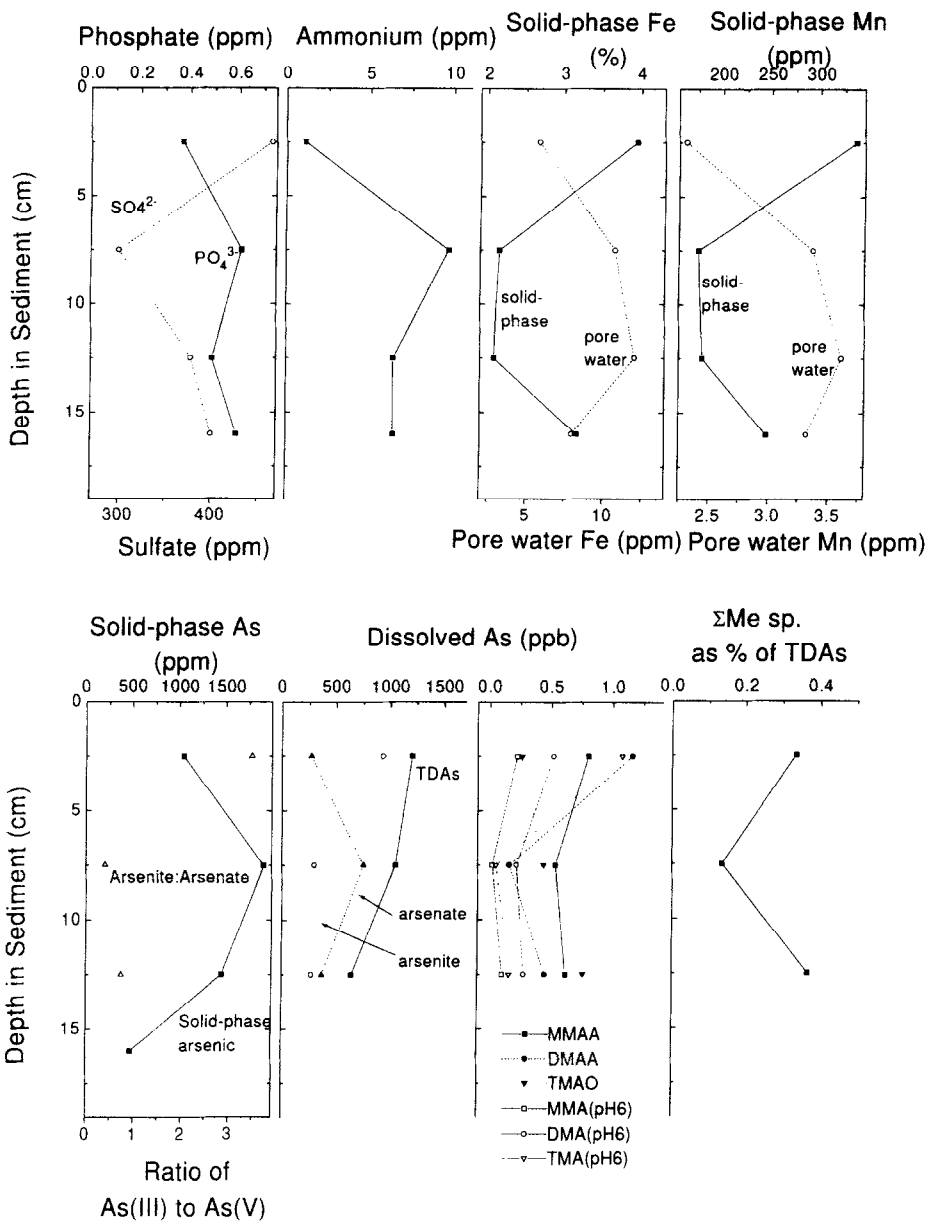

Fig. 10. Vertical profiles in a sediment core collected from Keg Lake, N.W.T., of various metabolites influences by the decomposition of detrital organic matter by heterotrophic bacteria. Vertical profiles of inorganic and methylated arsenicals are shown for comparison.

As was also correlated with arsenite concentration in pore water (Pearson $r = 0.62$; $P < 0.001$).

Vertical gradients in the chemistry of recent sediments are strongly influenced by the metabolic activity of heterotrophic bacteria. The vertical profiles of many chemical ions or compounds in pore water are the net result of heterotrophic bacterial decomposition of organic detritus. With increasing sediment depth, bacterial communities utilize oxygen, manganese-reduction, iron-reduction, and sulfate-reduction for provision of a terminal electron receptor during respiration. Vertical profiles of phosphate, sulfate and ammonium ion in pore water from Kam Lake, Meg Lake and Keg Lake are shown in Figs. 8–10, and compared to the vertical distributions of solid-phase arsenic and various arsenicals.

The lake bottom at all of the coring sites has undoubtedly been subjected to strong, often punctuated, temporal variation in the sedimentation regime owing to seasonal variations in hydrology associated with the subarctic climate, and the small size of the lakes in terms of both area and depth. Anthropogenic inputs into the Kam Lake and Meg/Keg/Peg Lake drainage systems have also undergone punctuated change over time [14]. Vertical profiles of several metabolites, therefore, tended to be erratic, especially for Meg, Keg (Figs. 9,10) and Peg Lakes. Nonetheless, the core profiles of iron and manganese in the pore water or solid-phase sediment are useful in defining the sediment depth at which sediments were reduced at the time of field collections.

In the Kam Lake core (Fig. 8), the sediment tended to be reduced in the 10- to 15-cm zone as indicated by iron and manganese dissolution. Assuming that there was a sufficient concentration of sulfate in the pore water, this zone would also have marked the minimum depth at which sulfate-reduction occurred as the dominant pathway of detrital carbon decomposition. The progressive accumulation of NH_4^+ with sediment depth is a direct consequence of nitrogen release from organic detritus. The depth at which the maximum ΣMeAs concentration occurred was also below 10 cm, rather than at the surface.

The redox potential of the sediment and dominant pathway for organic C breakdown should also be identifiable by the arsenite/arsenate ratio in pore water. In increasingly suboxic sediments, the arsenite/arsenate ratio should increase. As shown in Figs. 8–10, this was not the case. One possible explanation for this is the secondary adsorption/precipitation of dissolved arsenic, especially arsenite, through mineralization or complexation in a form hidden to our analytical methods. There is little information on mineralogical controls of arsenic-solubility in sulfide-rich environments [1]. Another possible explanation for the arsenite/arsenate ratio maximum is the localized presence of a redox minimum in the sediment, which is attributable to the onset of anthropogenic inputs.

There was a marked similarity between the phosphate and ΣMeAs species profiles in the Kam Lake core. Phosphate, like arsenic, has a strong affinity for particulate iron- and manganese-hydroxides in oxidized surface sediments [27]. While it might be tempting to assume some relationship between phosphate availability and the rate of arsenic methylation by sediment bacteria, it is more likely that the relationship between the two was a spurious result of redox conditions, which enhanced the solubility of phosphate in pore water on the one hand, and enhanced microbial arsenic methylation on the other. No relationship between phosphate and methylarsenical concentrations was observed in any of the other cores (for example, Meg and Peg Lake cores: Figs. 9 and 10, respectively).

The dissolution of iron or manganese in Meg Lake (Fig. 9) or Peg Lake (Fig. 10) sediment indicates that the depth cut-off between the redox zones dominated by heterotrophic sulfate-reducing bacteria and iron or manganese reducing bacteria was 10–15 cm and 5–10 cm, respectively in the two cores. The depth of the maximum ΣMeAs concentration in the Meg Lake core occurred below the zone of iron-reduction; the situation for the Peg Lake core is less clear due to two local ΣMeAs concentration maxima: one in the 0- to 5-cm zone and one at 10–15 cm.

Vertical profiles of sulfate concentration in cores from all collection sites did not bear any apparent relationship to the concentrations of methylated arsenicals. In addition, microbial sulfate reduction in lacustrine systems is often sulfate lim-

ited, in contrast to marine systems, and methanogenesis is generally the major contributor to organic detritus breakdown in suboxic environments [27]. It was previously shown [14] that sulfate porewater concentrations were anthropogenically enhanced in surficial sediments from Meg, Keg, Peg Lake and Kam Lake, with concentrations that were one to two orders of magnitude higher than in natural lake water. The probable source of elevated sulfate levels was addition of ferric sulfate to mine tailings (see 'Sample collection and processing', above) as well as atmospheric deposition of sulfuric acid derived from atmospheric SO_2 emissions from the roasting of gold-bearing ores which contained arsenopyrite. The sulfate levels in sediment pore water, therefore, were probably more indicative of the variation in the rate of input through sedimentation and downward diffusion than of instantaneous rates of sulfate-reduction. Hence, the sulfate profiles in sediment pore-water would not be expected to reflect either sulfate-reduction rates or methylarsenic production rates.

If the overall pore water data set is considered, as shown in Table 1, it is interesting to note that MMAA and MMA (pH 6) were significantly correlated (Pearson $r = 0.46$; $P = 0.02$), as were DMAA and DMA (pH 6) (Pearson $r = 0.47$; $P = 0.02$). This lends additional support to the hypothesis that the pH 6 arsenicals are reduced methylated arsinothiols, since a facile interconversion between As(V) methylarsenicals and methylated arsinothiols has been demonstrated [19,22]. This also suggests that direct bacterial methylation may not necessarily account for all methylated arsenicals in pore water. Dimethylarsinothiols, for example, could be a result of exocellular production from DMAA in the presence of reducing conditions (and sulfides) and/or thiolic compounds. Conversely, DMAA could be produced during the upward diffusion of porewater containing dimethylarsinothiols into a more oxic environment.

TMA (pH 6) and MMA (pH 6) tended to co-occur in all sediment pore-water samples (Pearson $r = 0.66$; $P = 0.003$), whereas a significant correlation between the other methylated species was not observed. The variable composition of methylated arsenicals between cores and with sediment depth can probably be attributed to microscale variations in the heterotrophic bacterial communities present, and associated differences in the extent and particulars of arsenic methylation/demethylation.

4. General discussion

In this study, we have demonstrated that a wide range of methylarsenic compounds occurs in the water column and pore water of freshwater environments near Yellowknife, N.W.T. Depth profiles of ΣMeAs compounds in sediment cores, along with corroborative evidence from arsenic methylation by anaerobic microbial cultures [14], indicate that sulfate-reducing bacteria play a role in the arsenic methylation. The results presented here exhibit a strong similarity to recent studies of methylarsenic distributions in marine sediments [4].

Aggett and O'Brien [29] proposed a detailed model of arsenic cycling in lacustrine sediments, and largely discounted earlier hypothetical models by Ferguson and Gavis [30] and Wood [31] which emphasized the role of methylarsenical production in controlling the upward remobilization of arsenic from sediments to the water column. The role of inorganic chemical processes, especially of iron- and manganese-oxyhydroxides, in controlling arsenic distributions in aqueous systems has subsequently been emphasized by a large number of researchers. However, recent research on the presence of MMAA and DMAA in lacustrine and marine waters [1,8], as well as the dominance of organoarsenicals over inorganic arsenic in multicellular aquatic organisms [1,2], suggest an importance of organoarsenicals in the fate and effects of arsenic inputs. The emphasis by most researchers on inorganic arsenic species in examining environmental arsenic cycling is, in part, an artefact of the currently available analytical techniques. This study and research by others [11,12], have provided evidence of exocellular arsenicals that are hidden to hydride-generation techniques, at concentrations in pore water that can exceed the concentration of arsenate + arsenite by a factor of three or more. Clearly, exocellular organoarsenicals and other hidden arsenic species potentially

represent a substantial portion of the total arsenic budget in some ecosystems; the geochemical fate and bioavailability of organoarsenicals has not been clearly elucidated.

Studies undertaken within the last decade of methylarsenic distribution in aquatic environments have emphasized the role of algae as methylators [5,6,8,26] and have focused on the concentrations of arsenite, arsenate, MMAA and DMAA in the water column. It is becoming increasingly clear, however, that the de novo production by bacteria may account for a significant proportion of methylated arsenic levels in aquatic systems, and that arsenic methylation in sediments bears a similarity to mercury methylation [28]. There is no reason to dismiss the role of bacterial arsenic methylation in the photic zone of the water column either.

Acknowledgements

This study was supported by an NSERC-administered Visiting Fellowship in Government Labs to D. Bright and a Department of National Defence Academic Research Program Grant to K. Reimer. We appreciate the assistance of the personnel of the Department of Indian and Northern Affairs, Water Resources Laboratory, Yellowknife — in particular, Bill Coedy. Assistance in the field was also provided by Bill Dushenko, Bernard Lakowski and Catherine Vardy. We thank an anonymous reviewer for helpful suggestions.

References

[1] W.R. Cullen and K.J. Reimer, Arsenic speciation in the environment. Chem. Rev., 89 (1989) 713–764.

[2] S. Maeda, Biotransformation of arsenic in the freshwater environment, in J.O. Nraigu (Ed.), Arsenic in the Environment, Part I: Cycling and Characterization, 1994, J. Wiley and Sons, Inc., N.Y. pp. 155–187.

[3] W.R. Cullen and J.C. Nelson, The biotransformation of monomethylarsonate and dimethylarsinate into arsenobetaine in seawater and mussels. Appl. Organomet. Chem., 7 (1993) 319–327.

[4] K.J. Reimer, The methylation of arsenic in marine sediments. Appl. Organomet. Chem., 3 (1989) 475–490.

[5] J.G. Sanders, Arsenic geochemistry in Chesapeake Bay: Dependence upon anthropogenic inputs and phytoplankton species composition. Mar. Chem., 17 (1985) 329–340.

[6] J.G. Sanders, Arsenic cycling in marine systems. Mar. Environ. Res., 3 (1980) 257–266.

[7] M.O. Andreae, Distribution and speciation of arsenic in natural waters and some marine algae. Deep Sea Res., 24 (1978) 391–402.

[8] L.C.D. Anderson and K.W. Bruland, Biogeochemistry of arsenic in natural waters: the importance of methylated species. Environ. Sci. Technol., 25 (1991) 420–427.

[9] T. Takamatsu, R. Nakata, T. Yoshida and M. Kawashima, Depth profiles of dimethylarsinate, monomethylarsonate and inorganic arsenic in sediment from Lake Biwa. Jpn. J. Limnol., 4 (1985) 93–99.

[10] W.R. Cullen and M. Dodd, The photo-oxidation of solutions of arsenicals to arsenate: a convenient analytical procedure. Appl. Organomet. Chem., 2 (1988) 1–7.

[11] A.G. Howard and S.D.W. Comber, The discovery of hidden arsenic species in coastal waters. Appl. Organomet. Chem., 3 (1989) 509–514.

[12] A.M.M. de Bettencourt and M.O. Andreae, Refractory arsenic species in estuarine waters. Appl. Organomet. Chem., 5 (1991) 111–116.

[13] K.J. Reimer, M. Dodd, W.T. Dushenko and D.A. Bright, Antimony Cycling and Bioavailability in Lakes near Yellowknife, Northwest Territories, 75th Canadian Society of Chemistry Conference, Edmonton, Alberta, 1993.

[14] D.A. Bright, W. Coedy, W.T. Dushenko and K.J. Reimer, Arsenic transport in a watershed receiving gold mine effluent near Yellowknife, Northwest Territories. Canada, Sci. Total Environ., 155 (1994) 237–252.

[15] D.A. Bright, S. Brock, W.R. Cullen, G.M. Hewitt, J. Jafaar and K.J. Reimer, Methylation of arsenic by anaerobic microbial consortia isolated from lake sediment. Appl. Organomet. Chem., 8 (1994) 415–422.

[16] J. Jafaar, The Interaction of Sediment Bacteria with Arsenic Comounds, M.Sc. Thesis, University of British Columbia, 1992, 59 pp.

[17] M.O. Andreae, Determination of arsenic species in natural waters. Anal. Chem., 49 (1977) 820–823.

[18] R.S. Braman, D.L. Johnson, C.C. Forback, J.M. Ammons and J.L. Bricker, Separation and detection of subnanogram quantities of inorganic arsenic and methylarsenic compounds. Anal. Chem., 49 (1977) 621–625.

[19] W.R. Cullen, B.C. McBride and J. Reglinski, The reaction of methylarsenicals with thiols. Some biological implications. J. Inorg. Biochem., 21 (1984) 179–184.

[20] D. Hocking, P. Kuchar, J.A. Plambeck and R.A. Smith, The impact of gold smelter emissions on vegetation and soils of a sub-arctic forest-tundra transition ecosystem. J. Air Pollut. Cont. Assoc., 28 (1978) 133–137.

[21] W.R. Cullen, B.C. McBride and J. Reglinski, The reduction of trimethylarsine oxide to trimethylarsine by thiols: a mechanistic model for the biological reduction of arsenicals. J. Inorg. Biochem., 21 (1984) 45–60.

[22] X.-C. Le, Speciation of Arsenicals, Ph.D. Thesis, University of British Columbia, 1993, 210 pp.

[23] X.-C. Le, W.R. Cullen and K.J. Reimer, Decomposition

of organoarsenic compounds by using a microwave oven and subsequent determination by flow injection-hydride generation-atomic absorption spectrometry. Appl. Organomet. Chem., 6 (1992) 161–171.

[24] P. Thalabalasingam and W.F. Pickering, Arsenic sorption by humic substances. Environ. Pollut. Ser. B, 12 (1986) 233–246.

[25] R.P. Kiene, Evidence for the biological turnover of thiols in anoxic marine sediments. Biogeochemistry, 13 (1991) 117–135.

[26] M.O. Andreae, Arsenic speciation in seawater and interstitial waters: the influence of biological-chemical interactions on the chemistry of a trace element. Limnol. Oceanogr., 24 (1979) 440–452.

[27] N. Caraco, J. Cole and G.E. Likens, A comparison of phosphorus immobilization in sediments of freshwater and coastal marine systems. Biogeochemistry, 9 (1990) 277–290.

[28] C.C. Gilmour, E.A. Henry and R. Mitchell, Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol., 26 (1992) 2281–2287.

[29] J. Aggett and G.A. O'Brien, Detailed model for the mobility of arsenic in lacustrine sediments based on measurements in Lake Oharuki. Environ. Sci. Technol., 19 (1985) 231–238.

[30] J.F. Ferguson and J. Gavis, A review of the arsenic cycle in natural waters. Water Res., 6 (1972) 1259.

[31] J.M. Wood, Biological cycles for toxic elements in the environment. Science, 183 (1974) 1049.