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Dynamics of organic and inorganic arsenic in the solution phase of an acidic fen in Germany

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Abstract

Wetland soils play a key role for the transformation of heavy metals in forested watersheds, influencing their mobility, and ecotoxicity. Our goal was to investigate the mechanisms of release from solid to solution phase, the mobility, and the transformation of arsenic species in a fen soil. In methanol–water extracts, monomethylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, arsenobetaine, and two unknown organic arsenic species were found with concentrations up to 14 ng As g^{-1} at the surface horizon. Arsenate is the dominant species at the 0–30 cm depth, whereas arsenite predominated at the 30–70 cm depth. Only up to 2.2% of total arsenic in fen was extractable with methanol–water. In porewaters, depth gradient spatial variation of arsenic species, pH, redox potentials, and the other chemical parameters along the profile was observed in June together with high proportion of organic arsenic species (up to 1.2 μg As L^{-1}, 70% of total arsenic). Tetramethylarsonium ion and an unknown organic arsenic species were additionally detected in porewaters at deeper horizons. In comparison, the arsenic speciation in porewaters in April was homogeneous with depth and no organic arsenic species were found. Thus, the occurrence of microbial methylation of arsenic in fen was demonstrated for the first time. The 10 times elevated total arsenic concentrations in porewaters in June compared to April were accompanied by elevated concentrations of total iron, lower concentrations of sulfate and the presence of ammonium and phosphate. The low proportion of methanol–water extractable total arsenic suggests a generally low mobility of arsenic in fen soils. The release of arsenic from solid to solution phases in fen is dominantly controlled by dissolution of iron oxides, redox transformation, and methylation of arsenic, driven by microbial activity in the growing season. As a result, increased concentrations of total arsenic and potentially toxic arsenic species in fen porewaters were found in the growing season, suggesting an enhancing risk of arsenic transport of ground- and surface-waters under these conditions.

1. Introduction

Arsenic is a ubiquitous trace metalloid and is found in virtually all environmental media through natural and anthropogenic processes (Adriano, 2001). Arsenic occurs mainly inorganic in the environment with the dominance of arsenate (As(V)) under aerobic and arsenite (As(III)) under anaerobic environments. Methylation of inorganic As species by aerobic and anaerobic microorganisms produce monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO) (Cullen and Reimer, 1989; Sadiq, 1997; Bentley and Chasteen, 2002). Tetramethylarsonium ion (TETRA), arsenobetaine (AsB), arsenocholine (AsC), and arsenosugars, are thought to be originated from biosynthesis, e.g., by alga or microorganisms (Pongratz, 1998; Geiszinger et al., 2002). The toxicity and mobility of As species depends on their chemical forms: inorganic As species are more toxic and less mobile than organic As species (Wauchope, 1975; Holm et al., 1980; Chiu and Hering, 2000; Mandal and Suzuki, 2002). Thus, investigation on the total As (As_{total}) is not sufficient for risk assessment of As in the environment.

The release of As into ground water is thought to be linked to the redox chemistry of iron and sulfur. Under oxic conditions, the release of As may be driven by dissolution of As-rich pyrites through oxidation (Chatterjee

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et al., 1995; Zheng et al., 2004). The occurrence of sulfide under anoxic condition may immobilize As by its high affinity to sulfide minerals or by the formation of As-sulfide minerals (Bostick and Fendorf, 2003). Under anoxic conditions, the release of As associated with Fe and Mn oxides may be important (Nickson et al., 1998; Bhattacharya et al., 2003). Under oxic conditions, As species are immobilized by Fe and Mn oxides by adsorption and coprecipitation (Wilkie and Hering, 1996; Bednar et al., 2005). Wetland soils are characterized by high content of organic matter. The probable interactions of organic matter with As are through the mobilization and immobilization of As species due to the microbial transformation of Fe and S species as controlled by availability of organic matters (Kirk et al., 2004; O'day et al., 2004), through the probably strong affinity of As species to organic matters (Thanabalasingam and Pickering, 1986; Anawar et al., 2003) and through fueling the redox transformation and methylation of As by microorganisms (Oremland et al., 2004). However, the mechanisms of As release and accumulation in wetland soils are still unknown.

In the case of Hg, wetland soils are of special importance for transformation reactions (Weber et al., 1998; Roulet et al., 2001). Reduction or methylation resulting in the formation of methylmercury or volatile compounds like Hg^0 and dimethylmercury influence strongly the mobility and export of organic Hg species from terrestrial catchments (Wallschläger et al., 1995; Lindberg et al., 2001). Methylation of As in soils is a strictly biological processes (Turner, 1949; Wood, 1974) and can be influenced by abiotic factors, such as pH and temperature (Cox, 1975; Huysmans and Frankenberger, 1991) and several methylated As species and AsB were detected in soils (Takamatsu et al., 1982; Tlustoš et al., 2002; Geiszinger et al., 2002). Methylation of As was demonstrated for different aerobic and anaerobic microorganisms, i.e., methanogenic and sulfate-reducing bacteria (Bentley and Chasteen, 2002; Kühnelt and Gössler, 2003). Therefore, the activity of methanogenic and sulfate reducing bacteria in wetland soils (Horn et al., 2003; Küsel and Alewell, 2004) suggests the occurrence of organic As species.

The chemistry of As in the aqueous environment is complex because of its multiple oxidation states and its association with a variety of minerals through adsorption and precipitation. The lack of knowledge about the behavior of As in wetland soils makes it difficult to estimate the transformation between organic and inorganic As species and mobility of As species in wetland soils. Therefore, this investigation focuses on As speciation in the solid phase and in porewaters in a fen soil with the objectives (i) to estimate the occurrence of As methylation, (ii) to identify the release mechanisms of As, (iii) to evaluate the mobility of As species, and (iv) to estimate the environmental relevance of As in wetland soils.

2. Materials and methods

2.1. Site description

The investigated site, Schlöppnerbrunnen I, is a part of Lehstenbach catchment (4.2 km^2 size) in the German Fichtelgebirge Mountains, located at an elevation of 700–880 m a.s.l. at 50° 08′ N, 11° 52′ E. The mountains are located in North East Bavaria. They are North East of Bayreuth and close to the border with the Czech Republic. Mean annual air temperature is 5 °C, and mean annual precipitation is about 1150 mm. The Lehstenbach catchment is dominated by Norway spruce (*Picea abies* [L.] Karst.) stands of different age. Approximately, 30% of the catchment is covered with wetland areas (Gerstberger et al., 2004). The background concentrations of As_{total} in the upland soil at Lehstenbach catchment are low (up to 24.4 μg As g^{-1}, Huang and Matzner, unpublished).

The fen at Schlöppnerbrunnen I is classified as Fibric Histosol (FAO classification) covered with patches of *Sphagnum* mosses and Norway spruce trees. The concentrations of As_{total} range between 3.3 and 4.2 μg As g^{-1} and vary little with depth. The fen is slightly acidic with soil $pH_{(H_2O)}$ of approximately 5 and the carbon content is close to 40% down to 70 cm depth (Table 1). The Al_{total} and Fe_{total} concentrations increase to 35 and 5.1 mg g^{-1} with depth, respectively, whereas the concentrations of Mn_{total} are highest at surface and deep horizons (ca. 66 μg g^{-1}). The oxalate extractable Al, Fe, and Mn is the lowest at 20–30 cm depth and increases with depth.

2.2. Sample collection and preparation

Soil samples from the fen were taken as mixed samples from three profiles at about 1 m distance from each other. Samples were taken with 10 cm intervals to 70 cm depth at the beginning of July, 2004 and sieved to 5 mm. For As speciation, fresh samples (10.0 g) were homogenized and crushed mechanically (Ultra Turrax, IKA Words, USA). After ultrasonic treatment for 10 min, the samples were centrifuged (8800 g) and the supernatant was analysed with HPLC–ICP–MS.

A dialysis chamber made of Plexiglas was used to collect porewaters in high spatial resolution (Fig. 1) (Loy et al., 2004). The dialysis chamber contains 60 small sample chambers with 1 cm distance and with 5 ml volume each. To sample the porewater, each sample chamber was filled with boiled and Ar-purged Milli-Q water, then covered with acetate–cellulose filter (0.2 μm, Schleicher and Schüll) as diffusion membrane and closed with silicon band. To avoid the contact of each chamber with air during the transport, the whole surface of the diffusion chamber was covered with polyethylene membrane and then fastened. The dialysis chamber remained in the fen for 14 days in order to reach an equilibrium state with the porewaters. The dialysis chamber was then taken back to laboratory by covering again with polyethylene membrane. Sampling in fen

Table 1
Carbon contents, pH, total Al, Fe, and Mn concentrations and oxalate extractable Al, Fe, and Mn concentrations in fen soils

Fen	Depth (cm)	$pH_{(H_2O)}$	C (%)	Al_{total} (mg g^{-1})	Fe_{total} (mg g^{-1})	Mn_{total} (μg g^{-1})	Al_{oxo} (mg g^{-1})	Fe_{oxo} (mg g^{-1})	Mn_{oxo} (μg g^{-1})
1	0–10	4.9	39.1	11.2	2.94	67.1	8.82	1.79	45.7
2	10–20	5.1	44.3	11.8	2.38	53.3	7.77	1.14	32.7
3	20–30	5.2	42.6	11.0	2.11	36.9	6.09	0.80	18.0
4	30–40	5.1	37.1	13.1	2.55	43.0	6.22	1.08	19.0
5	40–50	5.1	38.9	12.4	2.65	41.4	6.12	1.23	20.4
6	50–60	5.1	39.3	20.2	3.69	61.9	8.87	1.42	30.8
7	60–70	5.1	34.3	35.2	5.11	65.8	8.47	0.98	22.9

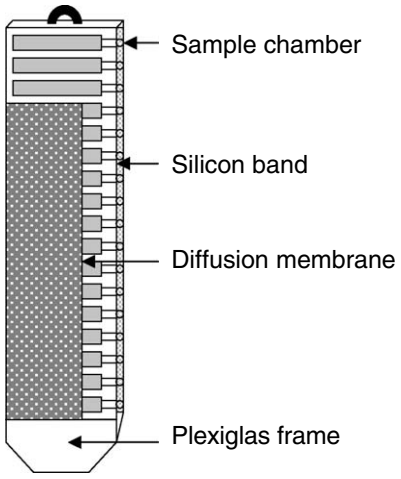

Fig. 1. Dialysis chamber for sampling porewaters of fen (Loy et al., 2004).

at the same place at the same location took place at the end of June, 2004 and the beginning of April, 2005 represented the drying period in June and a period of snow melting, respectively.

For analysis of the porewaters in each chamber, ca. 4.5 ml solution was taken from each chamber with a plastic syringe under Ar atmosphere. One milliliter solution was taken for As speciation using HPLC–ICP–MS (see Section 2.3) and for determination of Cl^-, SO_4^{2-}, NO_3^-, and PO_4^{3-} concentrations using ion chromatography (Dionex 2000i- SP). The remaining solution was moved to a 15 ml polyethylene centrifuge tube for Eh measurements using commercially available Pt-redox electrode fitted with a Ag/AgCl reference electrode (SenTix ORP, WTW) under Ar atmosphere. Afterwards, the solution was used for analysis of pH (glass electrode, WTW pH 90), concentration of Fe_{total}, Mn_{total}, and Al_{total} (ICP-MS, Agilent 7500c, Japan) and NH_4^+ (FIA, MLE, Germany). Dissolved organic carbon (DOC) was determined as CO_2 after combustion (Elementar, HighTOC).

For As speciation in field-moist samples and porewaters, the sampling, preparation and analysis together were finished in 48 h to prevent further transformation of As species (Garcia-Manyes et al., 2002). The mobile fraction of As in fen is defined as methanol–water soluble fraction in this study. The use of 20% (v/v) methanol in the extract was to enhance the extraction of organic As species. At the same time, the methanol–water (20%, v/v) extractable As may also represent the water soluble fraction, because little variation between water and methanol–water extraction was found (Pizarro et al., 2003).

The C-content of the solid phase was determined, using a CHN-analyzer (CHN-O-Rapid, Elementar Germany) and pH using a pH electrode in a 1:2.5 soil/water ratio. For determination of oxalate extractable Al, Fe, and Mn, 1.0 g freeze-dried fen soil was extracted with 0.2 M NH_4-Oxolate solution at pH 3 by shaking in the dark for 2 h. The suspension was then filtered and the concentrations of Al, Fe, and Mn were measured with ICP–OES (Vista Pro, Variant).

2.3. Speciation of arsenic species

Arsenate (As(V)), arsenite (As(III)), and dimethylarsinic acid (DMA) were purchased from Merck. Arsenobetaine (AsB) was obtained from Fluka and monomethylarsonic acid (MMA), arsenocholine (AsC), trimethylarsine oxide (TMAO), and tetramethylarsonium ion (TETRA) as iodide from Argus Chemicals, Italy. De-ionized water used throughout the work was purified in a Milli-Q system (Millipore, Milford, MA). Individual stock solutions (50 mg As L^{-1}) of As(III), As(V), MMA, DMA, AsB, AsC, TMAO, and TETRA were prepared in Milli-Q water and stored at 4 °C in the dark. A multi-compound working solution with a total concentration of 40 μg As L^{-1} was prepared before each use by dilution of the stock solutions with Milli-Q water.

An high performance liquid chromatograph (HPLC) instrument (BIOTEK Instruments, USA), consisting of a gradient pump (System 525), capillary PEEK tubing (0.25 mm i.d.) and a 200-μl injection loop (Stainless Steel), and a HPLC autosampler 465 (Kontron Instruments, Germany) was connected to an anion-exchange column (IonPak AG7 and AS7, both Dionex) and coupled to an ICP-MS (Agilent 7500c, Japan), equipped with a concentric nebulizer (Glass Expansion, Australia) and a Scott-type glass spray chamber. The separation was performed at a flow rate of 1 ml min^{-1}, using a nitric acid gradient between pH 3.4 and 1.8. Dipotassium salt of benzene-1,2-disulfonic acid (0.05 mM) was added to the eluent as an ion-pairing reagent. At the outlet of the separation column, an internal standard (10 μg Ge L^{-1} in 0.01 M nitric acid) was

added by means of an Y-connector. Quality control of As speciation was conducted by using reference material DORM-2 (National Research Council, Canada) with 100% and 92% recovery for water extractable AsB and TETRA, respectively.

2.4. Analysis of total arsenic, aluminum, iron, and manganese

Soil samples (0.5 g dried samples) were first digested with 3 ml nitric acid (65%) and 1 ml hydrochloric acid (30%) by High Pressure Accelerated Solvent (HPA-S, Anton Paar, Austria). In the 3-step program, a first heating to 80 °C, is followed by heating to 170 °C and finally to 270 °C, lasting for 90 min. The supernatant was then filtered with membrane filter, diluted to 25 ml with Milli-Q water for further analysis with ICP-MS (Agilent 7500c, Japan). Recovery of total As from the certificated tomato leaf 1573a (NIST) was 95%.

3. Results

3.1. Arsenic speciation in fen

The methanol–water extractable concentrations of all As species were highest at the surface horizons with concentrations of 83 ng As g^{-1}, representing 2.2% of As_{total} concentrations (Fig. 2). The percentage of methanol–water extractable As was lowest at 70 cm depth (0.5%).

Inorganic As species predominated in the methanol–water extracts (Fig. 2). Arsenate was the dominant As species at the upper 30 cm, whereas As(III) dominated at the deeper horizons. Therefore, the ratios of As(III) to As(V) at the 0–30 cm depth were apparently lower than those at deeper horizons. The concentrations of extractable As(V) were highest at the 0–10 cm depth (ca. 50 ng As g^{-1}) and decreased strongly down to 30 cm depth to 16 ng As g^{-1}. Under 30 cm depth, the concentrations of As(V) varied little. In contrast, the concentrations of extractable As(III) showed little spatial variation at the upper 30 cm with concentrations around 16 ng As g^{-1}. The concentrations of As(III) was highest at 30–50 cm depth (50 ng As g^{-1}) and decreased with depth under 50 cm depth.

Monomethylarsonic acid, DMA, TMAO, and two unidentified organic As species were detected in the methanol–water water extractable fraction (Fig. 2). The concentrations of DMA, AsB and "Unknown 2" were highest at the 0–10 cm depth and decreased with depth. Arsenobetaine was the most abundant organic As species (up to 14 ng As g^{-1}). The concentrations of DMA were much lower compared to AsB (up to 1.6 ng As g^{-1}). Trimethylarsine oxide was mostly found at the 0–50 cm depth with concentrations between 1 and 2 ng As g^{-1}. Monomethylarsonic acid was only observed at the 0–10 cm depth with 0.67 ng As g^{-1} (Data not shown). "Unknown 1" was found at 20–40 cm depth with the highest concentration of 0.6 ng As g^{-1}. The total amounts of organic As species were highest at the 0–10 cm depth, representing 24% of total extractable As. At the deeper horizons, the relevance of organic As species was close to zero.

3.2. Arsenic species and other chemical parameters in porewaters

The As_{total} concentrations in porewaters were quite identical around 0.2 μg As L^{-1} in April (Fig. 3). In June, the concentrations of As_{total} in porewaters varied largely along the fen profile (0.4–2.5 μg As L^{-1}) and were higher than those in April. The As_{total} concentration peaked at the surface with values of 1 μg As L^{-1}. At the 50–60 cm depth, the concentrations of As_{total} increased steeply to 2.4 μg As L^{-1}. The vertical distribution of As(III) was similar to that of As_{total}.

The concentrations of As(III) in porewaters increased slightly with depth from 0.04 to 0.09 μg As L^{-1} in April. The As(III) concentrations in June were an order of magnitude higher related to those in April and peaked at the surface, 10–20 cm depth and 59–60 cm depth with concentrations up to 0.66 μg As L^{-1}. The concentrations of As(V) ranged mostly between 0.1 and 0.3 μg As L^{-1} and decreased slightly with depth. The concentrations of As(V) varied little between the two sampling events, and were higher in June than in April only at the 0–10 cm and 55–60 cm depth. In April, the ratios of As(III) to As(V) increased slightly with depth in porewaters with all values <1. In contrast, the As(III) to As(V) ratios were >1 in most cases in June with the highest values of 4 at the 10–20 cm and 50–60 cm depth.

Organic As species, including MMA, DMA, TMAO, TETRA, AsB, and three unidentified organic As species, were only found in porewaters in June (Figs. 4 and 5). Monomethylarsonic acid was the dominant organic As species with maximum concentrations of 1.2 μg As L^{-1}. Dimethylarsinic acid, AsB and "unknown 1" were the second abundant organic As species with concentrations up to 0.1 μg As L^{-1}. All organic As species showed steeply increased concentrations at the 50–60 cm depth. Monomethylarsonic acid, DMA and TMAO had increased concentrations at the 0–10 cm depth than those at the 10–50 cm depth. In comparison, "unknown 1" and AsB were not detected at the 0–10 cm depth, whereas TETRA (57–60 cm depth), "unknown 2" and "unknown 3" were only observed at the 50–60 cm depth. The proportion of organic As species of As_{total} reached up to 70% at the 59–60 cm depth and were between 30 and 50% at the 0–10 cm and 30–50 cm depth.

A large spatial variation of pH, redox potentials, the other chemical parameters were observed in porewaters in June (Fig. 6). Conversely, these parameters varied little along the profile in April. In June, pH of porewaters had values around 6.0 at the surface and the 10–50 cm depth. The pH values decreased to 5.0 at the 5 cm depth and to 4.5 at the 59–60 cm depth. The redox potentials of porewa-

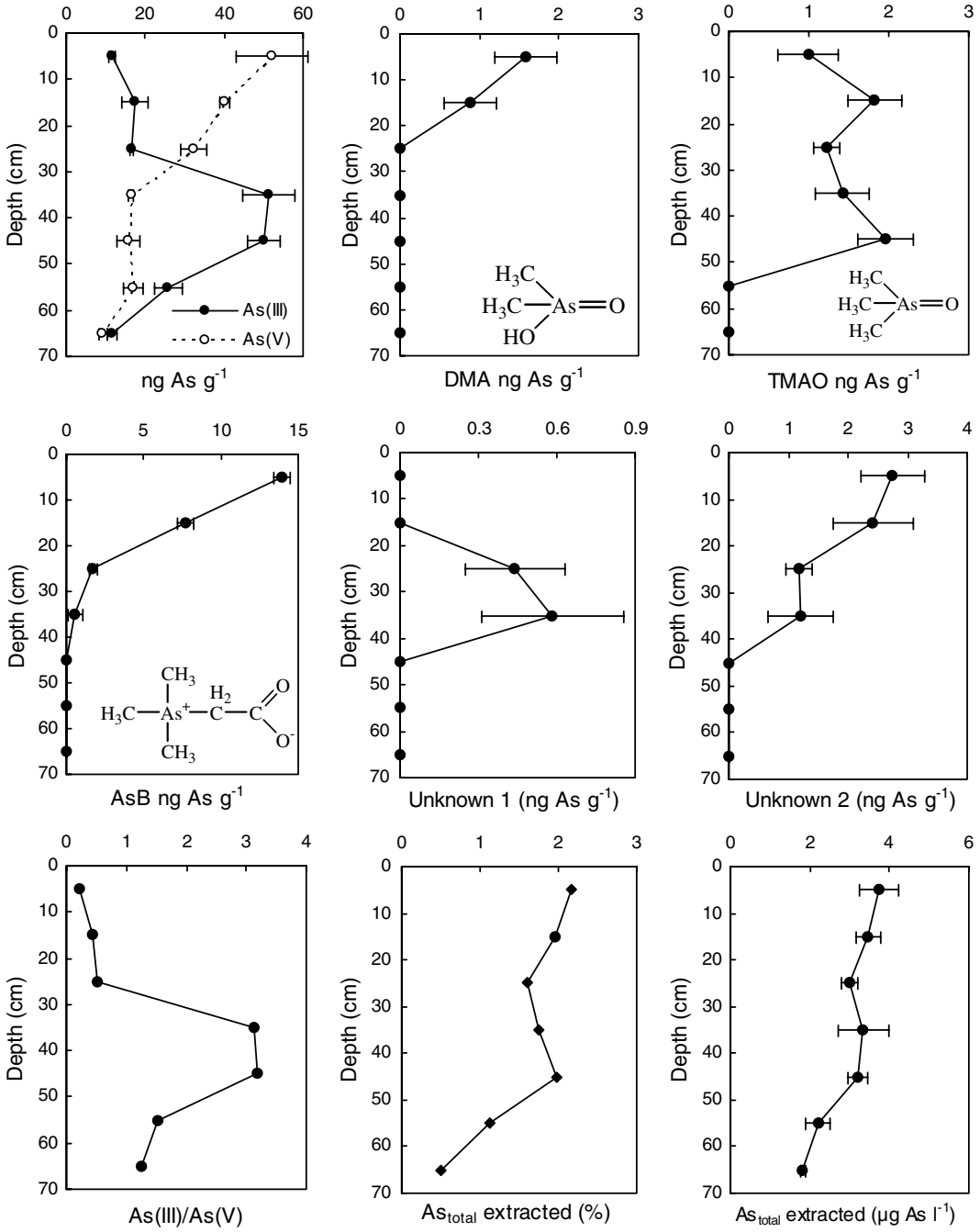

Fig. 2. Concentrations of methanol–water (20% v/v) extractable arsenate (As(V)), arsenite (As(III)), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), arsenobetaine (AsB), and two unidentified organic arsenic species and ratios of extractable arsenite to arsenate, percentage of extracted total As and concentrations of total extractable arsenic in methanol–water extracts in fen. Mean values and SD of three extraction and analytical replicates are shown.

ters increased from 225 mV at the surface and to 400 mV at the 20 cm depth. At 30 cm, 42–45 cm, and 59–60 cm depth, the redox potentials were under 200 mV. In April, pH and redox potentials of porewaters were around 5.0 and 400 mV, respectively. The concentrations of Fe_{total} in April were 20 µg Fe L^{-1}. The concentrations of Fe_{total} in porewaters in June were 2–3 orders of magnitude higher than those in April, ranging between 200 and 800 µg Fe L^{-1} at the first 50 cm, however, increased significantly to 3200 µg Fe L^{-1} at the 59–60 cm depth. The concentrations of Al_{total} and Mn_{total} in fen porewaters in April were around 200 and 50 µg Al, Mn L^{-1}, respectively. In the case of Al_{total}, the concentrations at the 40–60 cm depth in June were higher than those in April. The Mn_{total} concentrations in April were mostly higher than those in June except those at the 55–60 cm depth.

The concentrations of SO_4^{2-} in porewaters in June (up to 1.6 mg L^{-1}) were an order of magnitude lower than in

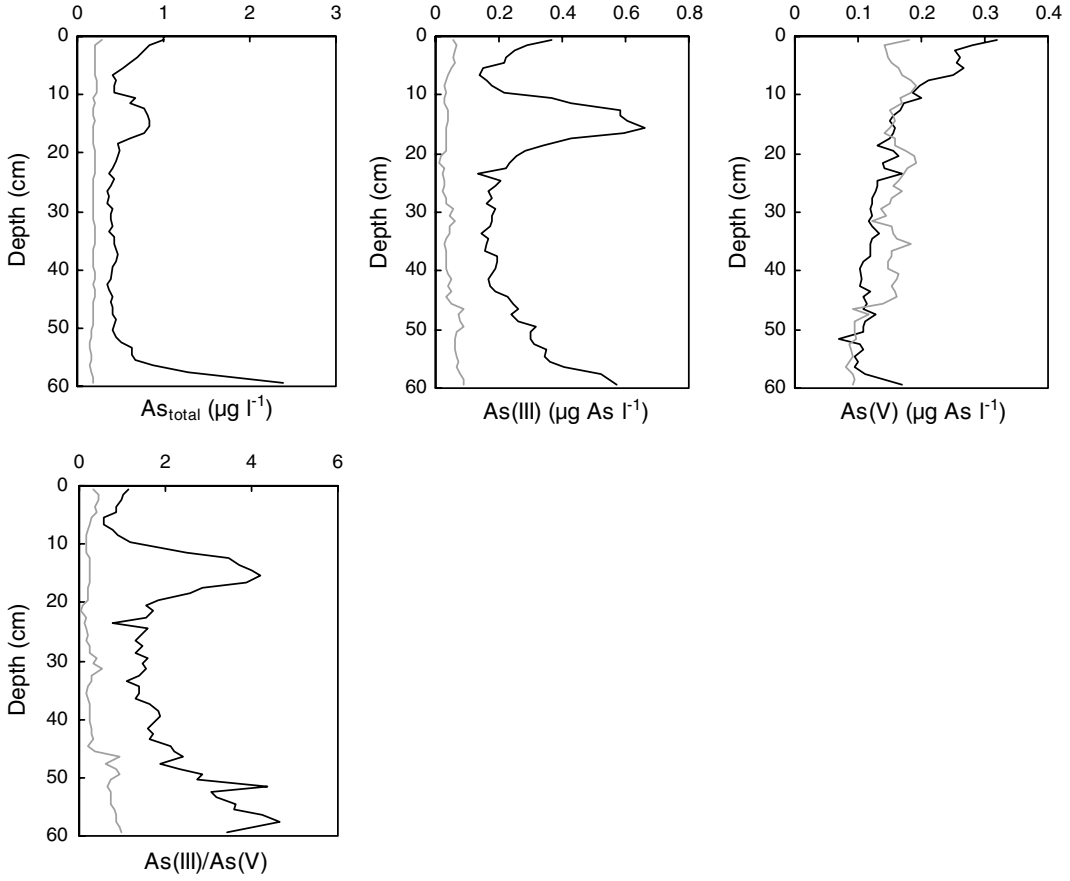

Fig. 3. Vertical profiles of total arsenic, arsenite (As(III)), arsenate (As(V)), and ratios of arsenite to arsenate (As(III)/ As(V)) in fen porewaters. ——: June, 2004, ——: April, 2005.

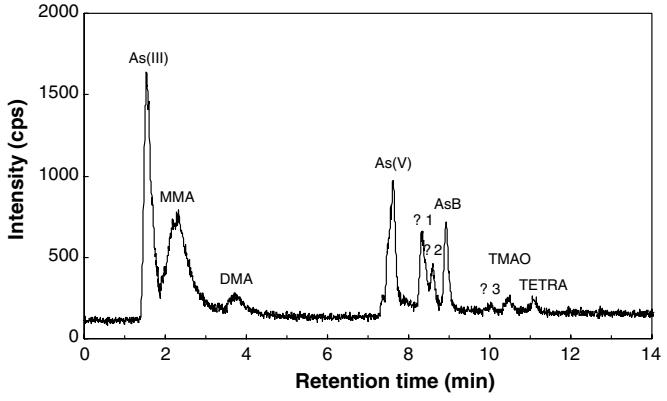

Fig. 4. HPLC-chromatograms of arsenic species in the fen porewater at the 59–60 cm depth in June, 2004.

April. Nitrate was only found in April with concentrations up to 5 mg L^{-1}, whereas PO_4^{3-} and NH_4^+ were detected only in June. The concentrations of DOC were similar in porewaters in June and in April, which were mostly close to the detection limit (=20 mg L^{-1}, based on dilution of 1 ml sample to 20 ml). Only at the 50–60 cm depth, the DOC concentrations in June were apparently higher than those in April.

4. Discussion

4.1. Methylation of arsenic in fen

The methylation of As in fen is demonstrated here for the first time by the abundance and variety of organic As species in porewaters. The absence of NO_3^-, an order of magnitude lower concentrations of SO_4^{2-} and the much higher concentrations of Fe_{total} in porewaters in June compared to those in April reflect the microbial activity for consumption and oxidation of organic matters, resulting in anoxic conditions (O'day et al., 2004). The pH values close to neutral in porewaters in June, which are caused by denitrification and reduction of SO_4^{2-} and NO_3^- with the consumption of protons (Tiedje et al., 1984; Küsel and Alewell, 2004), may favor As methylation (Bissen and Frimmel, 2003). The reduction of SO_4^{2-} in porewaters in June points to the activity of sulfate-reducing bacteria (Loy et al., 2004), which are able to mediate As methylation (Michalke et al., 2000). Besides, the order of abundance of methylated As species (MMA > DMA > TMAO) supports As methylation, since methylation of As follows the pathway: inorganic As species → MMA → DMA → TMAO (Cullen and Reimer, 1989). In comparison, no organic As species was detected in porewaters

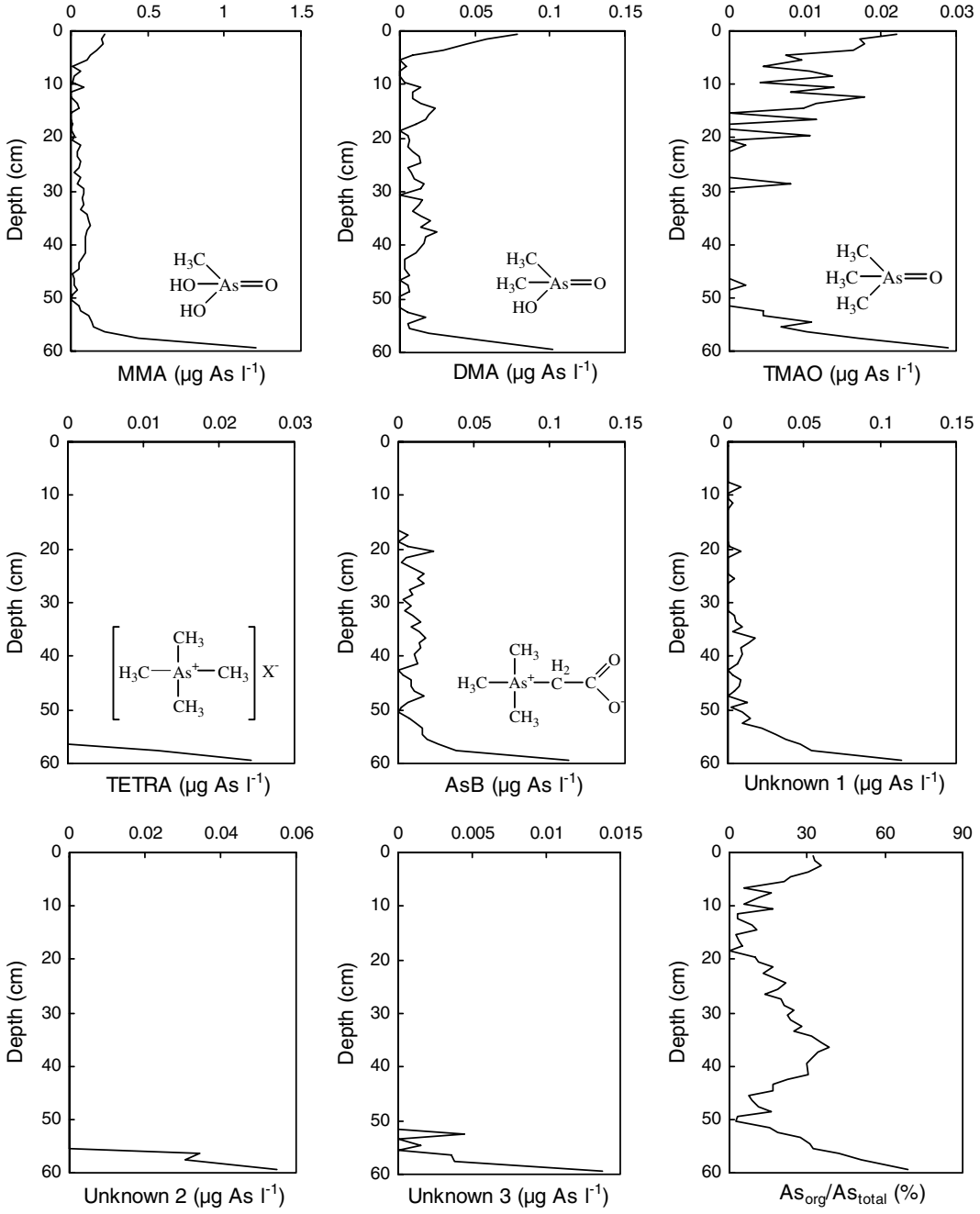

Fig. 5. Vertical profiles of organic arsenic species, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), tetramethylarsonuim ion (TETRA), Arsenobetaine (AsB), three unidentified organic arsenic compounds and percentages of organic arsenic to total arsenic in fen porewaters. ——: June, 2004. No organic arsenic species were detected in the sampling in April, 2005.

in April, indicating the biological nature of As methylation. The occurrence of higher concentrations of SO_4^{2-} related to those in June point to the limiting activity of sulfate-reducing bacteria during the cold period (Küsel and Alewell, 2004).

For the first time, TETRA and AsB were detected in natural waters. The occurrence of TETRA and AsB was so far demonstrated in organisms, like microorganisms and marine and terrestrial plants and animals (Cullen and Reimer, 1989; Kühnelt and Gössler, 2003; Dembitsky and Levitsky, 2004). The occurrence of TETRA, AsB, and the other three unknown organic As species in fen porewaters suggests not only their biosynthesis (Hanaoka et al., 1996) but also the potential release by microorganisms into the solution. The detection of TETRA in porewaters gives a strong support for in situ microbial methylation of As in fen. At our site, we have already detected MMA, DMA, TMAO, AsB, and all three unknown organic As species in different compartments, including throughfall and bulk precipitation (Huang and

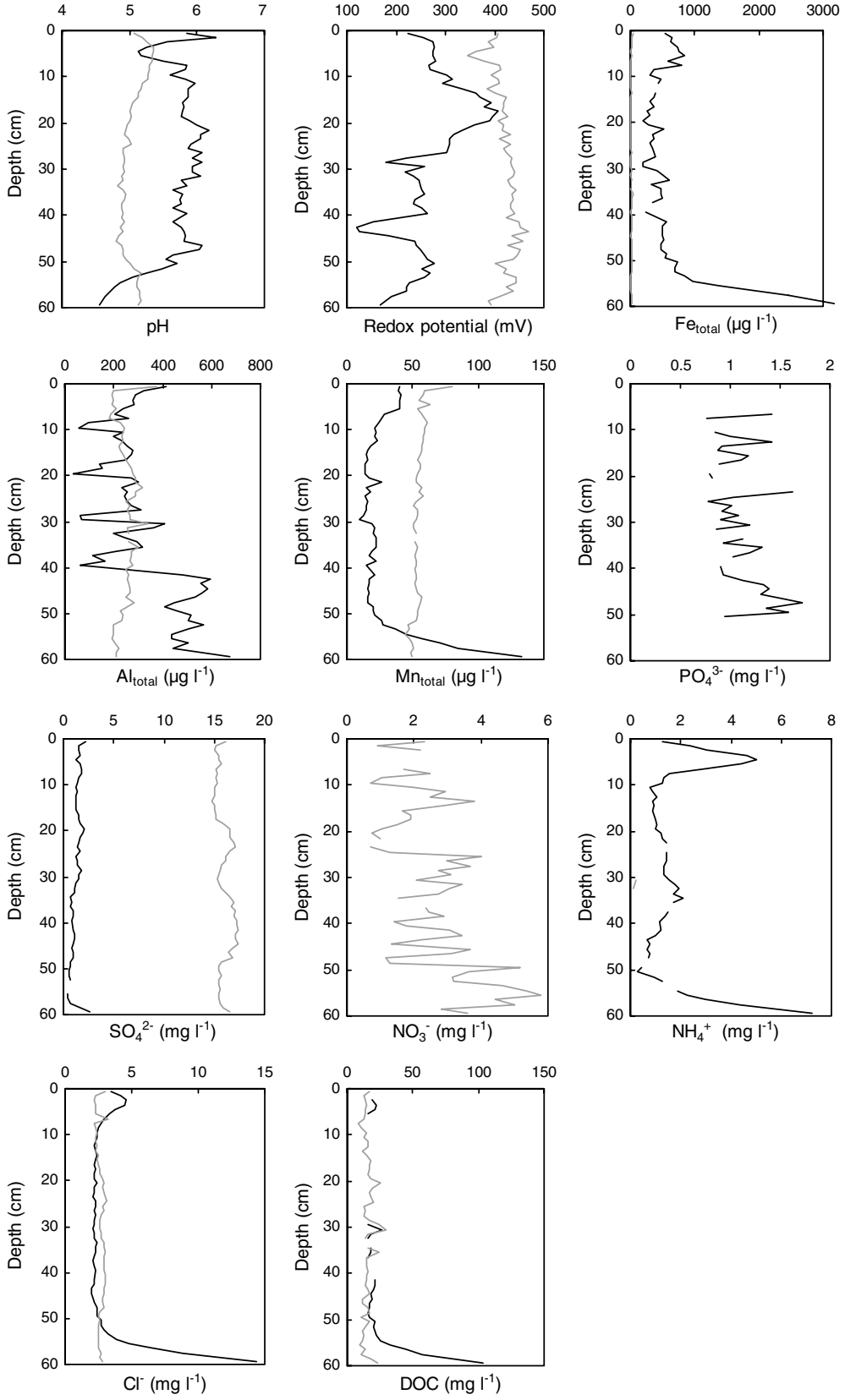

Fig. 6. Vertical profiles of pH, redox potentials and concentrations of Fe_{total}, Mn_{total}, Al_{total}, PO_4^{3-}, SO_4^{2-}, NO_3^-, NH_4^+, Cl^- and dissolved organic carbon (DOC) in the fen porewaters. ——: June, 2004, ——: April, 2005.

Ilgen, 2006; Huang and Matzner, unpublished), but never TETRA.

The in situ methylation of As is additionally demonstrated by the high proportion of organic As species at >55 cm depth. This can not be explained by atmospheric deposition of organic As species. The increased concentrations of Fe_{total}, Mn_{total}, NH_4^+, and DOC at 55–60 cm depth suggest increased microbial activity than the upper horizons. Subsequently, this results in 10 times increased concentrations of total organic As species and their proportion may reach up to 70% of As_{total} in porewater. Besides, three additional organic As species at the depth >55 cm were detected, indicating the high methylation potential of these horizons. Methanogenic bacteria may also be responsible for the methylation potential due to their ability to produce methylated As species (Michalke et al., 2000). The occurrence of methanogenic bacteria at our site was reported (Horn et al., 2003), however, the occurrence of SO_4^{2-} in porewaters in June suggests low activity of methanogenic bacteria (Kirk et al., 2004).

4.2. Redox transformation of arsenic in fen

The spatial and temporal variation of As(III) and As(V) concentrations in fen points out the As redox transformation. The measured redox potentials may be in a good agreement with redox potentials calculated based on As(III) and As(V) concentrations in waters (Yan et al., 2000; Bednar et al., 2005). However, there is no significant correlation between the redox potentials measured and the ratios of As(III) to As(V) in porewaters at both events, suggesting that the ratio of As(III) to As(V) in porewaters did not follow thermodynamic equilibrium. The elevated As(III) to As(V) ratios in porewaters in June indicate the reduction of As(V) during the growing season and the biological nature of As(V) reduction (Jackson et al., 2003; Meng et al., 2003). The different ratios of As(III) to As(V) in methanol–water extracts and porewaters may reflect that As redox transformation occurs mainly in solution phase rather than at the solid phase (Langner and Inskeep, 2000; Zobrist et al., 2000). The dominance of As(V) in porewaters in April indicates the oxidation of As(III) in porewaters (Jackson et al., 2003; Meng et al., 2003). Beside slow rates of As redox reactions in the aquatic environment (Meng et al., 2003), the redox disequilibrium of As species in porewaters may be additionally due to the methylation and demethylation, atmospheric input, drainage of porewaters, and ad-desorption of As species. For example, the slightly higher concentrations of As(V) at surface horizons than those at deep horizons suggest the input of As from snow, since As(V) is highly dominant in snow (Huang and Matzner, unpublished).

4.3. Release of arsenic from solid to solution phases in fen

The results of this study suggest that the release of As from solid to solution phases in fen is determined by (1) dissolution of Fe oxide, (2) redox transformation between As(III) and As(V), (3) different sorption of As species to Fe oxide, and (4) methylation of As.

The concentrations of both As_{total} and Fe_{total} in porewaters were apparently higher in June than in April, suggesting the microbial dissolution of Fe oxide and subsequent release of oxide bounded As. Since the transformation of Fe and As species is strongly associated with microbial activity (Kirk et al., 2004), the release of As in fen seems to be seasonal dependent. The low microbial activity in the cold period limits the dissolution of Fe oxide and the release of As. Under oxic conditions at pH around 5, the occurrence of Fe oxide in the fen profile may provide strong adsorption for As species (Smedley and Kinniburgh, 2002), leading to low As_{total} concentrations with little variation in porewaters. The dissolution of Mn and Al oxides seem to be less important for As release from solid to solution phases, while there is much less difference of Mn_{total} and Al_{total} concentrations in porewaters between two sampling events related to Fe_{total}. The occurrence of PO_4^{3-} in fen porewaters in June seems to reflect the reduction of Fe oxides onto which both As species and PO_4^{3-} are adsorbed (Zheng et al., 2004). Competitive sorption of PO_4^{3-} onto limited Fe oxides surface may also contribute to the release of As.

The elevated concentrations of As_{total} in porewaters in June were partly due to the occurrence of organic As species. Lafferty and Loeppert (2005) investigated the sorption behavior of methylarsonous acid, dimethylarsinous acid, MMA, DMA, As(V), and As(III) on goethite and ferrihydrite. Increased methyl substitution of As species resulted in both decreased adsorbed As at low As concentrations in solution phases and increased As release from the Fe oxide surface. The adsorption of As species to Al oxides, was As(V) > As(III) > MMA = DMA (Xu et al., 1991) In aerobic soils, the order of sorption was As(V) = MMA > DMA (Wauchope, 1975) and in aerobic river sediment, the order of sorption was As(V) > MMA > DMA (Holm et al., 1980). Arsenic methylation can be thus regarded as a process of As mobilization in soils and sediments.

The redox transformation of As can significantly affect the toxicity and mobility of As in soils, sediments and natural waters. More specifically, reduction of As(V) to As(III) may elevate As mobility and toxicity (Cullen and Reimer, 1989). The elevated concentrations of As_{total} in porewaters at surface horizons in June reflect the mobilization of As by reduction of As(V) to As(III) under low redox potential (Eh = 200–300 mV). The increase of As_{total} concentrations in fen porewaters in June at the 10–20 cm depth is mainly due to the increase of As(III) concentrations. However, the redox potentials at the 10–20 cm depth were the highest (Eh = 300–400 mV) along the profile. The apparent predominance and elevated concentrations of As(III) at 10–20 cm depth in the more oxidized porewaters may be due to As(V) being more easily removed than As(III) from the dissolved

phase by adsorption/coprecipitation mechanisms on Fe oxides (Wilkie and Hering, 1996; Bednar et al., 2005).

It was indicated that microbial reduction of SO_4^{2-} may limit the As concentrations in waters (Moore et al., 1988; Kirk et al., 2004). However, elevated concentrations of As_{total} in porewaters were still observed under sulfate-reducing conditions, reflecting the precipitation of As with sulfide is less effective than As release by reductive dissolution of Fe oxides in fen.

4.4. Mobility of arsenic species in fen

The very low percentages of As extracted with methanol–water (<2%) suggests a generally low mobile fraction of As species in fen. The concentrations of As_{total} in methanol–water extracts were higher than those in porewaters, suggesting the additional release of As species with ultrasonic treatment in methanol–water extraction. This also indicates adsorption of organic As species to solid phases in fen. However, the adsorbed organic As species were important only at the upper horizons. The potentially mobile fraction of As in fen is mainly inorganic As species, which coincide with the predominance of inorganic As species in porewaters in most cases. The dominance of As(III) under 30 cm depth reflects decreasing availability of oxygen with depth and the potential higher mobility of As at the deep horizons (Chiu and Hering, 2000; Wolthers et al., 2005).

We have demonstrated the generally low mobility of As species in fen. The mobility of As in fen is strongly linked to the microbial activity, following redox transformations of Fe and As and the methylation of As. Ten times elevated concentrations of As_{total} in fen porewaters in the growing season point to the risk of As transport into surface waters.

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References

Adriano, D.C., 2001. *Trace elements in the terrestrial environments: biogeochemistry, bioavailability and risks of metals*, second ed. Springer, New York.

Anawar, H.M., Akai, J., Komaki, K., Terao, H., Yasjioka, T., Ishizuka, T., Safiullah, S., Kato, K., 2003. Geochemical occurrence of arsenic in groundwater of Bangladesh: sources and mobilization processes. *J. Geochem. Explor.* **77**, 109–131.

Bednar, A.J., Garbarino, J.R., Ranville, J.F., Wildeman, T.R., 2005. Effects of iron on arsenic speciation and redox chemistry in acid mine water. *J. Geochem. Explor.* **85**, 55–62.

Bentley, R., Chasteen, T.G., 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiol. Mol. Biol. R.* **66**, 250–271.

Bhattacharya, P., Tandukar, N., Mukherjee, A.B., Valero, A.A., Jacks, G., 2003. Redox chemistry of arsenic in groundwaters of Terai Belt of Nepal. Oral. 7th international conference on biogeochemistry of trace element, Uppsala, Sweden (June 2003), vol. 2, pp. 10–11.

Bissen, M., Frimmel, F.H., 2003. Arsenic—a review. Part I: occurrence, toxicity, speciation, mobility. *Acta Hydrochim. Hydrobiol.* **31**, 9–18.

Bostick, B.C., Fendorf, S., 2003. Arsenite sorption on troilite (FeS) and pyrite (FeS_2). *Geochim. Cosmochim. Acta* **67**, 909–921.

Chatterjee, A.D., Das, D., Mandal, B.K., Chowdhury, T.R., Samanta, G., Chakraborty, D., 1995. Arsenic in ground water in 6 districts of West Bengal, India—the biggest arsenic calamity in the world. A. Arsenic species in drinking water and urine of the affected people. *Analyst* **120**, 643–650.

Chiu, V.Q., Hering, J.G., 2000. Arsenic adsorption and oxidation at manganite surfaces. 1. Method for simultaneous determination of adsorbed and dissolved arsenic species. *Environ. Sci. Technol.* **34**, 2029–2034.

Cox, D.P., 1975. Microbiological methylation of arsenic. In: Woolson, E.A. (Ed.), Arsenical pesticides, ACS Symp. Ser 7, 81–96.

Cullen, W.R., Reimer, K.J., 1989. Arsenic speciation in the environment. *Chem. Rev.* **89**, 713–764.

Dembitsky, V.M., Levitsky, D.O., 2004. Arsenolipids. *Prog. Lipid Res.* **43**, 403–448.

Garcia-Manyes, S., Jiménez, G., Padró, A., Rubio, R., Rauret, G., 2002. Arsenic speciation in contaminated soils. *Talanta* **58**, 97–109.

Geiszinger, A., Gössler, W., Kosmus, W., 2002. Organoarsenic compounds in plants and soil on top of an ore vein. *Appl. Organomet. Chem.* **16**, 245–249.

Gerstberger, P., Foken, T., Kalbitz, K., 2004. The Lehstenbach and Steinkreuz catchments in NE Bavaria, Germany. *Ecol. Stud.* **172**, 15–44.

Hanaoka, K., Dote, Y., Yosida, K., Kaise, T., Kuroiwa, T., Maeda, S., 1996. Arsenic compounds accumulated in sedimentary microorganisms cultivated in media containing several arsenicals. *Appl. Organomet. Chem.* **10**, 683–688.

Holm, T.R., Anderson, M.A., Stanforth, R.R., Iverson, D.G., 1980. Influence of adsorption on the rates of microbial-degradation of arsenic species in sediments. *Limnol. Oceanogr.* **25**, 23–30.

Horn, M., Matthies, C., Küsel, K., Schramm, A., Drake, H.L., 2003. Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl. Environ. Microb.* **69**, 74–83.

Huang, J.-H., Ilgen, G., 2006. Factors affecting arsenic speciation in environmental samples: sample drying and storage. *Int. J. Environ. Anal. Chem.* **86**, 347–358.

Huysmans, K.D., Frankenberger Jr., W.T., 1991. Evolution of trimethylarsine by a *Penicillium* sp. isolated from agricultural evaporation pond water. *Sci. Total Environ.* **105**, 13–28.

Jackson, C.R., Jackson, E.F., Dugas, S.L., Gamble, K., Williams, S.E., 2003. Microbial transformation of arsenite and arsenate in natural environments. *Recent Res. Dev. Microbiol.* **7**, 103–118.

Kirk, M.F., Holm, T.R., Park, J., Jin, Q., Sanford, R.A., Fouke, B.W., Bethke, C.M., 2004. Bacterial sulphate reduction limits natural arsenic contamination in groundwater. *Geology* **32**, 953–956.

Kühnelt, D., Gössler, W., 2003. Organoarsenic compounds in the terrestrial environment. In: Craig, P.J. (Ed.), *Organometallic Compounds in the Environment*. Wiley, NY, pp. 223–275.

Küsel, K., Alewell, C., 2004. Riparian zones in a forested catchment: hot spots for microbial reductive processes, Germany. *Ecol. Stud.* **172**, 377–395.

Lafferty, B.J., Loeppert, R.H., 2005. Methyl arsenic adsorption and desorption behavior on iron oxides. *Environ. Sci. Technol.* **39**, 2120–2127.

Langner, H.W., Inskeep, W.P., 2000. Microbial reduction of arsenate in the presence of ferrihydrite. *Environ. Sci. Technol.* **34**, 3131–3136.

Lindberg, S.E., Wallschläger, D., Prestbo, E.M., Bloom, N.S., Price, J., Reinhart, D., 2001. Methylated mercury species in municipal waste landfill gas sampled in Florida, USA. *Atmos. Environ.* **35**, 4011–4015.

Loy, A., Küsel, K., Lehner, A., Drake, H.L., Wagner, M., 2004. Microarray and functional gene analyses of sulfate-reducing prokaryotes in low-sulfate, acidic fens reveal cooccurrence of recognized genera and novel lineages. *Appl. Environ. Microbiol.* **70**, 6998–7009.

Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: a review. *Talanta* **58**, 201–235.

Meng, X.G., Jing, C.Y., Korfoatis, G.P., 2003. A review of redox transformation of arsenic in aquatic environments. In: Cai, Y., Braid, O.C. (Eds.), *Biogeochemistry of Environmentally Important Element*. American Chemical Society, Washington, DC, pp. 70–83.

Michalke, K., Wickenheiser, E.B., Mehring, M., Hirner, A.V., Hensel, R., 2000. Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. *Appl. Environ. Microbiol.* **66**, 2791–2796.

Moore, J.N., Flcklin, W.H., John, C., 1988. Partitioning of arsenic and metals in reducing sulfidic sediments. *Environ. Sci. Technol.* **22**, 432–437.

Nickson, R., McArthur, J., Burgess, W., Ahmed, K.M., Ravenscorft, P., Rahman, M., 1998. Arsenic poisoning of Bangladesh groundwater. *Nature* **395**, 338.

O'day, P.A., Vlassopoulos, D., Root, R., Rivera, N., 2004. The influence of sulfur and iron on dissolved arsenic concentrations in the shallow subsurface under changing redox conditions. *Proc. Natl. Acad. Sci. USA* **101**, 13703–13708.

Oremland, R.S., Stolz, J.F., Hollibaugh, J.T., 2004. The microbial arsenic cycle in Mono lake, California. *FEMS Microbiol. Ecol.* **48**, 15–27.

Pizarro, I., Comez, M., Camara, C., Palacios, M.A., 2003. Arsenic speciation in environmental and biological samples. Extraction and stability studies. *Anal. Chim. Acta* **495**, 85–98.

Pongratz, R., 1998. Arsenic speciation in environmental samples of contaminated soil. *Sci. Total Environ.* **224**, 133–141.

Roulet, M., Guimaraes, J.-R.D., Lucotte, M., 2001. Methylmercury production and accumulation in sediments and soils of an Amazonian floodplain—effect of seasonal inundation. *Water Air Soil Pollut.* **128**, 41–60.

Sadiq, M., 1997. Arsenic chemistry in soils: an overview of thermodynamic predictions and field observations. *Water Air Soil Pollut.* **93**, 117–136.

Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* **17**, 517–568.

Takamatsu, T., Aoki, H., Yoshida, T., 1982. Determination of arsenate, arsenite, monomethylarsenate and dimethylarsinate in soil polluted with arsenic. *Soil Sci.* **133**, 239–246.

Thanabalasingam, P., Pickering, W.F., 1986. Arsenic sorption by humic acids. *Environ. Pollut. B* **12**, 233–246.

Tiedje, J.M., Sexstone, A.J., Parkin, T.B., Revesbech, N.P., Shelton, D.R., 1984. Anaerobic processes in soil. *Plant Soil* **76**, 197–212.

Tlustoš, P., Gössler, W., Száková, J., Balík, J., 2002. Arsenic compounds in leaves and roots of radish grown in soil treated by arsenite, arsenate and dimethylarsinic acid. *Appl. Organomet. Chem.* **16**, 216–220.

Turner, A.W., 1949. Bacterial oxidation of arsenite. *Nature* **164**, 76–77.

Wallschläger, D., Hintelmann, H., Evans, R.D., Wilken, R.D., 1995. Volatization of dimethylmercury and elemental mercury from river Elbe Flood plan soils. *Water Air Soil Pollut.* **80**, 1325–1329.

Wauchope, R.D., 1975. Fixation of arsenical herbicides, phosphate, and arsenate in alluvial soils. *J. Environ. Qual.* **4**, 355–358.

Weber, J.H., Evans, R., Jones, S.H., Hines, M.E., 1998. Conversion of mercury(II) into mercury(0), monoethylmercury cation, and dimethylmercury in saltmarsh sediment slurries. *Chemosphere* **36**, 1669–1687.

Wilkie, J.E., Hering, J.G., 1996. Adsorption of arsenic onto hydrous ferric oxide: effects of adsorbate-adsorbent ratios and cooccurring solutes. *Colloid Surface A* **107**, 97–124.

Wolthers, M., Charlet, L., van der Weijden, C.H., van der Linde, P.R., Rickard, D., 2005. Arsenic mobility in the ambient sulfidic environment: sorption of arsenic(V) and arsenic(III) onto disordered mackinawite. *Geochim. Cosmochim. Acta* **69**, 3483–3492.

Wood, J.M., 1974. Biologocal cycles for toxic elements in the environment. *Science* **183**, 1049–1052.

Xu, H., Allard, B., Grimvall, A., 1991. Effects of acidification and natural organic materials on the mobility of arsenic in the environment. *Water Air Soil Pollut.* **57–58**, 269–278.

Yan, X.-P., Kerrich, R., Hendry, M.J., 2000. Distribution of arsenic(III) and arsenic(V) and total arsenic in porewaters from a thick till and clay-rich aquitard sequence, Saskatchewan, Canada. *Geochim. Cosmochim. Acta* **62**, 2637–2648.

Zheng, Y., Stute, M., van Geen, A., Gavrieli, I., Dhar, R., Simpson, H.J., Schlosser, P., Ahmed, K.M., 2004. Redox control of arsenic mobilization in Bangladesh groundwater. *Appl. Geochem.* **19**, 201–214.

Zobrist, J., Dowdle, P.R., Davis, J.A., Oremland, R.S., 2000. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. *Environ. Sci. Technol.* **34**, 4747–4753.