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Microbial effects on the release and attenuation of arsenic in the shallow subsurface of a natural geochemical anomaly

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ABSTRACT

Critical factors leading to arsenic release and attenuation from the shallow subsurface were studied with multidisciplinary approach in the natural gold–arsenic geochemical anomaly at Mokrsko (Czech Republic). The results show that microbial reduction promotes arsenic release from Fe(III) (hydr)oxides and Fe(III) arsenates, thereby enhancing dissolved arsenic in the shallow groundwater at average concentration of 7.76 mg/L. In the organic-rich aggregates and wood particles, however, microbial sulfate reduction triggers the formation of realgar deposits, leading to accumulation of As in the distinct organic-rich patches of the shallow subsurface. We conclude that precipitation of realgar in the shallow subsurface of soil/sediment depends on specific and non-trivial combination of water and rock chemistry, microbial community composition and spatial organisation of the subsurface zone, where speciation in saturated environments varied on a centimeter scale from reduced (decomposed wood, H_2S and realgar present) to oxidized (goethite and arsenate minerals are present).

1. Introduction

With elevated levels of arsenic (As) being detected in groundwater, most notably in Bangladesh, West Bengal and other Southeast Asian countries (Charlet and Polya, 2006), United States (Welch et al., 2000), and Europe (Lindberg et al., 2006), As has emerged as a serious health concern worldwide.

Among the many different mechanisms proposed to explain As mobilization into the groundwater, reductive dissolution of As-bearing Fe(III) (hydr)oxides has gained particular attention, with several studies providing evidence that dissimilatory Fe(III)-reducing microorganisms can play a critical role in mediating As mobilization (Islam et al., 2004; Smedley and Kinniburgh, 2002). The amount of As released into solution due to the microbial reductive dissolution of Fe(III) minerals has been shown to depend on the As oxidation state and Fe(III) mineralogy (Campbell et al., 2006; Islam et al., 2004; Tufano et al., 2008; Zobrist et al., 2000). Furthermore, several microbiological studies, e.g. Campbell et al. (2006), Kocar et al. (2006), Tufano et al. (2008) and Zobrist et al. (2000), have suggested that As release via the reductive dissolution of As-bearing Fe(III) (hydr)oxides is negligible under abiotic laboratory simulations, but significant through direct enzymatic reduction of sorbed As(V). Their observations indicated that As retention and release from Fe(III) (hydr)oxides is controlled by complex pathways of Fe biotransformation and that reductive dissolution of Fe(III) (hydr)oxides can promote As sequestration rather than desorption and release into the solution. Direct reduction of As(V) to As(III) is another mechanism by which As may be mobilized. Arsenate is usually not reduced abiotically by any other reductant than sulfide (Rochette et al., 2000), and the presence of As(III) in anoxic, but sulfide-depleted, waters has been attributed to microbial As(V) reduction (Nicholas et al., 2003; Páez-Espino et al., 2009). There are two known microbial pathways for direct reduction of As(V) to As(III). The respiratory pathway (*arrA* pathway) couples the oxidation of an organic substrate with As(V) reduction, resulting in cell growth (Malasarn et al., 2004). Alternatively, microbial reduction of As(V) may occur through a detoxification pathway (*arsC* pathway), during which organisms do not acquire energy for growth (Nicholas et al., 2003; Páez-Espino et al., 2009).

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Somewhat less attention has been paid to factors that remove As from solution under anoxic conditions, such as precipitation with sulfide, a reaction observed in laboratory studies of microorganisms and in shallow aquifers (Kirk et al., 2004; Ledbetter et al., 2007; McCreadie et al., 2000; O'Day et al., 2004). For these reasons, As concentrations in groundwaters can also be lowered by precipitation with sulfide produced by microbial sulfate reduction (O'Day et al., 2004; Héry et al., 2010; Kirk et al., 2004), and here evolutionarily highly conserved *dsrB* functional gene offers a potentially useful genetic marker for sulfate-reducing microorganisms.

Given the complexity of As release into solution, and the numerous parameters that can regulate it, a multidisciplinary approach using complementary microbiological, mineralogical and geochemical techniques has been used in the present study to identify critical factors promoting this process in the shallow subsurface of the natural geochemical anomaly in the central Czech Republic. The study site is a natural As–Au geochemical anomaly at Mokrsko (central Czech Republic) that is well known to have high concentrations of As present in shallow groundwater (0.13–1.14 mg/L) (Drahota et al., 2009).

The aims of the study were to: (i) explore the association of As with the solid phase in two contrasting shallow subsurface environments; (ii) characterize the microbial communities present, with particular emphasis on their functions (including chemoautotrophic As(III) oxidation, As(V) detoxification, dissimilatory As(V) respiration, and sulfate reduction); and (iii) investigate the biogeochemical processes associated with As mobilization/attenuation by developing suitable microcosm experiments.

2. Methods

2.1. Sample collection

The sampling site was located in the vicinity of the village of Mokrsko, Czech Republic, at N 49°44′42.93″ and E 14°20′0.69″, in area known to have elevated concentrations of As present in shallow groundwaters (Drahota et al., 2009). To represent the characteristic end-members of the range of the shallow subsurface conditions found in the surroundings of the village, two contrasting samples were chosen for this study. They were collected in triplicate from a depth of one meter. Sample R (regolith) was disintegrated granodiorite material within the range of water table fluctuations, whereas sample S was organic-rich stream sediment from the permanently saturated hyporheic zone. Samples were immediately transferred from the auger to N_2-filled sterile polyethylene bags, de-aerated with a stream of N_2, packed under ice, and transported to the laboratory where they were immediately homogenized using an acid-washed 2 mm nylon sieve in a glove bag under N_2 atmosphere. For microcosm experiments and DNA extraction, both samples were processed within two hours after their removal from the auger. For mineralogical and geochemical characterization, samples were freeze-dried and stored frozen in an N_2 atmosphere until geochemical and mineralogical data collection (within 1 month).

One-meter-long suction lysimeters were installed directly in the holes previously excavated for regolith and sediment samples and left to settle for more than six months. Groundwater pH, Eh, and specific conductivity were measured in the field, following stabilization, with calibrated portable multimeters (WTW). The field-measured Eh values were corrected to be presented relative to the standard hydrogen electrode. Groundwater samples were divided into precleaned HDPE or glass bottles and analyzed within four days, unless noted otherwise.

2.2. Microcosm experiments

Microcosms were set up by mixing 50 g of wet solid samples with 1 L of sterile synthetic groundwater (16 mg/L KNO_3, 240 mg/L $CaCO_3$, 110 mg/L $MgSO_4 \cdot 7H_2O$, 0.72 mg/L KH_2PO_4, with pH adjusted to 7.1 with $NaHCO_3$) in 1-L bottles. Five different types of microcosms were prepared for regolith and sediment samples: (i) aerobic with no amendment, (ii) aerobic with no amendment and poisoned with azide (10 mM sodium azide); (iii) anaerobic with no amendment, (iv) anaerobic with 50 mM glucose amendment, and (v) anaerobic with 50 mM glucose amendment and poisoned with azide (10 mM sodium azide). Aerobic conditions were maintained by the clamps with a 0.2 μm membrane to allow the free oxygen into the microcosm. Anaerobic conditions were maintained by sealing the bottles with a butyl rubber stopper, sealed with clamps and stored in a glove box under N_2 atmosphere. Each microcosm was performed in triplicate and incubated at 23 ± 2 °C in the dark for 29 days.

Slurry samples (c. 10 mL) were removed from the bottles for analysis on days 0, 2, 5, 9, 14, 19, 24, and 29 using sterile stainless steel hypodermic needles and syringes. Arsenic, Fe, and S in filtered (0.45-μm) and stabilized (2% HNO_3, Suprapure, Merck) samples was determined by ICP OES; changes in solid-phase As and Fe speciation after 29 days of incubation were tracked by sequential extraction.

2.3. Analysis

2.3.1. Regolith/sediment samples

Solid phase concentrations of major and minor elements were determined using an X-ray fluorescence spectrometer (XRF; ARL 9400 XP^+, Thermo ARL). The total organic carbon (TOC) and S were quantified using an ELTRA CS 530 total carbon analyzer and ELTRA CS 500 total inorganic carbon. All analyses were conducted in triplicate to assess the reproducibility, which was found to be within 11% and 3% for XRF and ELTRA, respectively. In order to characterize As-bearing minerals in the regolith and sediment, heavy minerals were separated using bromoform diluted with 1,4-dioxane ($d = 2.81$ g/cm^3) in the 0.1–0.5 mm fraction. Mineral identification of separated particles was undertaken by X-ray diffraction (XRD) using a PANalytical X'Pert Pro diffractometer with CuKα radiation (40 kV, 30 mA, step scanning at 0.02°/200 s in the range 5–70° 2θ). A scanning electron microscope (SEM; CamScan S4) equipped with an energy dispersive spectrometer (EDS; Oxford Link) was used to image the heavy mineral fraction and semi-quantitatively analysed mineral grains. The distribution of As, Fe, and S within different fractions of the solid phases was determined by sequential extraction, adapted from the method detailed by Wenzel et al. (2001). Extractants were used in the following order: (i) 0.05 M ammonium sulfate; (ii) 0.05 M ammonium dihydrogen phosphate; (iii) 0.2 M ammonium oxalate, pH 3 in the dark; and (iv) 0.2 M ammonium oxalate, pH 3 at 80 °C. To minimize sample oxidation, extractions were performed in a glove box under N_2 atmosphere. All extractions were conducted in triplicate to assess the reproducibility, which was found to be within 27% (avg. 10%).

2.3.2. Aqueous samples

Major cations (Ca, Mg, K, Na, Mn, Al, Fe) were analyzed by ICP OES (IRIS Intrepid II XPS), after the stabilization by 2% HNO_3 (Suprapure, Merck). Field and laboratory duplicates indicated a relatively high level of reproducibility (<8%). Groundwater As species were determined by HPLC-ICP MS (Agilent 7700x ICP MS with Agilent 1200 Series HPLC isocratic pump). The precision of the results was approximately 5%. Groundwater H_2S was preserved with Zn acetate prior to determination by the methylene blue method (APHA, 1998). Aliquots of groundwater were added to 1,10-phenanthroline solutions for Fe^{2+} determination (APHA, 1998). Sulfide and Fe^{2+} concentrations were determined within three hours after their collection on a Cintra 3300 UV–Visible Spectrophotometer (GBC) with a precision of ±15%. Anions (SO_4^{2-}, Cl^-, NO_3^-) were analyzed by ion chromatography (HPLC, column Dionex ICS-2000), with a precision of about ±8%; dissolved organic carbon (DOC) was determined by a TOC-VW analyzer (Shimadzu); the alkalinity was determined by titration (TitroLine Easy, Schott) with 0.1 M H_2SO_4.

2.3.3. Microbiological samples

Approximately 250 mg of each sample was collected from 50 g of homogenized material for DNA extraction. Genomic DNA was isolated using a ZR soil microbe DNA kit (Zymo Research). An approximately 860 bp-long fragment of 16S rDNA from *Bacteria* and *Archaea* was amplified following Turner et al. (1999). A 160–200 bp-long fragment of dissimilative arsenate reductase gene (*arrA*) was amplified following the touchdown protocol of Malasarn et al. (2004). An approximately 540 bp-long fragment of arsenite oxidase gene (*aioA*) was amplified following Inskeep et al. (2007). A 350 bp fragment of respiration sulfite reductase (*dsrB*) was amplified using the semi-nested PCR protocol of Foti et al. (2007). LA DNA polymerase (Top-bio) was used for all the PCR reactions. For primer sequences see Supplementary Information (Table S1).

PCR products were purified using the Zymoclean gel DNA recovery kit (Zymo Research) and TA cloned into pGEM-T easy vector (Promega) using chemocompetent *Escherichia coli* TOP10 cells. Approximately 50 randomly selected clones of 16S rDNA and 20 clones of each metabolic gene from each sample were reamplified and sequenced on a 3100 Genetic Analyzer (Applied Biosystems).

The newly determined sequences were deposited in the GenBank database under accession numbers KC149573 to KC149744. The newly determined sequences of *arrA* gene are listed in the Supplementary information (Table S2).

2.4. Data analysis

2.4.1. Equilibrium modeling calculation

Equilibrium aqueous speciation and mineral saturation states were calculated using the Geochemist's Workbench software package (Bethke and Yeakel, 2010), version 8.0.12, with a modified version of the thermodynamic database (thermo.dat) by Kirk et al. (2010). We added data for the sulfate form of green rust from Bourrie et al. (1999). The table of reactions for aqueous As species and Fe and As minerals (Table S3) included in the modified dataset is available in the Supplementary information.