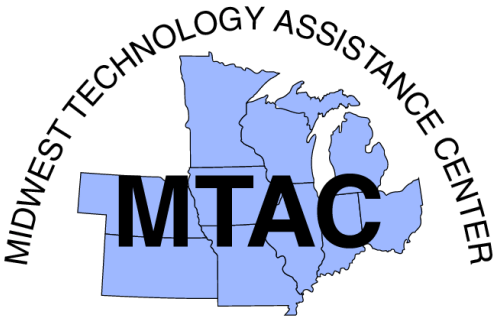

Fate of Arsenic in the Mahomet Aquifer; The Influence of Added Sulfate and Nitrate

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Fate of Arsenic in the Mahomet Aquifer: The Influence of Added Sulfate and Nitrate

Final Report
Midwest Technology Assistance Center

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Introduction

Material from the Mahomet aquifer, known to have pockets of high arsenic concentration, was evaluated for different microbiological activities that could influence the mobility of arsenic in groundwater. Project activities included a thorough assessment of core material collected in a region of the aquifer known for high arsenic concentration and flow-through column experiments evaluating the potential nature of arsenic mobilization in the subsurface.

Materials and Methods

Core material derived from both Glasford and Mahomet aquifer sands was collected in May 2007. A borehole was drilled just north of Minier, IL, using the Illinois State Geological Survey drill rig. Samples were collected from 25 intervals, including till layers above the Glasford and Mahomet aquifers. Representative material from each core was collected for microcosm studies, molecular work, and chemical extractions. The multiple samples allowed for the generation of a vertical library of sample types within the Glasford, Banner, and Sankoty sand formations. Table 1 summarizes the description and location of core materials collected. Sand material in all three sand formations looked similar except in one location. The core sample collected at the bottom of the Glasford formation (93-100 feet) contained three different types of material. All three were collected for further analysis. In the middle of the core we observed a 1- to 2-foot red (iron oxide rich) section of saturated sand, below which was a 1-foot section of gray sand (presumably reduced). The gray reduced sand layer occurred right above the boundary till layer. In general, when good core material was obtained, samples were collected for microbial analysis (frozen in the field), microbial culturing (placed on ice), and for bulk sediment collection to prepare for column experiments.

Table 1. Summary of Sediment Samples Collected from a Mahomet Aquifer Borehole

Formation	Interval ID	Depth taken (feet)	Sediment Description
Boundary		55-67	Till
		66	Till
Glasford	1	75-80	Sand
	2	80-85	Sand
		86	Sand
	3	86.5-87.5	Coarse to fine sand transition
	4	87.5-90	Sand
		89	Coarse gravel
		89.5	Sand
	5	90-93.5	Sand
	6,7	93-100	Sand (orange)

	8	93-100	Sand (gray)
		93-100	Transition to till
Boundary		93-100	Till just below Glasford
		158-161	Till
Banner	9	175-177.5	Sand and gravel
		180-185	Sand
		185-190	Sand
		195-200	Sand
		205-210	Sand
		210-215	Sand
		210-215	Sand
		217-225	Sand
Sankoty		230-235	Sand
		230-235 b/s	Sand

Microcosms

To evaluate the microbial activity potential associated with the aquifer sediments, microcosms containing core material, a small amount of acetate plus formate (<1 millimolar [mM] each) as electron donors, and either ferric iron (Fe(III)) or sulfate as electron acceptors, were prepared. Fe(III)-reducing microcosms were supplemented with ferric citrate (5 mM). Sulfate-reducing microcosms received 2 mM sodium sulfate. Duplicate microcosms were incubated at about 20°C for at least six weeks before being analyzed for ferrous iron (Fe(II)) generation or sulfate depletion. Active iron-reducing microcosms were transferred to a fresh anaerobic culture medium with amorphous ferrihydrite to develop active inocula for the arsenic mobilization column experiment. After several months, more electron donor and sulfate were added to evaluate the sustained ability of the reduction with different aquifer core materials.

Molecular Analysis

We planned to extract DNA from sediment subsamples retrieved during the drilling operation. Unfortunately, we were not able to obtain sufficient DNA from the core material to proceed with the planned analysis. As an alternative, we used the microcosms fed sulfate or ferric iron to determine if different microbial communities were responsible for the target activities with depth. Terminal restriction fragment length polymorphism (T-RFLP) microbial community fingerprint analysis was used to show the differences between the communities (Liu et al., 1997; Flynn et al., 2008).

Column Study

A flow-through column experiment was performed in this study to better simulate conditions in an aquifer. The experiment consisted of two cylindrical glass columns with an inner diameter of 1 cm and a length of 15 cm. Each column was filled with sand coated with ferric-oxide and arsenate (As(V)). Groundwater was pumped upward through

the columns using a syringe pump, into sample collection tubes (Figure 1). PEEK Tubing with a 0.0625-inch outer diameter and a 0.03-inch inner diameter (Grace Davison Discovery Sciences, Deerfield, IL) was used to connect the syringes and sample tubes to the columns. Columns and syringes were covered with aluminum foil to prevent photosynthetic growth as well as photoreduction of ferrous iron. The experiment was conducted in a room with an average temperature of 23°C.

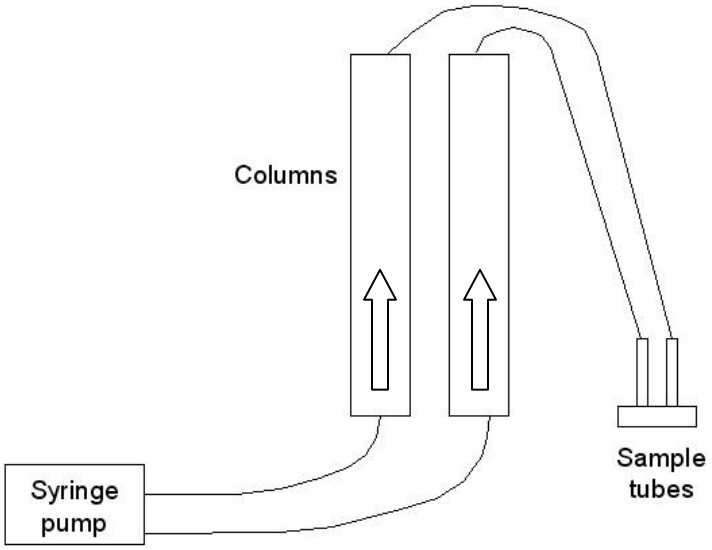

Figure 1. A simplified schematic of the flow-through column experiment. The gray lines are tubing and the arrows indicate direction of flow.

Mahomet aquifer sand was coated with ferrihydrite and arsenate prior to the start of the study. The presence of ferric iron and arsenate on the sand was verified through a bottle experiment. We prepared three serum bottles with 100 milliliters (mL) of filtered groundwater and 5 grams of sand loaded with ferric iron and arsenate. Two bottles were inoculated with iron-reducing bacteria, and the third was left as a control. All three bottles incubated at 30°C for three months. Iron reduction and arsenate release were verified using Kostka and Nealson's (1998) ferrozine method and ion chromatography (Metrohm-Peak, Houston, TX), respectively. We found the sand to be coated with sufficient ferric iron and arsenate, allowing the next phase of column preparation to begin.

For the column experiment, we used groundwater collected from the Illinois State Water Survey Mahomet aquifer monitoring well IRO-95A (Iroquois County, IL). The sample was stored in a Nalgene container at 4°C prior to use. The groundwater was filtered through 0.45 micrometer (μm) filter paper (Millipore Corporation, Bedford, MA) prior to experiments using a vacuum pump, then degassed in a serum bottle by bubbling with nitrogen gas, and stored at 4°C until use.

Columns were filled with sand coated with ferric-oxide and arsenate by first pouring a little groundwater in, filling the column with sand up to the level of the water,

and repeating the process until the column was completely filled with sand (~20 g). This method was used to prevent air bubbles from being trapped between sand grains. Anaerobic filtered groundwater was then pumped through the columns at a rate of 5 milliliters per hour (mL/hr) for two hours to equilibrate the system to anoxic conditions. Over the course of the experiment, groundwater was pumped through the two columns at a rate of 0.083 mL/hr (or ~2 mL/day) for five days. Effluent from the columns was collected in a 15 mL falcon tube and stored at 4°C.

After the initial three samples per column were taken, the column was inoculated with 10 mL of the iron-reducing bacterial culture at a rate of 5 mL/hr. The iron-reducers were cultivated from sediments taken from a core sample from the Glasford Formation at a depth of 87.5 feet. The microbes were then incubated in serum bottles containing hydrous ferric oxide (HFO) or with sands coated with ferric-oxide to confirm iron reduction. Once the inoculation was complete, the groundwater was amended with 200 micromolar (μM) acetate and 400 μM formate to promote microbial growth, and then pumped through the columns at a rate of 0.083 mL/hr (or ~2 mL/day) for five days. Column effluent was collected in 15 mL falcon tubes during those five days, and then stored at 4°C once the five days had ended. We repeated this process three times for a total of 15 days and three samples per column (Table 2). All samples were tested for arsenate using ion chromatography.

To preserve ferrous iron in the effluent, three 3 mL samples were preserved in 0.5 N HCl during the sample period of five days. We repeated this procedure twice for each column (Table 2).

Table 2. Timetable Showing When Each Type of Sample was Taken During the Column Experiment

When	Type of sample	Time (days) 5 10 15 20 25 30 35 40
before inoculation	3 10-mL samples, no acid	← →
after inoculation	3 10-mL samples, no acid	← →
	6 3-mL samples in 0.5N HCl	← →

Note: This sampling process was done for each column. "No acid" indicates those samples taken for arsenate analysis and samples collected with acid for ferrous iron analysis.

Chemical Extractions

Core sections from nine depth intervals were collected for chemical extractions to determine the concentrations and chemical forms of arsenic in the solid aquifer material (Table 1). During drilling, a 1-oz. (30 mL) HDPE bottle was filled with material from the center of the core and ambient groundwater. The bottle was tapped to allow any air bubbles to escape, filled so that there was no headspace, and tightly capped. A waterproof label with a description of the core was applied and the bottle was submerged in a Mason jar full of anoxic groundwater and stored on ice in a cooler.

Sediment samples were extracted using the method of Wenzel et al. (2001) in which samples are extracted with increasingly aggressive reagents (Table 3). The method is based on extraction schemes for phosphate, which is chemically similar to As(V). Although the various extraction steps have been tested using well-defined minerals, for

"real" samples the fractions are operationally defined. The extractions were carried out in polypropylene centrifuge tubes. The amount of sediment was 1.00 ± 0.05 grams and the extractant volume for each step was 25 mL. In the third step and the wash of the fourth step, tubes were wrapped in aluminum foil to prevent photo-reduction of iron oxides. At the end of each step, the samples were centrifuged and the supernatants filtered (0.2 μm).

Table 3. Sequential Extraction Procedure, after Wenzel et al. (2001)

Extractant	Time (hr)	Temp (°C)	Other	
$(NH_4)_2SO_4$, 0.05M	4.0	20		
$NH_4H_2PO_4$, 0.05M	16.0	20		
$(NH_4)_2C_2O_4$, 0.2M	4.0	20	pH 3.25, dark	combine
$(NH_4)_2C_2O_4$, 0.2M	0.2	20	pH 3.25, dark, wash step	
$(NH_4)_2C_2O_4$, 0.2M Ascorbic acid, 0.1M	0.5	95	pH 3.25	combine
$(NH_4)_2C_2O_4$, 0.2M	0.2	20	pH 3.25, dark, wash step	

Arsenic in the extracts was determined by graphite furnace atomic absorption spectrophotometry (GFAAS) using palladium as a matrix modifier (Welz et al., 1988). The method detection limit (Glaser et al., 1981) was ~2 μg/L. The $NH_4H_2PO_4$ and $(NH_4)_2C_2O_4$ solutions caused interferences in arsenic determination by GFAAS, and the extracts had to be diluted by a factor of 10 for quantitative analyses.

Results

Microcosms

Combined results of the sulfate and iron-reducing microcosms are shown in Table 4. Microbially mediated ferric-iron reduction was observed in samples from all formations; however, the most extensive reduction occurred at the boundary layer below the Glasford formation (Figure 2). We observed similar results with sulfate-reducing microcosms even though the groundwater analyzed from an adjacent monitoring well was very low in sulfate (Figure 3). Activity in all microcosms was confirmed when they were re-fed with more electron donor and acceptor (data not shown). Select iron-reducing microcosms were chosen for use as inocula for column experiments.

Several ferric-iron reducing microcosms were selected for transfer to fresh iron-reducing media. These included microcosms with sediments from depths of 77.5, 87.5, and 232.5 feet. Instead of ferric citrate, these cultures were tested with amorphous ferrihydrite and crystalline lepidocrosite. All enrichment cultures reduced iron, although with clear differences in rate and extent. For example, the enrichment derived from the 232.5-feet sample showed very rapid iron reduction even with the more crystalline lepidocrosite showing complete reduction within 72 hours (data not shown). This is much more rapid than the other enrichments from the Glasford aquifer.

Table 4. Summary of Results from Duplicate Microcosms Amended with 2 mM Sulfate or 5 mM Ferric Citrate.

Formation	Depth (feet)	SO_4^{2-} reduction microcosms			Fe(III) reduction microcosms		
		SO_4^{2-} (mM)		Average SO_4^{2-}	Fe(II) (mM)		
		Rep 1	Rep 2	Consumed mM	Rep 1	Rep 2	Average
Boundary	61	1.9	0.16	0.97	0.29	0.55	0.42
Glasford	77.5	0.18	0.14	1.84	0.01	0.16	0.085
	82.5	0.3	0.43	1.635	0.11	0.06	0.085
	88.75	0.54	0.13	1.665	0.43	0.51	0.47
	94.5	0.14	0.19	1.835	0.12	0.09	0.105
	97	0.14	0.12	1.87	0.13	0.13	0.13
Boundary	99	0.13	0.14	1.865	2	2	2
Banner	182.5	0.15	0.14	1.855	0	0.02	0.01
	187.5	0.14	0.13	1.865	0.11	0.11	0.11
	207.5	0.27	0.15	1.79	0.01	0.37	0.19
	221	0.16	0.14	1.85	0.05	0.06	0.055
Sankoty	232.5	0.11	0.12	1.885	0.39	0.04	0.215

Note: For sulfate-reducing microcosms, the average concentration consumed (= mM added – mM observed) is shown. For Fe(III) reduction, the average ferrous iron concentration is shown.

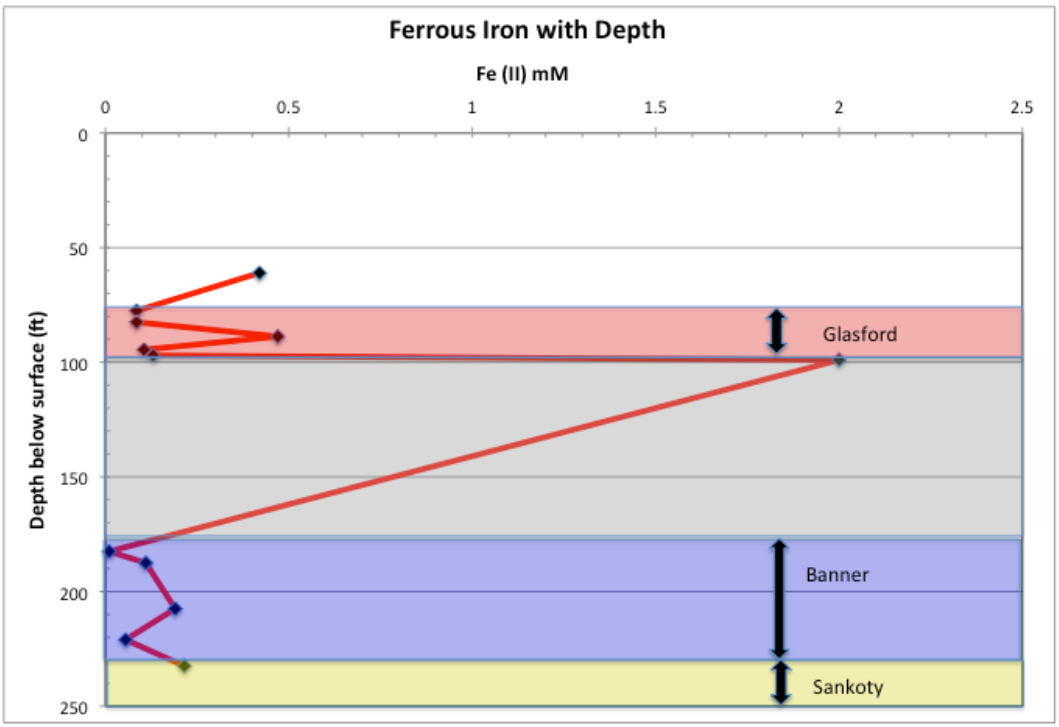

Figure 2. Map of relative iron reduction activity in microcosms as a function of depth in the Mahomet aquifer

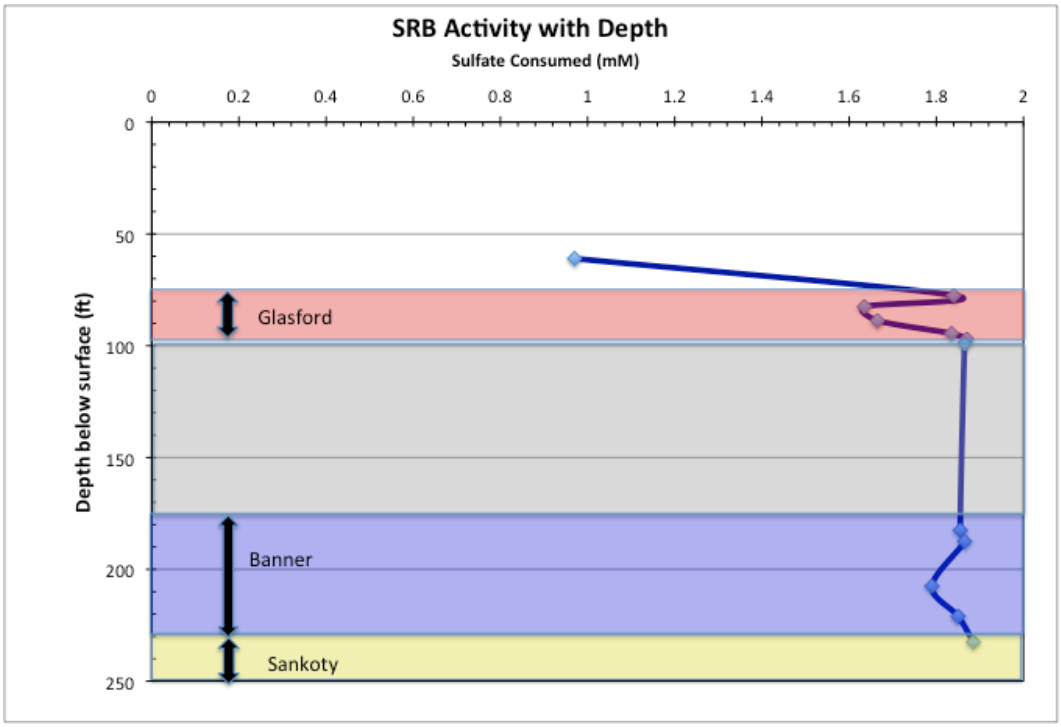

Figure 3. Map of relative sulfate consumption activity in microcosms as a function of depth in the Mahomet aquifer

Molecular Analysis

We obtained a bacterial community profile for sulfate-reducing microcosms with depth (Figure 4). Unique bacterial populations associated with sulfate reduction were present as a function of the parent sediment location. For example, in the case of the Mahomet aquifer sample taken at 232.5 feet, one population represented by a peak size of 330 is unique to this depth. Our efforts to characterize the ferric-iron reducing community failed because of inhibitory effects of reduced iron species on DNA extraction.

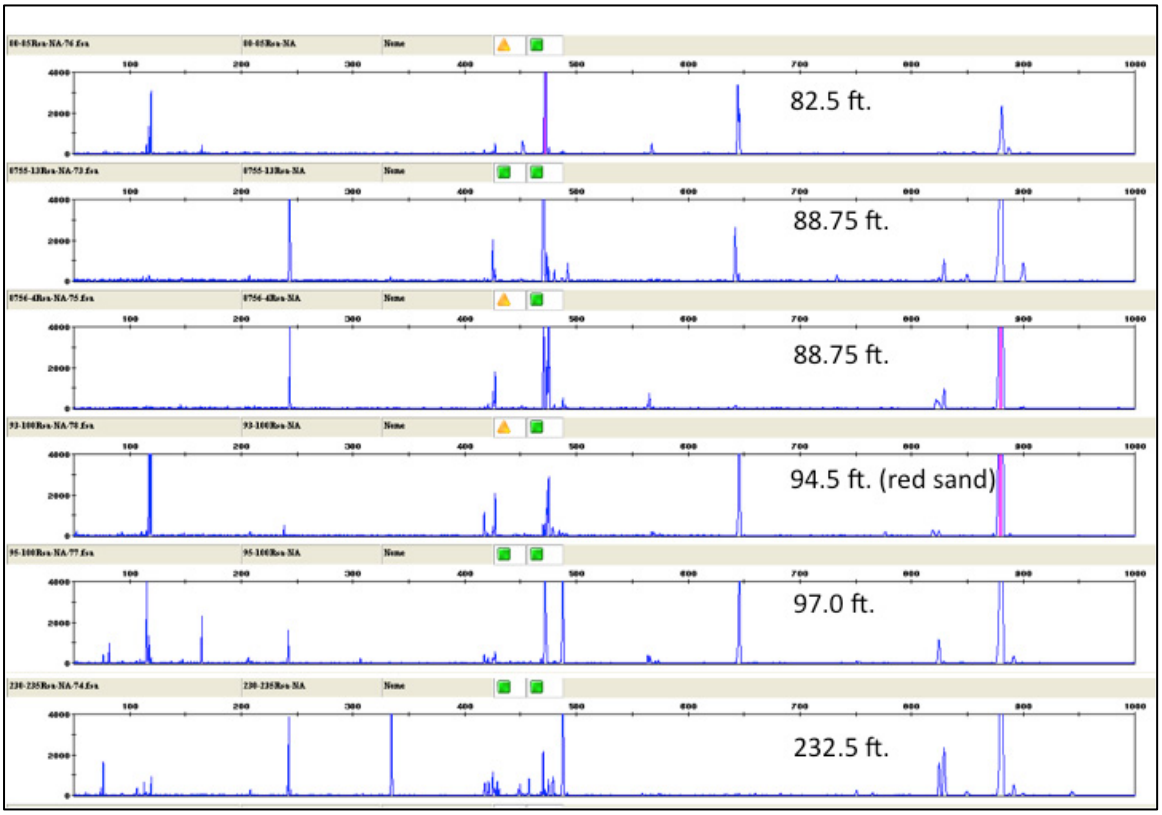

Figure 4. Microbial community profile relative to depth as found in sulfate amended microcosms from Glasford and Mahomet aquifers. Each peak represents at least one bacterial population type. Relative size of peaks correlates to relative abundance of 16S gene in the microcosm.

Column Experiment

Prior to the addition of an inoculum of aquifer-derived ferric-iron reducing bacteria, we detected no arsenic (<10 µg/L) as arsenate in the column effluent (Figure 5). In the presence of the bacterial culture, arsenate concentrations increased in the effluent to as high as 460 micrograms per liter (µg/L). Ion chromatography detects only arsenate and was not configured to detect reduced arsenite-arsenic (As(III)), so the occurrence of arsenic reduction could not be monitored. Although iron-reducing bacteria were added, we found no detectable ferrous iron in any effluent sample (data not shown). Due to the obvious continued presence of an iron-oxide coating on the sand, we assumed that ferrous iron adsorption to Fe(III) mineral phases occurred. To test this hypothesis, we added a

different Mahomet aquifer-derived iron-reducing bacterial enrichment culture as an inoculum to the columns. The inoculum was allowed to sit stagnant within the column for 48 hours prior to reinitiating flow. We observed some increase in ferrous iron concentration in the effluent over the additional two-month period of column flow; however, concentrations again fell below detection limits even with continuous feeding of an acetate-formate mixture as electron donors (data not shown).

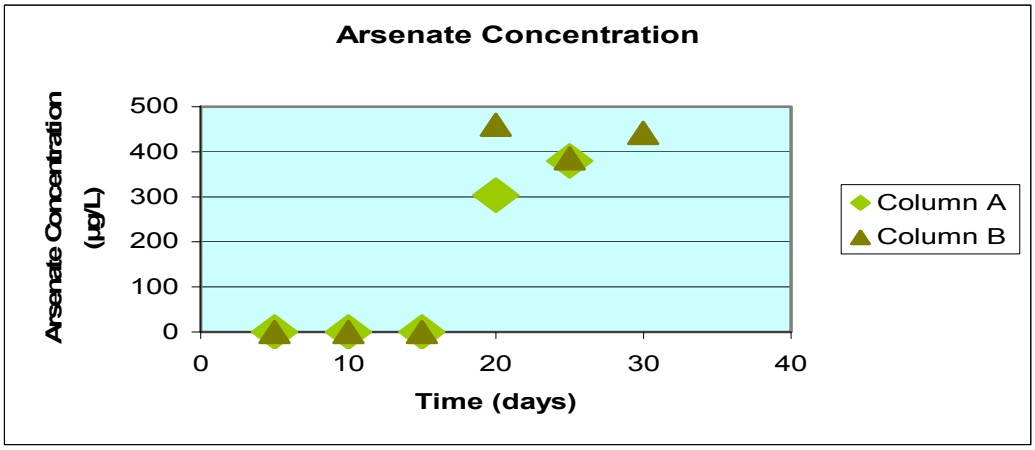

Figure 5: Arsenate release after the addition of iron-reducing bacteria in duplicate columns. Detection limit is 10 µg/L. Column A does not have a sixth value due to system error.

Chemical Extractions

The highest arsenic concentrations for each fraction were found in the reddish layer at the base of the Glasford formation (Figure 6). Arsenic was detected in at least one fraction for all samples from the Glasford aquifer. The arsenic concentration in the $(NH_4)_2SO_4$ fraction (non-specifically sorbed) of the sediment sample from the top of the Mahomet aquifer had arsenic at just above the detection limit. For all other extracts in all sections of the Mahomet aquifer, arsenic was undetectable.

For every sediment sample, the $(NH_4)_2SO_4$ fraction had either the lowest arsenic concentration or arsenic was undetectable. The $NH_4H_2PO_4$ fraction (specifically sorbed) had detectable arsenic in just two samples. The $(NH_4)_2C_2O_4$ (room temperature, dark) fraction (amorphous iron and manganese oxides) had detectable arsenic in all Glasford samples. The $(NH_4)_2C_2O_4$/ascorbic acid fraction (crystalline iron and manganese oxides) had detectable arsenic in three of the Glasford samples, but had the highest arsenic concentration of all fractions for those samples. These results are consistent with published results of sequential extractions. The amorphous and crystalline iron oxide fractions had most of the arsenic in aquifer sediments (Guo et al., 2008; Postma et al., 2007; von Brömssen et al., 2008) and soils (Doušová et al., 2008; Gault et al., 2005; Smith et al., 2008).

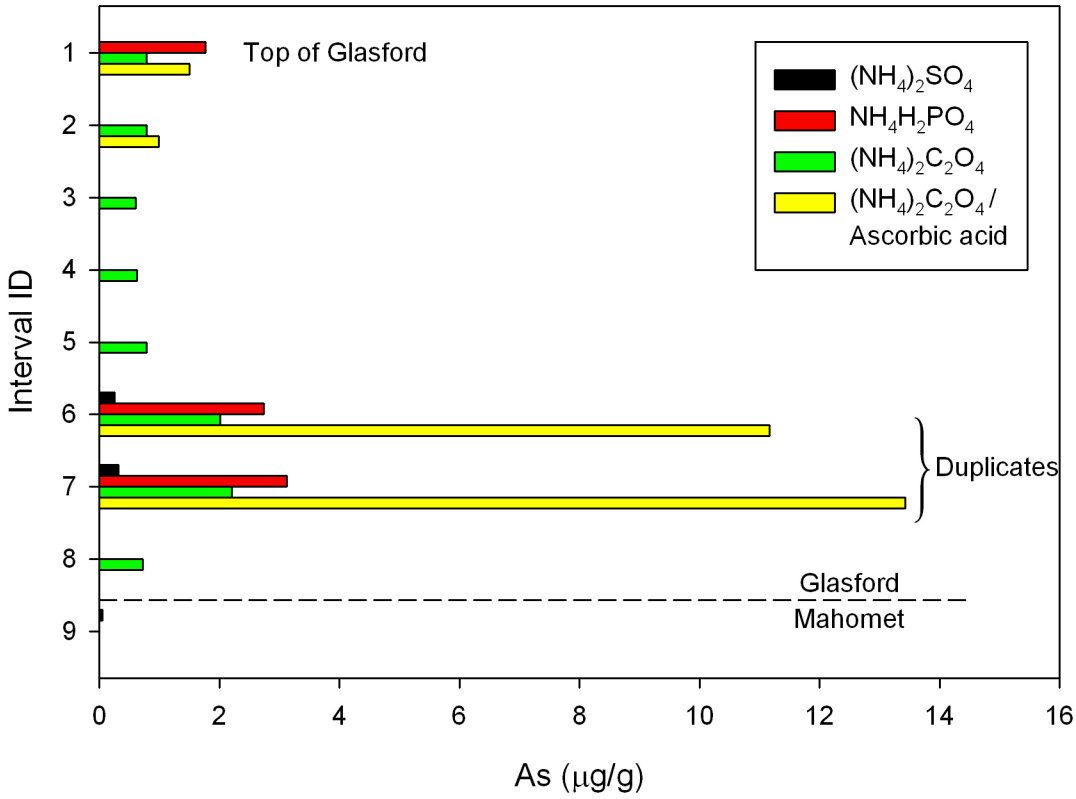

Figure 6. Arsenic concentrations in sequential sediment extractions. See Table 1 for interval ID information.

Discussion and Conclusions

Our experiments showed vertical spatial variability in microbial activity and bacterial community composition in aquifer sediments taken in the Glasford and Mahomet aquifers. Since different microbial populations responsible for either sulfate reduction or ferric iron reduction are distributed with depth, the characteristic groundwater chemistry and sediment chemistry profile may also vary. Other studies have shown considerable variability in groundwater chemistry in these aquifers (Kelly et al., 2005; Holm and Wilson, in prep.). We also showed that despite the very low natural sulfate concentration, sulfate-reducing bacteria are uniformly distributed throughout both anoxic aquifers. We believe this is consistent with a coexistence model for iron-reducing bacteria and sulfate reducers occurring in groundwater (Park et al., 2006; Bethke et al., 2008).

Column experiment results were surprising in the sense that although little or no iron-reduction activity was apparent by traditional ferrous iron analysis, arsenic release was dramatic once an inoculum was added. This iron-reducing culture had been shown to reduce the iron-oxide coating the sand along with releasing the arsenic in batch experiments (data not shown). The column experiment appears to have changed the dynamic association between iron-reducing bacteria and the detection of ferrous iron; however, there appears to be little impact on the extent of arsenic release. Thus there appears to be a bacterial-mediated arsenic release that precludes its readsorption to the abundant iron-oxides remaining in the column. Perhaps the accumulated ferrous iron interferes with this process.

A good future study would be to run the columns with some sulfate in the groundwater to test if sulfate reduction activity helps sequester arsenic released from iron-oxide coated sands. We have found evidence for this in our previous research on the Mahomet aquifer (Kirk et al., 2004).

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