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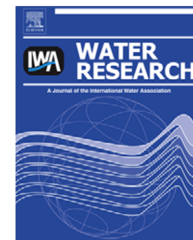

Characterization of geochemical constituents and bacterial populations associated with As mobilization in deep and shallow tube wells in Bangladesh

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ABSTRACT

While millions of people drink arsenic-contaminated tube well water across Bangladesh, there is no recent scientific explanation which is able to either comprehensively explain arsenic mobilization or to predict the spatial distribution of affected wells. Rather, mitigation strategies have focused on the sinking of deep tube wells into the currently arsenic-free Pleistocene aquifer. In this study, Bangladesh shallow tube wells identified as contaminated and uncontaminated, as well as deep tube wells, were analyzed for geochemical and *in situ* microbiological composition. Whereas arsenic was detected in all Holocene aquifer wells, no arsenic was found in wells accessing the Pleistocene aquifer. Bacterial genera, including Comamonadaceae, *Acidovorax*, *Acinetobacter*, and *Hydrogenophaga*, associated with tolerance of high arsenic concentrations, rather than dissimilatory Fe(III) or As(V) reduction, were identified in shallow tube wells, indicating that mobilization may not occur at depth, but is rather due to drawdown of surface contaminated water. Deep tube wells contained microbes indicative of aerobic conditions, including the genera *Aquabacterium*, *Limnobacter*, and *Roseomonas*. It is concluded that through drawdown of arsenic or organic matter, further utilization of the Pleistocene aquifer could result in contamination similar to that observed in the Holocene aquifer.

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1. Introduction

In order to combat the occurrence of diseases associated with the consumption of untreated surface water, shallow tube wells were and continue to be installed throughout Southeast Asia as a source of pathogen-free drinking water (Caldwell et al., 2003). Owing to their low cost, minimal maintenance, and convenience, it is currently estimated that in Bangladesh over 97% of the rural population utilizes 6–11 million government funded and privately sunk wells (Yu et al., 2003; Jakariya et al., 2007). However, elevated As concentrations have been found; it is estimated that over 100,000 people have developed skin lesions due to drinking As-contaminated water and that, without mitigation, excess deaths of 3000 per year should be expected (Yu et al., 2003).

Arsenic occurs naturally in Bangladesh sediments due to weathering of arsenopyrite from the Himalayas and subsequent deposition by the Ganges–Brahmaputra–Meghna River system

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(Acharyya et al., 2000). Solid phase As is found coprecipitated in or coadsorbed on a number of minerals in concentrations consistently below 10 μg/g in both the Holocene and Pleistocene aquifers (Swartz et al., 2004; Akai et al., 2004). In contrast, the concentration of dissolved As shows high depth variations and no clear pattern of spatial distribution (Swartz et al., 2004).

Several geochemical explanations for As release have been proposed (Acharyya et al., 1999; Chowdhury et al., 1999). However, correlations between contamination and reducing conditions have led to the broadly-accepted hypothesis that dissimilatory Fe oxyhydroxide reduction leads to the release of adsorbed and coprecipitated As (Nickson et al., 1998, 2000; BGS and DPHE, 2001; McArthur et al., 2001; Harvey et al., 2002; Dowling et al., 2002). Respiration on organic carbon present in the aquifer (McArthur et al., 2001, 2004), or infiltrating from the surface (Harvey et al., 2002) leads to anoxic conditions, under which other electron acceptors, such as Fe and As, are employed.

Although it appears that microbial activity leads to reducing conditions associated with As mobilization, to date investigations of bacterial communities have focused only on analyzing population shifts in incubation experiments utilizing environmental samples from contaminated sediments in Southeast Asia. Anoxic incubations of sediments from Bangladesh and West Bengal, India with electron donors yielded increased aqueous As concentrations, and molecular analysis indicated a shift in the bacterial community towards the Fe(III) reducers Geobacteraceae (Akai et al., 2004; van Geen et al., 2004; Islam et al., 2004). Similarly, the presence of organisms possessing the *arrA* gene encoding for As respiration has been confirmed by molecular analysis in incubation experiments with As-contaminated Cambodian sediments amended with acetate (Lear et al., 2007).

Whereas previous research supports the biogeochemical basis for As mobilization, such incubation experiments fail to identify the responsible *in situ* microbial populations. Additionally, as Fe(II) concentrations do not show a consistent correlation with As in either field studies (Swartz et al., 2004; Zheng et al., 2004, 2005) or incubation experiments (van Geen et al., 2004; Islam et al., 2004; Gault et al., 2005), it is yet to be concluded that Fe(III) reduction is responsible for As mobilization.

As a decisive explanation of and a solution to shallow tube well contamination is yet to be presented, recent mitigation strategies have led to the sinking of tens of thousands of thus far uncontaminated deep tube wells (Ahmed et al., 2004, 2006). It is assumed that the absence of organic carbon impedes microbial As mobilization in wells utilizing the oxic Pleistocene sediments, where increased weathering during the last glaciation has led to a higher concentration of iron oxides onto which As can absorb (McArthur et al., 2004). However, somewhat conflicting associations between Fe and As and assertions that organic carbon may not limit microbially mediated As mobilization argue against the aforementioned explanations (van Geen et al., 2004).

Although dissimilatory Fe reduction may play a role in As mobilization, no theory as yet is able to predict the location of contaminated wells or explain their inconsistent distribution. Rather, it is clear that As mobilization is a complex interplay of microbially mediated reactions and geochemical processes sensitive to site specific hydrology and sediment composition. In contrast to previous microbiological investigations of microbial population shifts, this study intends to identify *in situ* bacterial communities and geochemical constituents associated with As mobilization. Through analysis of water samples obtained from contaminated and uncontaminated shallow tube wells at close proximity to one another, this work aims to investigate As mobilization in wells with similar sediment composition and hydrology. In addition to identifying bacterial populations and chemical compositions associated with elevated As concentrations, geochemical and microbiological analyses of the insufficiently-investigated deep aquifer aim to provide confirmation of conditions under which contamination is not observed. The results presented here provide documentation of the chemical and microbial characteristics of water in As-affected and unaffected wells in Bangladesh.

2. Materials and methods

2.1. Site description and sample collection

Water samples were collected in April and May 2008 from deep tube wells (DTW) and shallow tube wells (STW) at five villages in Bangladesh: four locations in the Munshiganj district (samples DTW4, STW5, DTW6, STW7, DTW8, STW9, STW10, DTW11, STW12, STW13) and one location in the Jessore district (samples DTW1, STW2, STW3) (see Supplementary Data Table 1 for upzilla and village names). As this study did not include sediment sampling, information on lithology is gleaned from the literature. Swartz et al. and Polizzotto et al. provide a thorough core description for Munshiganj to a depth of 165 m (Swartz et al., 2004; Polizzotto et al., 2006). Briefly, a 3.5 m-thick clay layer covers the Holocene aquifer, which is composed of gray and greyish-green sands with interspersed silty–clay layers, to a depth of 119 m. The Holocene aquifer is separated from the Pleistocene aquifer, starting at 150 m depth, by 30 m of greenish clay. The As content in the solid phase is below 3 μg/g throughout the core (Swartz et al., 2004). Core analysis for Jessore district shows reducing sandy sediments to a depth of 61 m with muddy layers encountered at a depth of 3–9 m and in dispersed patches between 33 and 46 m (Akai et al., 2004). Solid phase As concentrations for the mud and sand layers are 7–16 μg/g and 2–5 μg/g, respectively.

Additionally, the British Geological Survey (BGS) has extensively investigated an area near Faridpur, which lies between the two sites investigated in this study (BGS and DPHE, 2001). The upper aquifer from 15 m to 44 m is composed of gray sand deposits with wood fragments observed between 25 m and 44 m. A few meters of silty clay layers exist at 45 m, followed by gray sand and gravel deposits to a depth of 134 m, the position of the sea level low stand of the last glaciation. Sediments from 134 m to 155 m are gray-brown sands deposited prior to the last glaciation.

At each site, water was collected from a deep tube well ($n = 5$, installed by the Arsenic Mitigation Research Foundation (AMRF)) and the nearest shallow tube well marked as As-contaminated by red paint ($n = 5$, within 20 m of each deep tube well). When available, water from a shallow tube well identified (with green paint) as having As concentration below the BDWS standard was also collected ($n = 3$, two wells (STW4, STW13) were within 20 m of the deep tube well, a third (STW10) was 400 m away). After inquiring into age, depth, and

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usage, the tube well was flushed by pumping until electrode measurements steadied (temperature, conductivity, pH and oxidation–reduction potential). Water samples from each well were collected and filtered (0.2 μm filter) or acidified, following the procedure described below:

(1) 50 mL sample without headspace was collected for alkalinity analysis, which was performed within 10 h (Hach Digital Titrator Test Kit);
(2) three HDPE screw cap bottles (Nalgene) were filled with 60 mL filtered sample, acidified to 1% (w/v) with suprapure HNO_3, and stored at 4 °C for trace metals and elements quantification;
(3) 5 mL filtered sample for dissolved organic carbon analysis (DOC) was collected in a glass tube and stored at −20 °C;
(4) three filtered samples of 2 mL each were collected in HDPE microcentrifuge tubes, acidified to 1% (v/v) with suprapure HCl, and stored at −20 °C for PO_4^{3-} and Si quantification;
(5) three filtered samples of 2 mL each were collected in HDPE microcentrifuge tubes without acid and stored at −20 °C for NO_3^-, NO_2^-, and NH_4^+ quantification;
(6) 60 mL unfiltered sample was prepared for As speciation analysis as described previously (Karori et al., 2006) and stored at 4 °C. Briefly, the sample was acidified to a final concentration of 0.01 M HAc and 0.5 mM EDTA, allowed to incubate for a few minutes, and then poured through a 10 mL chloride resin mini-column (Dowex 1 × 8). The final 40 mL of the sample from the column were analyzed for As concentration, which is reported here as As(III).
(7) 5 L sample collected in a polycarbonate jug was filtered by gravity within 10 h through a 0.2 μm hollow fiber filter (Spectrum labs, mediakap-5 hollow fiber filter) to concentrate the biomass. The filter was wrapped in Parafilm, placed in a 50 mL sterile tube, and stored at −20 °C until use for DNA extraction.

2.2. *Laboratory analytical methods*

Samples were analyzed for dissolved constituents in May 2008 at Utrecht University Geolab (Faculty of Geosciences, Utrecht, The Netherlands) using conventional methods. Trace metals and elements, including total As and As speciation analysis, as well as Al, B, Ba, Ca, Fe, K, Li, Mg, Mn, Na, P, S, Si, and Sr, were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Spectro CIROS CCD, Kleve, Germany). NH_4^+ and PO_4^{3-} were quantified by photometry at 660 nm and 880 nm, respectively (Bran and Luebbe autoanalyzer AA3, Europe). F, Cl, Br, NO_2^-, NO_3^-, and SO_4^{2-} concentrations were determined using ion chromatography (Dionex IonPac AS14, Benelux). DOC was measured by combustion on a TOC-5050A analyzer (Shimadzu's–Hertogenbosch, The Netherlands).

2.3. *Molecular techniques*

2.3.1. *DNA extraction*

DNA was extracted from the biomass collected on fiber filters at the University of Dhaka. Filters ($n = 13$) were first thawed on ice and then cut open in a sterile environment over a sterile petri dish. Both the water retained within the filter as well as the filter fibers themselves were retained in sterile tubes. DNA extraction was performed on a mixture of approximately 1 mL liquid and ¼ of the filter fibers using a soil DNA extraction kit (Mo Bio Laboratories Inc, Carlsbad) according to the manufacturer's protocol. DNA was quantified on a Nanodrop 1000 Spectrophotometer (Thermo Scientific, The Netherlands).

2.3.2. *PCR*

Purified DNA was transported on ice to Delft University of Technology for further analysis. Partial 16S rRNA gene sequences were amplified using the universal bacterial primer pairs 341F + GC and 907R (Schafer and Muyzer, 2001). 50 μL PCR mixtures containing Taq PCR master mix (Qiagen), DNA–RNA free water (Qiagen), 1 μM of each primer, and approximately 80 ng template DNA were run on a T1 thermocycler (Biometra, Goettingen, Germany) following a touchdown program (Schafer and Muyzer, 2001). PCR product was checked on a 1.5% agarose gel run at 100 V for 45 min. PCR products were obtained for all wells except STW13.

2.3.3. *Denaturing gradient gel eletrophoresis (DGGE)*

DGGE was performed on the bacterial 16S rRNA fragment using a 1 mm thick, 6% polyacrylamide gel with a urea–formamide gradient of 20–80%, as described previously (Schafer and Muyzer, 2001). Gels were run at 60 °C, 100 V, 43 mA for 16 h on a BIO RAD Dcode system, stained with Gel Green (Biotum, USA), and photographed using a blue light safe imager in a C-box doc system with accompanying Genesnap software (Syngene). Bands were cut using a sterile blade and incubated for 48 h at 4 °C in 15 μL 10 mM tris buffer (pH 8.5). The bands were reamplified using the solution as a template in the aforementioned PCR regimen and GC clamp free primers. Purification of 25 μL PCR product was performed using 1.6 μL Exo-Sap-IT (USB, Europe) according to the manufacturer's recommendations (30 min at 37 °C, 15 min at 80 °C). The final product was first checked for purity and concentration on a 1.5% agarose gel, diluted to 50 ng/μL and then sent away for commercial sequencing (Macrogen, Seoul, South Korea).

2.3.4. *Clone libraries*

Clone libraries for STW4, DTW5, and STW10 were constructed using pCR®4-TOPO cloning kit (Invitrogen), as described by the manufacturer. *Escherichia coli* colonies, grown on kanamycin plates to select for vectors containing an insert, were picked (95 per sample) and reamplified using the universal M13 primer pair (Invitrogen). The product was purified, quantified, and sequenced as described above for DGGE products.

2.3.5. *Sequence analysis*

Sequences were screened for purity and chopped to remove primers. The resulting sequences were compared to those in GenBank using Blast (Zhang et al., 2000; http://www.ncbi.nlm.nih.gov/BLAST). The sequin program was used to submit sequences for accession numbers (http://www.ncbi.nlm.nov/sequin). DGGE bands have accession numbers FJ196237–FJ196259 and FJ232946. Clones have accession numbers

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FJ204929–FJ205138. Additionally, sequences were loaded into ARB software, which was used for alignment and in the creation of a phylogenetic tree utilizing the neighbour-joining algorithm (Ludwig et al., 2004; http://www.arb-home.de). Finally, clone library coverage was determined using webLIBSHUFF, which estimates within a 95% confidence level the similarity of two sets of sequences (Henriksen, 2004; http://libshuff.mib.uga.edu).

3. Results

3.1. Chemical composition of wells

To assess the geochemical conditions in shallow and deep tube wells, water samples were analyzed for chemical constituents. All tube wells accessing the Holocene aquifer contained As concentrations above the BDWS of 50 μg/L, including those marked as being within safe limits during the testing campaign from 1999 to 2005. As concentrations were between 72 μg/L and 432 μg/L. (For the entire geochemical data set, see Supplementary Data, Table 1.) Dissolved As existed predominantly in the reduced species, As(III), making up on average 80% of the total concentration. Such partitioning between As(V) and As(III) species is consistent across all shallow tube wells ($R^2 = 0.836$ and 0.988, respectively; Fig. 1). As was not found in any deep tube well.

The concentrations of dissolved ions indicate a difference in geochemical conditions between shallow and deep tube wells (Fig. 2). Higher average NH_4^+ and PO_4^{3-} concentrations are found in shallow tube wells and show a correlation to As ($R^2 = 0.709$ and 0.558, respectively; Figs. 2 and 3). When the three data points from the geologically dissimilar Jessore are removed, this linear relationship is significantly more robust, with $R^2 = 0.964$ for NH_4^+ and 0.706 for PO_4^{3-}. NO_3^- was identified in DTW11, but was absent in all STW. Over ten times more dissolved Fe is found in samples from the Holocene aquifer compared to the Pleistocene aquifer (Fig. 2); however, no correlation with As is found ($R^2 = 0.302$). The higher concentrations of constituents associated with reducing conditions and microbial activity may be related to higher DOC concentrations observed in shallow tube wells, which on average contained 6.40 mg C/L, as opposed to deep tube wells, with an average of 1.96 mg C/L (Fig. 2). DOC measurements for

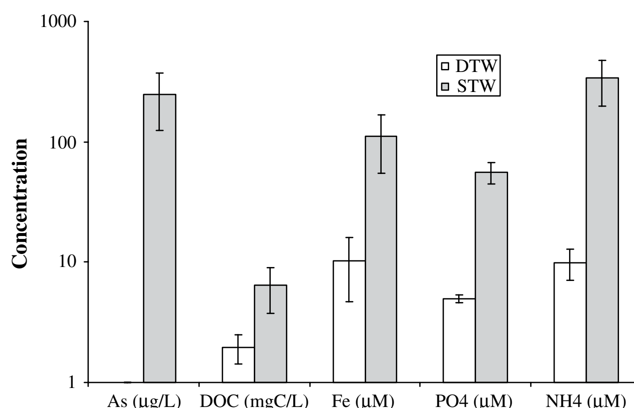

Fig. 2 – Average dissolved constituents for deep tube wells (white) and shallow tube wells (grey). Note that the Y axis is logarithmic. Error bars are one standard deviation.

Munshiganj are overall lower but of the same order of magnitude as those reported by Swartz et al. (2004). However, no correlation between organic carbon content and As concentrations is observed in shallow tube wells ($R^2 = 0.026$).

3.2. Microbial population analysis

To assess the bacterial diversity present in deep and shallow tube wells, 16S rDNA, concentrated and purified from water samples, was used for DGGE and in the creation of clone libraries. DGGE yielded 24 sequences (from 30 bands), of which 16 came from shallow tube wells and eight from deep tube wells (Fig. 4). Of these sequences, 84% are β-*Proteobacteria* (Table 1). The remaining 16% are made up of *Bacteroidetes* (one sequence), α-*Proteobacteria* (one sequence), and γ-*Proteobacteria* (two sequences). Similar distributions were observed in clone libraries of DTW4, STW5, and STW10 (Table 1). Overall, 75% of the 209 clones were β-*Proteobacteria*. Shallow tube wells had higher diversity than deep tube wells, as observed both in the number of DGGE bands and in the clone library diversity (Fig. 4, Table 1). Of the 79 clones for DTW4, 92% were β-*Proteobacteria*, in contrast to STW5 and STW10, in which around two-thirds of clones were of this class. An exception is observed in STW7, where reduced diversity may be related to

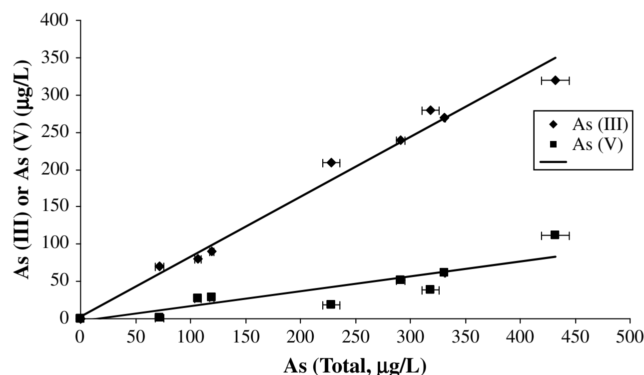

Fig. 1 – As(III) (◆) and As(V) (■) versus total As. Error bars, for total As only, are one standard deviation. $R^2 = 0.988$ for As(III) and $R^2 = 0.836$ for As(V).

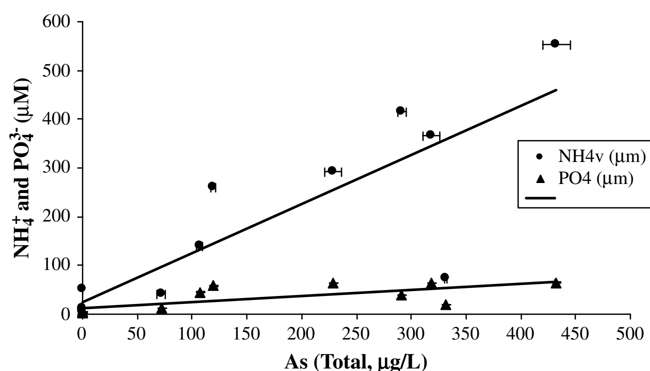

Fig. 3 – NH_4^+ (●) and PO_4^{3-} (▲) versus As concentration. Error bars are one standard deviation. $R^2 = 0.706$ for NH_4^+ and $R^2 = 0.558$ for PO_4^{3-}.

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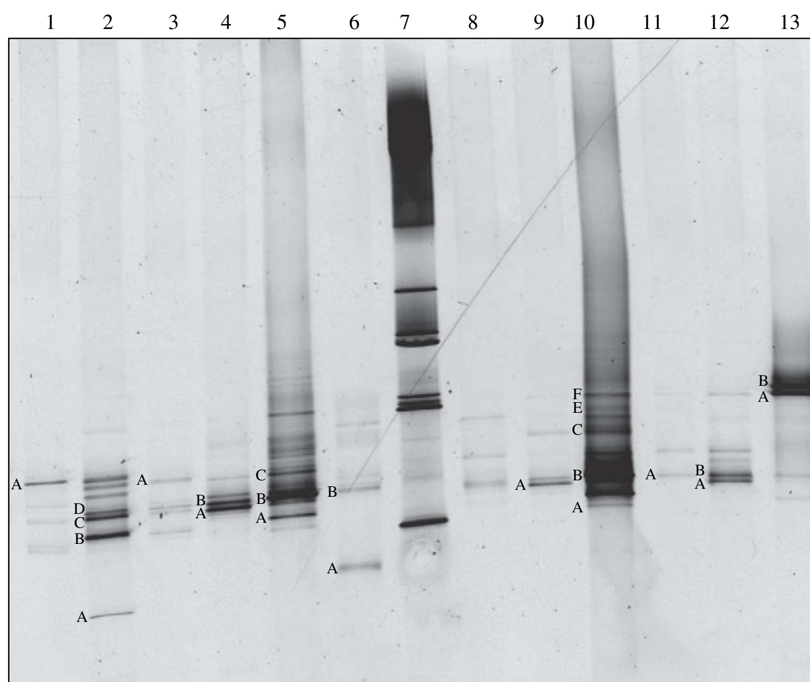

Fig. 4 – DGGE of bacterial 16S rRNA gene fragments from deep and shallow tube wells, labelled DTW and STW, respectively. Bands are labelled with a letter, which is used for reference in the text and Figs. 5 and 6. (+) denotes wells marked as being above the BDWS, (−) were marked as uncontaminated. Lane 1, DTW1; Lane 2, STW2 (+); Lane 3, STW3 (−); Lane 4, DTW4; Lane 5, STW5 (+); Lane 6, DTW6; Lane 7, Ladder; Lane 8, STW7 (+); Lane 9, DTW8; Lane 10, STW9 (+); Lane 11, STW10 (−); Lane 12, DTW11; Lane 13, STW12 (+).

the high As concentrations (430 μg/L) found. Deep tube wells show a limited microbial population (DTW1, DTW6, DTW8, DTW11), with DTW4 as a notable exception.

In addition to differences in diversity, the composition of clone libraries for shallow tube wells is statistically different from that of the deep tube well. Separate comparison of sequences from DTW4 with those from STW5 and STW10 produces p values of 0.001 for both XY and YX comparisons, indicating that the composition is significantly different within a 95% confidence interval (webLIBSHUFF). In contrast, the shallow tube well clone libraries are more similar, producing p values of 0.001 for YX comparison, but 0.043 for XY comparison.

The phylogenetic tree of sequences found in shallow tube wells in DGGE and clone library analysis shows similarity to species identified in previous incubation studies (Gault et al., 2005; Lear et al., 2007), found to sustain growth in As-contaminated environments, and, in some cases, associated with arsenite and Fe(II) oxidation (Figs. 5 and 6). DGGE bands are indicated by the well name (i.e. STW5) and the band letter (i.e. a) as observed in Fig. 4. Species of the family Comamonadaceae, found in STW5_a (FJ196255) and STW9_a (FJ196237), were prominent in the microbial populations both in the *in situ* and As(V)-amended incubation experiments with As-containing sediments from Cambodia (Lear et al., 2007); arsenate resistance has been noted (Ma et al., 2007). STW5_c (FJ232946) and STW10 clone library contained sequences showing similarity to *Acidovorax*, which was also found by Lear et al. (2007) to be prominent both before and after As(V) amendment and is known to be highly arsenite resistant. The two adjacent sequences found in STW12_a and STW12_b (FJ196245, FJ196246) show >99% sequence identity to *Acinetobacter junii* and *Acinetobacter baumannii*, which were also observed in the STW10 clone library, and which have been identified in West

Table 1 – Distribution of bacterial sequences obtained from DGGE or clone libraries over the different types of wells.

		DGGE			Clone libraries			
		Overall	DTW	STW	Overall	DTW4	STW5	STW10
No. of sequences		24	8	16	209	79	61	69
Class	% Bacteroidetes	4	0	6	1	1	0	1
	% α-Proteobacteria	4	13	0	4	1	0	10
	% γ-Proteobacteria	8	0	13	20	6	38	20
	% β-Proteobacteria	84	87	81	75	92	62	69

DTW, deep tube well; STW, shallow tube well.

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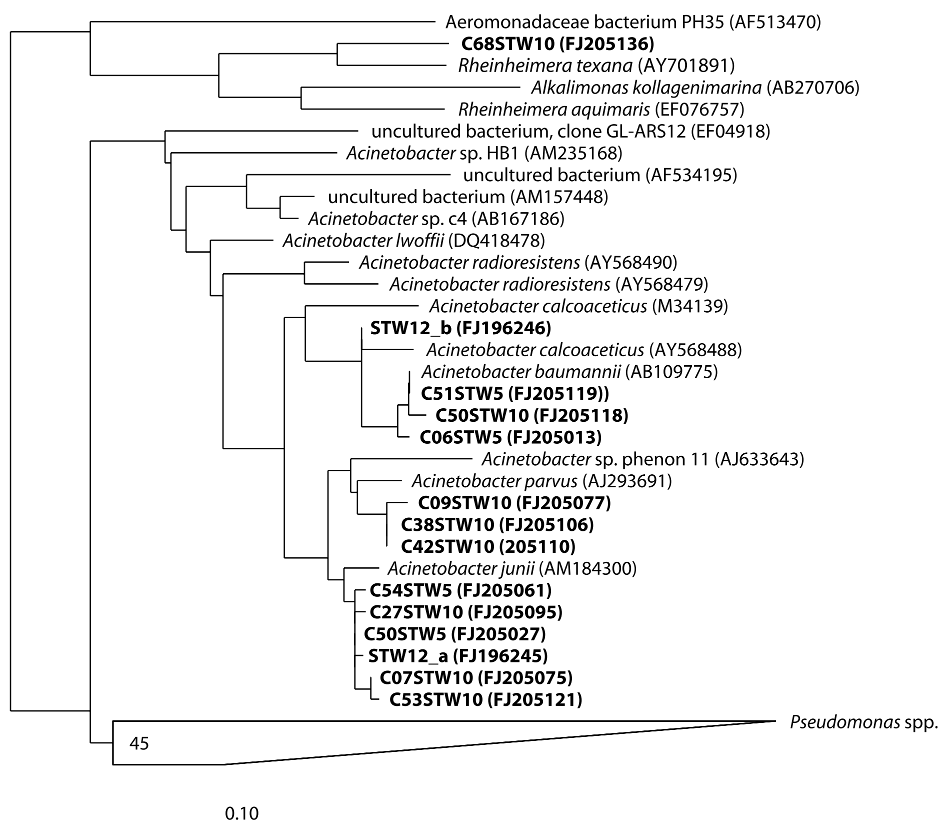

Fig. 5 – Phylogenetic tree based on 16S rRNA sequences of the γ-*Proteobacteria*. Sequences determined in this study are in boldface. Sequence accession numbers are shown in parentheses. The white triangle indicate compressed sections of the tree, with the ratio of sequences from this study to total sequences indicated. DGGE sequences are identified by the well name (STW12) and band letter (a). Clone library sequences are identified by the clone number (C68) and location (STW10).

Bengal sediment incubations (Gault et al., 2005). Arsenite oxidase activity has been confirmed for this genus (Fan et al., 2008). Similarly, a sequence found in STW2_b (FJ196249) showed >97% similarity to *Hydrogenophaga*, which has been identified in arsenic-oxidizing biofilms (Salmassi et al., 2006) and can oxidize arsenite, however only under oxic conditions (van den Hoven and Santini, 2004). Whereas no known Fe(III) reducers were identified, sequences showing >97% similarity to denitrifying Fe(II)-oxidizing bacteria were identified in STW2_d (FJ196251), STW5_b (FJ196256), and STW9_b and STW9_f (FJ196238, FJ196241) (Straub et al., 2004). These NO_3^- reducing bacteria allow anoxic Fe cycling in fresh water sediments. However, in this study, ferric iron reducers were not found in As-contaminated sediments.

DGGE and clone library analysis from deep tube well bacterial DNA were dominated by sequences showing similarity to *Aquabacterium* (Table 1). Specifically, *Aquabacterium hongkongensis*, identified in DTW4_a (FJ196253), DTW8_a (FJ196259), and DTW11_a (FJ196243), was prevalent (Fig. 6). Additionally, 39 of the 79 clones in the DTW4 clone library were of this genus. *Aquabacterium*, also associated with denitrifying Fe(II)-oxdizing sediments, are facultative aerobes (Straub et al., 2004). Sequences showing similarity to *Limnobacter* (DTW1_a, FJ196247) and *Roseomonas* (DTW6_a, FJ196257), were also identified in fresh water lake sediments (Spring et al., 2001; Jiang et al., 2006) and are obligate aerobes (Gallego et al., 2006).

4. Discussion

Indications of reducing conditions due to microbial activity, as seen in higher PO_4^{3-}, NH_4^+, and Fe concentrations and the absence of NO_3^-, were markedly more prominent in shallow tube wells as compared to deep tube wells. The higher DOC levels observed in the Holocene aquifer most likely support the increased bacterial diversity observed and associated higher As concentrations. STW7 shows limited diversity, which may be due to the exceptionally high As concentrations found here (431 μg/g). In contrast, low DOC concentrations are found in deep tube wells, which, in contrast to previous investigations of wells accessing the Pleistocene aquifer, did not contain As (Swartz et al., 2004) and showed a reduced assortment of species. More bands are observed in DTW4; however, as this well was installed a week before sampling, contamination during the drilling process may have instigated additional bacterial growth. These results support the role of prokaryotes in the creation of reducing conditions associated with As mobilization in the shallow subsurface.

Unfortunately, the shallow tube wells marked as uncontaminated that were sampled in this study contained As concentrations above the BDWS. Although this does reinforce the importance of continued and accurate test campaigns, we are unable to compare microbial populations in shallow tube

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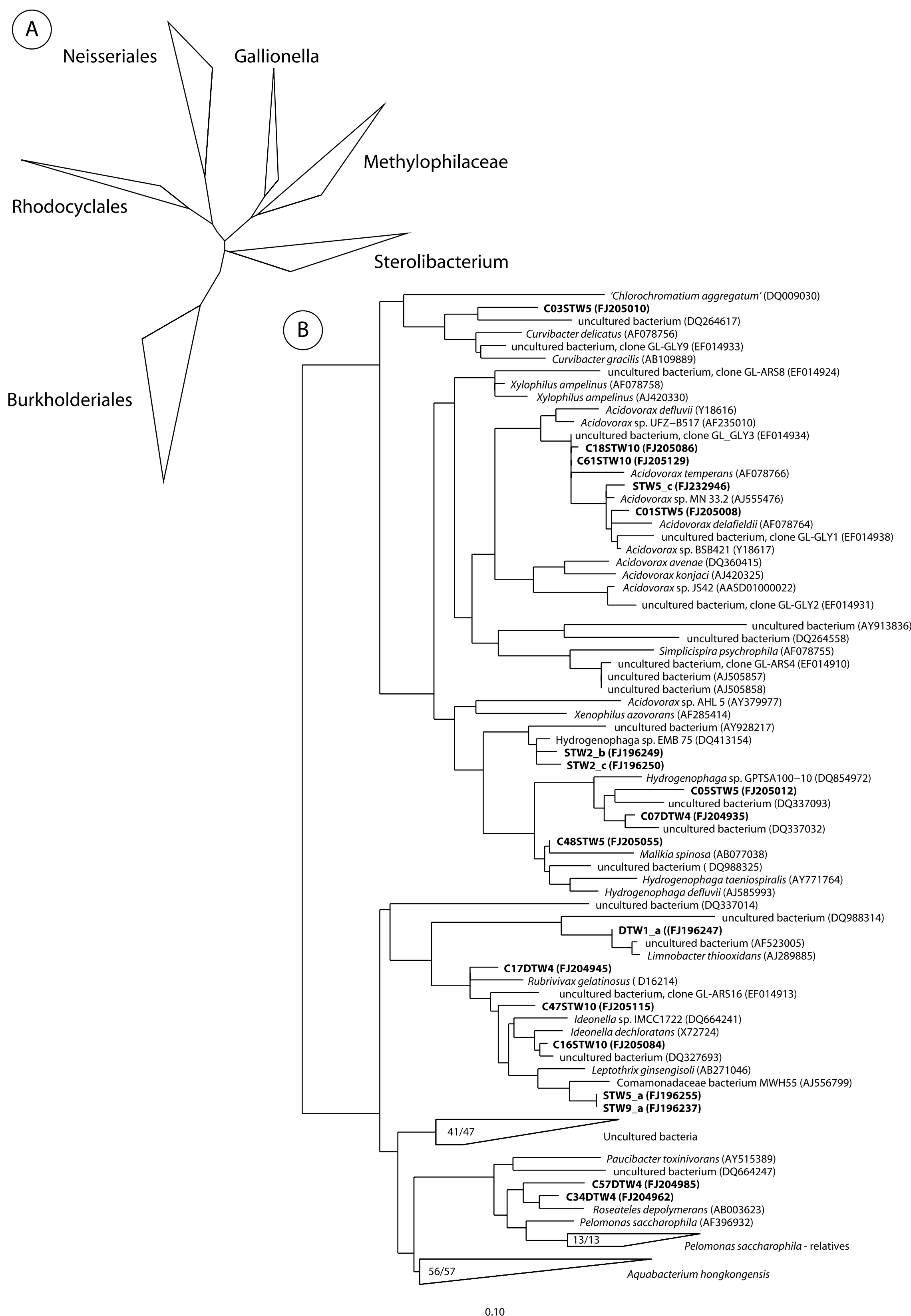

Fig. 6 – (A) Phylogenetic grouping based on 16S rRNA sequences of the β-*Proteobacteria*. (B) Expanded phylogenetic tree of sequences from the order Burkholderiales. Sequences determined in this study are in boldface. Sequence accession numbers are shown in parentheses. White triangles indicate compressed sections of the tree, with the ratio of sequences from this study to total sequences indicated. DGGE sequences are identified by the well name (STW12) and band letter (a). Clone library sequences are identified by the clone number (C68) and location (STW10).

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wells with and without As under similar geological and hydrological conditions.

4.1. Shallow tube well bacterial populations indicative of As tolerance, not mobilization

Analysis of microbial diversity from DGGE and clone libraries did not produce any sequences associated with Fe or As reduction, which questions the role of dissimilatory iron or arsenate reduction in As mobilization. Additional analysis targeting the functional genes for As and Fe reduction in both the water- and sediment-bound bacterial communities is required to make this statement more robust. However, as previous work did not successfully produce PCR amplification of the *arrA* gene for As reduction in native sediment samples (Lear et al., 2007), our study focused specifically on the *in situ* microbial diversity.

The bacterial community identified in the shallow tube wells studied shows similarity to those identified previously in As-contaminated sediments (Gault et al., 2005; Lear et al., 2007; Anderson and Cook, 2004); however, their prominence is more likely due to As resistance than function. Comamonadaceae, identified here in STW5 and STW9 with As concentrations of 318 μg/L and 107 μg/L, respectively, and *Acidovorax*, seen in STW5 and STW10 (119 μg/L), were also documented to dominate the microbial population in contaminated Cambodian sediments (Lear et al., 2007). Upon amendment with acetate and 10 mM arsenate (740 mg/L As), the proportion of these species relative to the total population increased, indicative of either As resistance or utilization. Considering that the *arrA* gene for As reduction was not found for this genus in work by Lear et al. (2007), the role of Comamonadaceae in As mobilization is unclear. Additionally, although *Acidovorax* does possess the *arsC* arsenate reductase gene, this functions as a form of As resistance rather than for dissimilatory growth. Whereas activation of this resistance pathway could cause some As mobilization, it is expected that the relative yield of reduced As is small compared to that produced by dissimilatory growth. Similarly, *Acinetobacter*, identified here in STW5, STW10 and STW12, the latter containing 229 μg/L As, was found in previous work with Bengal delta sediments (Gault et al., 2005) and at an As-contaminated site in New Zealand (Anderson and Cook, 2004). This genus shows exceptional As tolerance, able to sustain growth in the presence of 320 mM As(V) and 14 mM As(III), several orders of magnitude higher than the concentrations observed in this study (Achour et al., 2007). Although the species *Acinetobacter calcoaceticus* is able to mobilize As on copper arsenate-treated timber, this has only been observed under aerobic conditions (Clausen, 2000).

Sequences showing similarity to bacteria able to oxidize arsenite were also observed. Microbial As oxidation has been suggested as a mechanism in household filters for removal of As (Berg et al., 2006). *Acinetobacter* has been shown capable of As oxidation (Fan et al., 2008); however, the As resistance genes *ars*R and *ars*H are indicative of a survival strategy rather than a means of chemotrophic growth (Fournier et al., 2006). Species within the genus *Hydrogenophaga*, identified in STW2 with an As concentration of 332 μg/L, have been found in association with As-oxdizing biofilms (Salmassi et al., 2006).

In contrast to the commonly held theory that dissimilatory Fe reduction is responsible for As mobilization, only sequences similar to Fe(II) oxidizing bacteria (STW2, STW5, STW9) were found in this study (Straub et al., 2004). Such organisms could explain As mobilization in conjunction with anoxic Fe cycling. Under this scenario, As associated with Fe(II) biominerals produced through microbial activity (Islam et al., 2005) would be mobilized upon the structural changes associated with ferrous oxidation. Inconsistent correlations between Fe and As concentrations could be explained by the limited accessibility of Fe(II) atoms on the surface of the biominerals to microbial oxidation. However, in view of the fact that no Fe(III) reducers were found in this study, in contrast to the experiments with *Geobacter* and *Geothrix* where As affinity for Fe(II) biominerals was observed, this explanation is speculative.

4.2. Deep tube well microbial diversity indicative of oxic conditions and low DOC

Bacteria identified with DGGE in wells accessing the Pleistocene aquifer were indicative of oxic or suboxic conditions. Sequences showed >97% similarity to *Limnobacter* and *Roseomonas*, both obligate aerobes, and *Aquabacterium*, a facultative aerobe (Straub et al., 2004; Spring et al., 2001; Gallego et al., 2006). This finding supports previous geochemical work indicating that the Pleistocene is oxic. Zheng et al. found dissolved oxygen concentration up to 7 mg/L; and at 274 m, the depth analyzed here, 0.8 mg/L was detected (Zheng et al., 2004). As their investigation was performed on the uplifted Pleistocene Madhupur terrace, which would have been more exposed during the last glaciation, it is not surprising that in this study less oxic conditions were observed. Rather, the presence of *Aquabacterium*, which is a facultative aerobe able to use oxygen or nitrate as electron acceptors (Kalmbach et al., 1999), is consistent with the suboxic conditions found in Munshiganj Pleistocene sediments (Swartz et al., 2004).

The low concentration of organic matter found in this study prevents microbial respiration and the onset of reducing conditions. Although unable to grow autotrophically, *Limnobacter* (DTW1) is able to grow on very low organic carbon concentrations (Spring et al., 2001). Notably, this aerobe was only found in the deep tube well in Jessore with the lowest DOC concentration of this study (0.91 mg C/L). Pleistocene aquifer organic matter abundance in Jessore, an area which also may have undergone additional weathering during the low sea levels of the last glaciation, agrees with the Zheng et al.'s estimate of 1% for a similarly oxidized area (Zheng et al., 2004).

4.3. Theories of As mobilization

The absence of previously characterized Fe(III) or As(V) reducers in DGGE analysis does not preclude their presence in small numbers or role in As mobilization. However, this observation, in conjunction with the inconsistent community distributions seen across contaminated wells, leads us to conclude that As mobilization may not occur within the wells. Rather, as suggested by Polizzotto et al., reducing conditions caused by

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microbial activity in conjunction with redox cycling at the surface mobilizes As via chemical or biological processes, which is subsequently drawn down to well depth by pumping (Polizzotto et al., 2006). This conclusion is supported by the observed inverse relationship between well depth and As concentration ($R^2 = 0.520$, only shallow tube wells).

Well contamination due to recharge with water containing dissolved As explains a number of inconsistencies in this study. If mobilization occurs at the surface, the concentration of As in the well is not dependent upon the availability of DOC at that depth, as observed here ($R^2 = 0.026$ for DOC versus As correlation). Preferential usage and thus increased recharge at wells previously identified as being within BDWS limits would explain why this study found such wells to be contaminated. Additionally, the recent or sudden influx of As would initially reduce microbial diversity in these wells, as observed here, until a new community of As-resistant bacteria forms.

4.4. *Deep tube well sustainability*

The lack of investigations in depths similar to the deep tube wells examined here makes predictions of As contamination in wells accessing the Pleistocene aquifer challenging. Core analysis in the Munshiganj district is only to a depth of 165 m, whereas this study investigates 240 m deep wells (Swartz et al., 2004). Although this study indicates that current conditions are not favorable for As mobilization (oxic or suboxic with limited DOC), water extraction could change this. As theorized for the Holocene aquifer (Polizzotto et al., 2006), and observed in the Hanoi, Vietnam Pleistocene aquifer exploited for municipal drinking water (Berg et al., 2008), downward recharge could cause contamination in the deep tube wells studied here.

Whether drawdown could transport As or promote microbial activity through the introduction of DOC is a question of lithology and geology. Although a 30 m clay layer has been identified in the Munshiganj district (Swartz et al., 2004), similar confining layers were not identified in Jessore (Akai et al., 2004) or Faridpur (BGS and DPHE, 2001). The absence of dissolved As in the Pleistocene has been attributed to the absence of electron donors and the abundance of Fe(III) oxides available to adsorb As (Polizzotto et al., 2006; Stollenwerk et al., 2007). However, adsorption and modeling experiments utilizing sediments collected near Dhaka indicate that the solid phase buffer capacity is highly dependent upon the depth of the well screen relative to the confining layer, local lithology, and extraction practices (Stollenwerk et al., 2007). Considering that tens of thousands of deep tube wells have already been sunk without scientific investigation into the geological and hydrological constraints of the Pleistocene aquifer, future research must focus on illuminating the processes responsible for As contamination and determining the sustainability of deep tube wells in order to circumvent tragedies similar to those seen with Southeast Asian shallow tube wells.

5. Conclusions

In an attempt to illuminate the geochemical and biological conditions that lead to arsenic mobilization, water was analyzed from deep tube wells and shallow tube wells labelled as contaminated (red) and uncontaminated (green) during previous testing campaigns. Major chemical constituents were quantified and the bacterial community was analyzed using DGGE and clone libraries. The following conclusions were made:

1. Although labelled otherwise, all shallow tube wells had arsenic concentrations above the BDWS of 50 μg/L, indicating the need for continuous testing.
2. No arsenic was found in any of the five deep tube wells tested. The bacteria species identified were indicative of aerobic conditions and included members of the genera *Aquabacterium*, *Limnobacter*, and *Roseomonas*.
3. The microbial populations of shallow tube wells were dominated by species associated with arsenic tolerance and observed in previous investigations of arsenic contaminated environments, including Comamonadaceae, *Acidovorax*, *Acinetobacter*, and *Hydrogenophaga*. No known dissimilatory Fe(III) or As(V) reducers were identified.
4. Results including that: (1) no bacteria responsible for arsenic mobilization were identified; (2) the observed inverse correlation between well depth and arsenic concentration; and (3) no relationship between DOC and arsenic was seen, speaks in favour of the theory of Polizzotto et al., that contamination of shallow tube wells is due to drawdown of As-enriched surface water (Polizzotto et al., 2006). Therefore, further research should investigate the sustainability of deep tube wells for extraction of drinking and irrigation water.

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Supplemental material

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