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Mineral transformation and release of arsenic to solution under the oxidizing conditions of well disinfection

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ABSTRACT

The purpose of this project was to investigate geochemical and biogeochemical mechanisms of arsenic release to well water from arsenic-rich sulfide minerals and iron (hydr)oxides during *in situ* disinfection with chlorine. Guidance for disinfection of domestic wells in arsenic-sensitive areas of Wisconsin calls for a treatment at 20 percent of the chlorine strength and 10 percent of the contact time recommended for non-arsenic impacted wells. This guidance is based upon an assumption that the oxidizing strength of chlorine causes chemical oxidation of arsenic-rich sulfide minerals. However, microbially-mediated reductive dissolution of arsenic-bearing iron (hydr)oxides also contributes arsenic to groundwater in areas of Wisconsin. Therefore, a low-dose chlorination treatment may not be a preferred method of disinfection in all settings where groundwater and wells are arsenic-impacted.

In laboratory experiments, lower As concentrations occurred under strongly oxidizing compared to moderately oxidizing conditions, probably due to formation of differing types of Fe oxides, or differing rates of Fe oxide formation. Sulfide oxidation was enhanced (as evidenced by higher dissolved sulfate concentrations) in a sample of St Peter sandstone subjected to a 1200 mg/L chlorine solution compared to a sample subjected to 8 mg/L O_2 in nanopure water, at a pH of 8.5. Under exposure to high chlorine, initial release of dissolved As (30 µg/L) and Fe (250 µg/L) to solution was followed by rapid precipitation of Fe oxides, which occurred concomitantly with a reduction in arsenic concentration. The Fe oxides presumably adsorbed arsenic from solution. Increases in Fe and As concentrations in solution later in the 24-hour experiment suggest deflocculation of the Fe oxides resulted in release of As-Fe nanoparticles. Under the moderately oxidizing control treatment of 8 mg/L O_2, aqueous arsenic concentrations increased steadily over the 24-hour period to 58 µg/L, exceeding those measured under high-chlorine treatment.

The field experiments reported here support the conclusion that microbially facilitated reduction of arsenic-bearing iron (hydr)oxides contribute low but regulatory significant concentrations of arsenic to well water in areas of northeast Wisconsin. Water with a longer residence time in the well had higher concentrations of arsenic, iron and other trace metals, such as nickel and zinc. The lower-quality water correlated to increases in numbers of Fe(III), As(V)- and sulfate-reducing microorganisms in the well. *In situ* well disinfection with low-dose chlorine (as currently recommended in DNR guidance) caused strongly oxidizing conditions in the well bore for less than one day, but with no apparent detrimental effect on water quality. Water quality during routine pumping *without* chlorination was similar to that measured following disinfection treatment. Under both phases of routine pumping tested during this work, arsenic concentrations were lower than during non-pumping phases. Populations of microorganisms in well water returned to pre-treatment levels within three weeks of low-dose disinfection, suggesting that either fresh formation water re-inoculated the well or that biofilm and scale in the well harbored some microbes from the disinfectant.

These experiments do not support recommendation of low-dose chlorination in all arsenic-impacted areas of Wisconsin. In areas where the primary source of aqueous arsenic is reductive dissolution of Fe (hydr)oxides, such as glacial aquifers in southeast Wisconsin, imposing strongly oxidizing conditions over short time periods is unlikely to

promote arsenic release from aquifer solids because arsenic is not found in association with sulfide minerals. In these settings, high-dose chlorination may be more effective in ridding the well of pathogenic and nuisance bacteria.

In northeast Wisconsin, the St. Peter sandstone aquifer contains arsenic-bearing sulfide minerals and arsenic-bearing iron oxides. The complexity and variability in arsenic geochemistry and aquifer mineralogy in this region preclude a single preferred method for well disinfection. Where the aquifer is under confined conditions and aqueous arsenic is relatively low (about a few tens of µg/L), the source of arsenic is more likely attributable to reduction of iron hydr(oxides). In the test well, which is under reducing geochemical conditions, effectively ridding the well of Fe-reducing bacteria (though routine pumping or *in situ* chlorination) improved well water quality. In earlier work, Sonzogni et al. (2004) demonstrated that high-dose chlorination had no detrimental effect in this setting. Effective well disinfection may reduce arsenic in well water in settings where the arsenic is a result of biogeochemical reactions that occur within the well.

Where water levels in wells completed in the St. Peter aquifer indicate unconfined conditions (that is, where static water levels are close to the elevation of the top of the St. Peter Formation), and where aqueous arsenic concentrations are relatively high, the source of aqueous arsenic is likely oxidation of arsenic-bearing sulfide minerals. The current guidelines for low-dose chlorination are more appropriate under these conditions. The laboratory experiments reported here demonstrate that strongly oxidizing conditions imposed under high-dose chlorine treatment can increase the rate of sulfide oxidation. However, the experiments also indicated that strongly oxidizing conditions favor the formation of iron oxides, leading to complex cycling of iron and arsenic. These experiments were limited in nature and do not provide conclusive evidence of long-term geochemical impacts to water quality from high-dose chlorination.

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INTRODUCTION

Background

Arsenic release from naturally-occurring sources is the dominant cause of elevated arsenic concentrations in ground water (Nordstrom 2002; Welch et al. 2000). Geochemical mechanisms including reductive dissolution of iron (hydr)oxides, release of sorbed arsenic from mineral surfaces, and oxidation of arsenic-bearing sulfide minerals can release arsenic to natural waters. These reactions can be reversible, in chemical disequilibrium, and can be abiotic or microbially mediated. These processes can potentially occur simultaneously, complicating identification of a single mechanism of arsenic release in a particular environment. Spatial or temporal changes in geochemistry can transpose a geologic source of dissolved arsenic to an arsenic sink, and vice versa.

Naturally occurring arsenic is present in many aquifers in Wisconsin. In east-central Wisconsin, the primary source of arsenic is sulfide minerals in the St. Peter sandstone (Schreiber et al. 2000). Reduction of arsenic-bearing iron (hydr)oxides is likely a secondary source of arsenic to groundwater in this region where the aquifer is under confined conditions and the groundwater is reducing (Gotkowitz et al 2004). Up to 30 percent of wells in some parts of Winnebago County have arsenic concentrations greater than 5 μg/L. Arsenic also impacts groundwater quality in some glacially deposited sand and gravel aquifers in Wisconsin, where the lack of oxygenated recharge along deep flowpaths leads to reducing geochemical conditions and release of arsenic via reductive dissolution of Fe-(hydr)oxides (Root et al. 2005). These findings are similar to other glacial aquifers throughout the Midwest (Warner 2001; Thomas 2003; Kelly et al. 2005).

Domestic wells completed in bedrock aquifers are often constructed with long open intervals (10s to 100 of m) that provide a significant volume of well bore storage relative to typical domestic water use. Therefore, the quality of the well water can be affected by rock-water interactions that occur over a long residence time in the borehole. Although the potential for transport within the subsurface depends on aquifer water chemistry, reactions that occur in the borehole environment have the potential to control human exposure to arsenic via well water. Conditions in wells that may affect arsenic concentrations include changes in redox related to pump action, which introduces oxygen into well water, and the potential for the growth of microorganisms that facilitate a variety of biogeochemical reactions (Taylor et al. 1997).

Current Wisconsin Department of Natural Resources (DNR) guidelines for maintenance of domestic wells in arsenic sensitive areas suggest disinfect with a low-dose chlorine solution. This guidance is based upon anecdotal evidence suggesting that well disinfection treatments (e.g., chlorination) to control iron oxidizing microbes may increase concentrations of arsenic in well water (DNR 2002). In other areas of the state, the DNR recommends in situ well disinfection with a high chlorine concentration and longer contact time (DNR 1999; DNR 2005). At the foundation of the arsenic-sensitive guidance is the assumption that the oxidizing strength of chlorine disinfectant results in chemical oxidation of arsenic-rich sulfide minerals in the aquifer. However, in areas where microbially-facilitated reduction of iron (hydr)oxides contributes arsenic to groundwater, successfully ridding a well of iron-reducing bacteria through *in situ* chlorination may aid in minimizing the concentration of arsenic in well water. Central to the issue of providing homeowners and well-maintenance professionals with useful guidance is the likelihood that rock-water interactions and the role of microbes in well-bore geochemistry vary at temporal and spatial scales that preclude developing a simple, prescriptive approach to effective well disinfection in some areas of Wisconsin.

Previous work

In 2002, the Wisconsin State Laboratory of Hygiene collaborated with the DNR and the Wisconsin Geological and Natural History Survey (WGNHS) to evaluate three chlorine-based disinfection techniques (Sonzogni et al. 2004). The disinfection practices included a high dose ("shock") chlorination at a concentration of 1,200 mg/L Cl_2; an acid surfactant treatment (to aid in disintegration of microbial film deposits in the well) followed by "low dose" chlorination at 60 mg/L Cl_2; and the current DNR-recommended method of low dose chlorination at 100 mg/L Cl_2. All treatments resulted in a short-term increase in well water arsenic concentrations. However, once fully purged of the products of disinfection, arsenic concentrations and microbial populations decreased relative to those measured in the well water prior to treatment. There was no evidence that any of three chlorination practices caused a sustained, detrimental effect on well water quality. Concentrations of arsenic and iron in the well water returned to pre-experimental levels after several weeks of non-pumping. Testing for bacteria (including coliform bacteria by the Colilert™ test, heterotrophic bacteria by a plate count, Fe(II)-oxidizing bacteria identified and counted microscopically, and the presence or absence of sulfate reducing bacteria via selective media culture) demonstrated the resurgence of microbial populations in stagnant well water. Other recent research on the topic of *in situ* well disinfection and water quality (Seiler 2006) documented short-lasting but large increases in trace metals concentrations following chlorination of domestic wells in Nevada.

The experiments conducted by Sonzogni et al. (2004) were not intended to address all aspects of well disinfection and arsenic release. There was no differentiation between effects on microbially mediated arsenic release and abiotic arsenic release, and information gained on geochemical and biogeochemical processes in the well was indirect. Measurements of Eh, dissolved oxygen and pH of well water during and following treatment gave evidence of extremely oxidizing conditions within the well induced during chlorination. However, it was not possible in the *in situ* experiments to elucidate the effect of these conditions on the mineral surfaces exposed in the borehole.

Sonzogni et al. (2004) suggested additional work to determine the chemical and biological mechanisms affecting arsenic release under the conditions imposed by chemical oxidation. The project reported on here addressed this recommendation by: 1) investigating the effect of oxidizing conditions on abiotic mineral transformations in a laboratory setting and 2) conducting an additional field experiment to evaluate the effect of chlorine disinfection on microorganisms involved in Fe and As cycles.

Purpose and scope

The objective of this project was to investigate the effect of oxidizing conditions imposed by well disinfection on mineral transformations in the St Peter sandstone and on microbially-mediated arsenic release to groundwater. Improvements in understanding both short- and long-term effects of chlorination may inform guidance offered by the DNR for well chlorination in arsenic sensitive areas of Wisconsin.

There are two components to this project. One investigation focused on the effects of strongly oxidizing conditions arising from chlorination on arsenic-bearing minerals (sulfides, iron oxides) in a controlled, laboratory setting. This work addresses the potential for disinfection with a high concentration of chlorine to facilitate arsenic release from aquifer sediments by increasing the rate of sulfide mineral oxidation.

The second investigation focused on the effect of low-dose chlorine disinfection on microbial populations and water quality in a well containing arsenic-bearing sulfides and iron oxides. This work employed a field test of *in situ* disinfection at a well in northeast Wisconsin to investigate controls on microbes that may facilitate As release.

Hydrogeologic setting and well construction for field experiments

The hydrogeologic and geochemical conditions of arsenic contamination are well-characterized at the site selected for the field component of this study (Figure 1) (Schreiber et al. 2003). The hydrostratigraphy includes glacial and glaciolacustrine deposits underlain by a thick sequence of Paleozoic rocks. Most domestic water wells are completed in the upper 45 m of bedrock, within the Sinnipee Group dolomite and underlying sandstone of the St. Peter Formation. The St. Peter constitutes a regionally extensive aquifer of variable thickness that is generally confined in the area of the test well but may be locally unconfined due to variation in topography or dewatering from pumping. The hydraulic conductivity of the aquifer is on the order of 1 m/day (Gotkowitz et al. 2004). Where the St. Peter is absent, domestic water wells are typically completed in dolomite of the Sinnipee and underlying Prairie du Chein Groups.

The test well is constructed similarly to domestic wells in the area. The base of the Sinnipee dolomite and the upper portion of the St Peter sandstone are exposed in the uncased portion of the well, which extends from the base of casing at 18.9 m to a total depth of 26.0 m below ground surface. The aquifer is confined at this location, with a static water level at 8.9 m below ground surface. Borehole flow meter measurements indicate that natural gradients are insufficient to induce measurable flow into and out of the borehole under non-pumping conditions (Gotkowitz et al. 2004).

Site geochemistry

In the area surrounding the field site, solid-phase arsenic is associated with iron-sulfide minerals, including pyrite and marcasite, and iron (hydr)oxides. Solid-phase concentrations of arsenic range from 10s to 100s of parts per million (ppm) in minerals that form sulfide cement at the Sinnipee-St. Peter contact and in sulfide-rich nodules and veins disseminated within the St. Peter sandstone. Where sulfide minerals are not present in the sandstone, arsenic concentrations average less than 5 ppm. In some samples collected at this site, iron oxide weathering products of the sulfide minerals have higher associated arsenic concentrations than the iron-sulfide minerals (Gotkowitz et al. 2004).

Results of residential water well testing provided by the WDNR indicate that groundwater arsenic concentrations vary from non-detectable levels to hundreds of μg/L within a 4 km^2 area of the test well (figure 1). Groundwater in the St. Peter aquifer is Ca-

Mg-HCO_3 type, with a pH of 7.1 and low dissolved oxygen (DO) concentrations (about 0.35 mg/L). Groundwater is under reducing conditions with an oxidation reduction potential (ORP) of -50 mV (Gotkowitz et al. 2004).

Based on several pumping tests at the field site, Gotkowitz et al. (2004) attributed variability in groundwater arsenic concentrations to the residence time of groundwater in the well bore. During this previous cite characterization, As ranged from 1.8 to 22 μg/L in wells at the site, with higher concentrations related to longer residence times. Samples collected during a pumping regime intended to simulate domestic use (380 L, or about 1.3 well volumes, pumped every eight hours at a rate of 38 L/minute) ranged from about 3 to 6 μg/L. The well water became more reducing during this domestic pumping schedule than under fully purged conditions, with DO decreasing to 0.25 mg/L and ORP to -100 mV. These findings indicate that pumping at a rate and volume similar to domestic use does not fully purge the well of water with a long residence time in the borehole. While oxidation of sulfide minerals appears to release arsenic to ground water in zones within the aquifer, reduction of arsenic-bearing iron (hydr)oxides is a likely mechanism of arsenic release to water having a long residence time in the well borehole.

PROCEDURES AND METHODS

Laboratory studies of strongly oxidizing conditions

The impact of high-dose chlorination on mineral transformation and arsenic release was assessed through batch experiments conducted with two different samples of the St. Peter formation: one from the Skunk Hill (SH) quarry (located about two miles west of the Town of Freedom, Outagamie County, Wisconsin) and the other from the Leonard Road Michaels Materials (LM) quarry (figure 1).

For each sample, thin sections (12) were made (Spectrum Petrographics, Inc) for characterizing the mineralogy and elemental composition before and after chlorination. Because of the small surface area of the thin sections, additional experiments with crushed sample were conducted to allow for calculation of rates of oxidation and arsenic release. Portions of the crushed sample were also digested and analyzed for whole rock geochemistry (Actlabs, Inc).

Pre-characterization of the thin sections was completed at the Microbeam Lab at USGS in Reston, VA. Quantitative analysis of sulfide minerals was run on the JEOL 8900 EMPA at 20kV and 3x10-8 mA. Qualitative element maps were run at 20kV and 5x10-8 mA at a 30msec dwell time. Thin sections were characterized before and after 24-hr exposure to high (1200 mg/L) free chlorine (Cl_2 + HOCl + OCl^-) solutions.

Initial batch experiments using the SH material in high chlorine (1200 mg/L free chlorine) solution released very low concentrations of arsenic in solution; later digestion and analysis of this material revealed low (22 mg/kg) arsenic in the whole rock material. Thus, remaining experiments focused on the LM quarry material, which from previous analyses (sample LE-X; see Appendix A-2, Simo et al. 1997), showed higher concentrations (up to 500 mg/kg) of As in the whole rock sample.

The batch experiments were performed in a Metrohm 719 S Titrino pH stat. The solution pH was maintained at 8.5, a pH at which hypochlorite (OCl^-) should be the dominant chlorine species. The pH was maintained using 0.1M NaOH. To date, two experiments with the LM material have been conducted: in deionized, nanopure water ("MilliQ" by Millipore, Inc) with 8 mg/L dissolved oxygen (DO), and high (1200 mg/L) chlorine solution. The high chlorine solution was made by diluting newly opened bleach (Chlorox) with nanopure water. The concentration of free chlorine was estimated using CHEMetrics Vacuettes kits K-2505C.

The LM sample was crushed to particles between 250 and 500 microns in diameter. Five grams of the solid material was then reacted in 100 mL of solution over a 24 hour period. Two milliliters were sampled at variable time steps over the experimental period and split for metals (As, Fe) and anion (SO_4) analysis. Both sample aliquots were filtered (0.20 micron) to remove particles from solution. The metals samples were preserved with 0.1M HNO_3 to pH<2. Arsenic was analyzed using graphite furnace atomic absorption spectroscopy (Varian Spectra220Z with Zeeman background correction). Iron was analyzed using the Ferrozine method (Stookey, 1970). Sulfate was analyzed by ion chromatography (Dionex DX-120 Ion Chromatograph).

Field study of in situ disinfection

Well treatments

The experiment consisted of a control treatment (a period of pumping without disinfection) followed by chlorine disinfection and pumping. These two treatments were carried out sequentially in a single test well, from September 2006 to February 2007 (Figure 2). This experimental design, using only one test well, is not ideal because initial conditions in the well, such as the types and numbers of microbes, may not be identical for the control and treatment phases. However, an alternative approach, such as subjecting two wells to either control or treatment, would presumably suffer from differing initial conditions related to the small-scale variability in mineral assemblages and solid-phase arsenic concentrations at the site (documented in Schreiber et al. 2003). The single well approach used here is also preferred to a multi-well experimental design because of the differences in water quality in wells of similar design as noted by Schreiber et al. (2000).

Prior to the control phase, over 15,000 L (about 52 well volumes) were purged from the well and a pre-control stagnant period (no pumping) was imposed for 27 days. About 4,200 L were purged from the well (14.6 well volumes) to begin the control phase, which consisted of pumping with no chemical disinfection. The well was pumped at a rate and schedule simulating domestic water use for 28 days. The domestic pumping schedule consisted of pumping about 360 L of water three times per day, at a rate of 36 L per minute, resulting in a total daily withdrawal typical of a four-person residence, assuming a per capita residential water use rate of 255 L per day. Samples of well water were collected periodically throughout each experimental phase (Figure 2). Samples meant to characterize water quality under stagnant conditions were collected by purging only a few liters prior to sample collection, to flush water from the pipe and sample port. Samples collected to characterize the domestic pumping routine were collected during the first five minutes of a 10-minute pumping cycle, after purging several liters of water to clear the pump and piping.

Following the control phase, a pre-disinfection stagnant (non-pumping) period was imposed for 30 days. At the end of this period, the well was disinfected with a chlorine solution following DNR (2002) recommendations for this arsenic-impacted area in Wisconsin. The treatment was initiated by purging 1,245 L (4.2 well volumes) from the well. Chlorox® bleach containing 6% sodium hypochlorite was mixed with 378 L of well water to produce a solution of about 100 mg/L chlorine. The solution was poured into the well and recirculated through the well, pump and associated pipe for twenty-five minutes. A sample of the re-circulated treatment solution was collected for analysis. About 2.3 well volumes, 685 L, were purged to the sanitary sewer, until the water was free of chlorine odor. The well was pumped at the domestic schedule for 28 days, after which pumping was discontinued. Sampling was continued through a 28-day post-treatment stagnant period.

Field and laboratory procedures

Field parameters, including temperature, pH, DO, and ORP, were measured with a submersible Hydrolab Minisonde water quality sensor and data logger installed above the pump, near the base of the test well. Sensors were calibrated prior to deployment and were checked for drift following recovery of the unit from the well. Water levels in the well were monitored and recorded with a Solinst® Levelogger® and Barologger® system.

Aliquots of samples collected for total metals concentrations were left unfiltered and preserved with nitric acid to a pH <2 (0.5% HNO_3). Aliquots for analyses of dissolved fractions (As, Fe, Mn, Cu, Pb, Co, Zn, Cr, Mo and Ni) were filtered in the field (0.20 micron) prior to acid preservation. The suite of metals selected for dissolved analyses was based on those found at elevated concentrations in samples of aquifer solids collected while drilling the monitoring well (Gotkowitz et al. 2004). Samples analyzed for arsenite (As^{3+}) were speciated on-site using anion exchange cartridges (Le et al. 2000). Arsenate (As^{5+}) concentrations were determined by subtraction of arsenite from the total dissolved As result. Samples collected for DOC (filtered in the field to 0.2 micron) and TOC were preserved with H_2S0_4. Samples collected for sulfate were unpreserved. Samples collected for sulfide were preserved with ascorbic acid. Samples collected for analysis of ammonia and nitrate were filtered in the field (0.2 micron) and preserved with H_2S0_4. All samples were placed on ice following collection and transported to the Wisconsin State Laboratory of Hygiene for analysis.

Arsenite was determined by graphite furnace atomic absorption spectrophotometer (GFAAS) according to Method 3113B (APHA, 1999); total dissolved arsenic was determined by AA. Other metals were measured by inductively coupled plasma-optical emission spectrometer (ICP-OES) according to USEPA Method 200.7 (detection limit <0.1 mg/L). Sulfate was measured by methylthymol blue colorimetry according to USEPA Method 375.2 (detection limit 4.5 mg/L). Sulfide was determined by SM 4500G and ammonia by EPA 350.1.

Water samples for microbiological analyses were collected in a sterilized glass container and transported on ice to the Department of Geology and Geophysics at the University of Wisconsin - Madison. Upon arrival at the laboratory, 3 L of the water sample was filtered through 0.2 micron Whatman nylon membrane filter. The filter was preserved at $-80^{o}C$ for future Molecular analysis. An aliquot of unfiltered sample was aseptically transferred into sterile 160 ml serum bottles. The bottles were capped with sterile butyl rubber stoppers and used for following culture-dependent analysis.

Molecular analysis of well water was performed using standard 16S rDNA methodology, as previously described (Holmes et al. 2002). In brief, DNA was extracted from filters using PowerSoil DNA Kit for Soil (MOBIO Laboratories Inc., 2746 Loker Ave West, Carlsbad, CA). Aliquots of DNA from water samples collected at days 0, 14, 55, 85 and 128 were PCR amplified using 16S rRNA gene forward primer 8F (AGAGTTTGATCMTGGCTCAG) and the reverse primer 1492R (TACGGCTTACCTTGAGAGACG92). PCR reactions were performed in a BioRad thermal cycler as previously described (Shelobolina et al. 2007). PCR amplicons were cloned using the pGEM®-T vector and Escherichia coli JM109 competent cells according to the manufacturer's instructions (Promega, Madison, WI USA). Clones were sequenced and identified by BLAST analysis.

Culture dependent microbial community analysis was performed for aerobic microorganisms including acetate- and As(III)-oxidizing bacteria, and for anaerobic microorganisms including Fe(III)-reducing, sulfate-reducing and As(V)-reducing bacteria. Strict anaerobic techniques were used to cultivate anaerobic organisms as previously described (Shelobolina et al. 2007). For microbial enumeration, water samples were sequentially diluted into roll-tubes with agarized medium. The roll-tube method (Hungate 1969) utilizes a solidifying agent–containing medium prepared under O_2-free gas, and dispensed into culture tubes, which are sealed with the rubber stoppers. After inoculation, rapidly rolling the tubes horizontally results in a thin layer of solidified medium on the inner surface of the tubes. Electron donors and acceptors were added to corresponding tubes aseptically before the microbial analysis. 20 mM acetate was used as the electron donor for enumeration and isolation of aerobic heterotrophic, Fe(III)-, As(V)- and SO_4^{2-} -reducing microorganisms. For enumeration of As(III)-oxidizing organisms, 5mM As(III) was added as the electron donor. Sterile air was added to the gas phase of the tubes for aerobic and As(III)-oxidizing bacteria. 10 mM SO_4^{2-}, 100 mM hydrous ferric oxide (HFO), and 10 mM As(V) were added as the electron acceptors to the respective anaerobic cultures. Once colonies utilizing specific electron donors and acceptors developed in the tubes, the number of colonies (CFU) for each microbial group was determined by visual examination.

RESULTS AND DISCUSSION

Laboratory studies of strongly oxidizing conditions

Results of the SH characterization before and after exposure to chlorine are shown in Figures 3 and 4. Figure 3 contains images of the pre-reacted sample. In the back scattered electron (BSE) image, quartz grains appear as the round, darker gray particles. The pyrite appears as the brighter cement between the sand particles. The maps show relatively pure pyrite, cementing quartz sands. Results of quantitative WDS (wavelength dispersive spectral) analysis and whole rock digestion reveals low concentrations of arsenic and other trace metals such as Ni, Co and Cu in the pyrite (Table 1; Appendix 1). Lead concentrations are higher (up to 1.4 weight %; Table 1; Appendix 1) and occur with sulfur, indicating the presence of galena.

A potassium rich mineral occurs at some boundaries between the pyrite and quartz, and in cracks in the pyrite cement. This mineral appears in the BSE image as the darker material that rims the quartz sands and fills cracks in the pyrite. It is probable that this material is jarosite ($KFe_3(SO_4)_2(OH)_6$). This mineral is typically found in association with pyrite as it forms as a reaction product of pyrite oxidation.

Figure 4 shows EMPA element maps of the reacted (1200 mg/L “free chlorine” for 24 hours) SH thin section. The pyrite cements remain rather pure with potassium-rich jarosite filling cracks and contact boundaries. One notable difference in the post-reacted section is the appearance of iron oxide material on the surface. These iron oxides emerge from the pyrite surface as bulbous clusters of very fine particles, along cracks and boundaries where the pyrite surface has more reactive surface area. These oxides have very strong Fe signals and contain little else. The post-reacted WDS analyses (Table 2) reveal slightly lower trace metal concentrations in the pyrite surfaces than the un-reacted analyses. Although these differences could be related to release of trace metals from the pyrite surface after exposure to chlorine solutions, the differences are well within one standard deviation of the mean of both populations.

As mentioned in the methods section, results of the chlorination experiments with the SH material, containing 22 mg/kg As, revealed almost undetectable As in solution throughout 24 hours of exposure to the high chlorine levels. Experiments conducted with the LM material, which contains up to 500 mg/kg of As in bulk, released much higher As concentrations to solution. Concentrations of arsenic and sulfate measured over the 24 hour monitoring period from both the control (nanopure water at 8 mg/L DO) and high chlorine experiments, both at pH 8.5, are shown in Figures 5 and 6.

Figure 6 shows that arsenic concentrations peak at 59 µg/L, after 24-hour exposure to nanopure water. A similar pattern of increasing concentrations over time, reaching 300 mg/L, is observed in the sulfate data (Figure 7). Exposure of the LM crushed sample to high chlorine results in significantly higher concentrations of sulfate released to solution (Figure 7). Sulfate concentrations reached 700 mg/L after 24 hours, signifying increased sulfide oxidation.

Other evidence of sulfide oxidation during high chlorine reaction with the LM sample was the volume of NaOH added to maintain pH 8.5 in solution. As shown in reaction 1, sulfide oxidation decreases the pH. In the experiment with water, 8.4 mL of 0.1 M NaOH was added to maintain pH 8.5. The high chlorine experiment required 24 mL of NaOH to maintain the pH, which we attribute to the acidity generated from sulfide oxidation.

1) $2FeS_2 + 7O_2 + 2H_2O = 2Fe^{2+} + 4SO_4^{2-} + 4H^+$

During the high chlorine experiment, in contrast to the steady increase of sulfate, arsenic concentrations reach a peak of 30 µg/L at 5 minutes, decline over the next 7 hours, then increase to 22 µg/L at 15 hrs and 33 µg/L at 24 hours (Figure 6). Analysis of iron concentrations in the high chlorine experiment is shown in Figure 7. The pattern of iron release to solution mimics that of arsenic release – with the initial peak concentration at 5 minutes, declining to below detection until 15 hours, and then increasing to 24 hrs.

During the high chlorine experiments, we observed the formation of iron oxides at approximately 5 minutes after the experiment began. The iron oxides continued to increase during the experiment, forming a fluffy layer on top of the sulfide material. The formation of oxides was not observed during the reaction of the LM material with nanopure water. This observation is significant because Fe oxides play an important role in adsorbing arsenic. If the formation of Fe oxides is not favored in an aquifer or well bore, arsenic is more likely to remain in solution.

The similar patterns of As and Fe release during the high chlorine experiment, combined with knowledge of Fe solubility, suggest that the initial increase in As and Fe during the initial 5 minutes likely results from release of ferrous iron and associated As from pyrite oxidation (reaction 1). Observations of Fe oxide formation and measured decrease in As and Fe concentrations after five minutes of reaction are consistent with nucleation and precipitation of Fe oxides and their concomitant adsorption of As.

The increase of Fe and As in solution at 15 hours may be a result of deflocculation of the ferrihydrite into nanoparticles, which can pass through the 0.2 micron filter. We have observed this phenomenon in previous studies of the As-Fe system (Tadanier et al. 2005). Further experiments using ultrafiltration or ultracentrifugation to remove small particles are necessary to test this hypothesis. An alternative hypothesis that attributes the increase of Fe and As to reductive dissolution of the recently-formed iron oxides is not plausible because oxidizing conditions were maintained throughout these experiments.

Field study of ***in situ*** disinfection

Arsenic concentrations generally increased during non-pumping periods, reaching a maximum concentration of 13.7 µg/L in well water (table 3). An exception to this was the pre-disinfection non-pumping period, during which arsenic did not increase. Results of well water analysis during the pre-disinfection period suggested leaching of dye from rope was used to suspend the Hydrolab in the well during this period. The rope was subsequently tested by placing a portion of it in deionized water for several weeks. This water had elevated concentrations of aluminum, chromium, lead, copper, nickel and zinc compared to a field blank of deionized water. A different type of rope was used in the well during all other experimental phases.

Arsenic consistently decreased to 6 µg/L or less under the control and post-disinfection pumping periods (table 3 and Figure 8). This is consistent with previous results from this well that show higher arsenic concentrations in water with a long residence time in the well bore and lower concentrations of arsenic in groundwater that is representative of aquifer water (meaning that the well is fully purged when the sample is collected). Arsenic is primarily dissolved and present as arsenite in samples of well water with a long residence time in the borehole (stagnant conditions) and samples collected under fully or partially purged conditions that are representative of aquifer water.

Concentrations of dissolved iron correlate to those of arsenic, with iron exceeding 1 mg/L during non-pumping phases only. Dissolved manganese is also of interest because microbial reduction of manganese oxides can also provide arsenic to groundwater. Manganese remained between 44 to 69 µg/L with the exception of the first stagnant phase, during which dissolved Mn was 123 µg/L. Microbial activity requires a source of organic carbon. Both DOC and TOC are low but consistently present in the well and aquifer water, with concentrations ranging from 0.9 to 5.2 mg/L (table 4).

Concentrations of other metals remained very low in aquifer water and water with a long residence time in the well (tables 5 and 6). Dissolved nickel and zinc are positively correlated with arsenic and iron, with higher (but still very low) concentrations noted during periods of non-pumping (table 5). As indicated in table 5, results for the pre-disinfection stagnant period are not available for all parameters due to sample contamination.

Strongly reducing conditions developed in the well water during non-pumping phases, with DO less than the instrument detection limit of 0.2 mg/L and ORP decreasing to less than 400 mV after several days (Figure 9). During stagnant phases, pH increased asymptotically for a period of about 5 days (Figure 9). A similar asymptotic rise to pH of about 8 occurred in less than one day under both periods of routine pumping (Figures 10 and 11). The well water does not become strongly reducing during periods of routine (three times daily) pumping, with ORP stabilizing at about -100 (Figure 10). During chlorination, DO levels remained elevated for about 2.5 hours (0.1 day), and ORP was elevated for about 2 days (Figures 11 and 12). ORP values measured in the well during the pumping-only control phase are lower than those collected during post-disinfection pumping. Both sets of measurements indicate that periodic pumping from the well is sufficient to maintain a check on the reducing conditions that develop in the well during extended periods of non-pumping.

Microbiological populations

Results of microbial community analysis using culture-dependent methods are illustrated in Figure 13, and demonstrate that the number of all tested groups of microorganisms (including As(V)-reducing, Fe(III)-reducing, sulfate-reducing, As(III)-oxidizing and aerobic microorganisms) increased under stagnant regimes in the pre-control and pre-disinfection phases.

The counts of Fe(III), sulfate-, and arsenate-reducing bacteria might overlap. Several cultured sulfate- and Fe(III)-reducing bacteria are also capable of As(V) reduction (Stolz et al. 2006). The numbers of anaerobic bacteria including dissimilatory iron-reducing bacteria (DIRB), dissimilatory arsenate-reducing bacteria (DARB), and sulfate-reducing bacteria generally increase with increases in arsenic concentrations (Figures 8 and 13). This finding supports the hypothesis that As-rich microbially-facilitated reduction of As-rich iron (hydr)oxides is a source of arsenic to well water in this setting.

In situ treatment with low-dose chlorination almost completely ceased the growth of tested microorganisms, but these microbial communities returned to previous levels in the well bore within three weeks of treatment (Figure 13). This suggests that either fresh formation water re-inoculated the well or that biofilm and scale in the well harbored some microbes from the disinfectant; low-dose disinfection appears to have had no lasting positive or negative effect on microbial activity or on the quality of pumped water.

Routine pumping of the well primarily affects anaerobic organisms. The numbers of As(III)-reducing, Fe(III)-reducing and sulfate-reducing microorganisms decreased 1-1.7 orders of magnitude during the control pumping phase (Figure 13). In this setting, routine well use may improve well water quality by preventing the microbially-facilitated reactions that occur in the well and promote arsenic release.

Culture-independent analysis was performed for well water collected at days 0, 14, 55, 85 and 128 (Figure 14). Analysis of 16S rDNA sequences indicated that all of the water samples had a high degree of microbial diversity. The proportions of the groups at the division and subdivision levels varied after different well water treatments. The microbial community at T0 represents the microbial community of the groundwater and was composed of 13.4% *Firmicutes,* 29.9 % *Proteobacteria*, 29.9 % of bacteria of the *Chloroflexi* environmental cluster and 26.9% of other bacteria including phyla *Nitrospira*, *Actinobacteria*, *Spirochaetes*, *Bacteroidetes*, and *Verrucomicrobia*.

At 14 days under stagnant conditions, culture-independent results show a decrease in the population of Gram-positive bacteria (*Firmicutes*, 1.4%) and a significant increase in the number *Proteobacteria* (to 74.3%). The composition of the clones in *Proteobacteria* phylum changed as well. Delta Proteobacteria become dominated by *Desulfobulbaceae* clones. Cultured *Desulfobulbus* spp were shown to be capable of both sulfate- and Fe(III)-reducing activity (Holmes et al. 2004). Another cultured *Desulfobulbaceae* family member has recently been shown to reduce As(V) with sulfide surving as the electron donor (Hoeft et al. 2004). Beta Proteobacteria became dominated by the clones of *Gallionella ferruginea* and *Dechloromonas* spp, to which Fe(II)-oxidizing activity can be attributed. *Gallionella ferruginea* is often found in As-contaminated sites (Ohnuki et al. 2004);(Bruneel et al. 2006);(Battaglia-Brunet et al. 2006) but its direct role in As(III) oxidation has not been investigated. It also yet remains to be determined if *Dechloromonas* spp are capable of As(III) oxidation.

The microbial counts showed significant increase in the number of As(III)-oxidizing bacteria at day 55, which was at the end of the control-phase pumping period. This sample also documented further increase in the proportion of Beta Proteobacteria in the clone library, which became almost completely dominated by *Gallionella ferruginea* clones.

The well water sample collected on day 85, at the end of the 30-day stagnant period prior to chlorination, is characterized by an increase in the proportion of Delta-proteobacteria, including representatives of known Fe(III)-reducing bacteria belonging to genera *Geobacter* and *Anaeromixobacter*, along with the members of the family *Desulfobulbaceae*. Other Fe(III)-reducing bacteria detected at day 85 were in the class Beta Proteobacteria (*Rhodoferax* spp) and in the phylum Acidobacteria (*Geothrix fermentans*). Molecular analysis of the well water collected during the final stagnant regime (day 128) shows absolute dominance of Proteobacteria with the highest proportion of Beta Proteobacteria (70%) including clones of bacteria of iron cycle belonging to *Galionella*, *Rhodoferax* and *Dechloromonas* spp.

The results of culture-dependent and culture-independent analyses demonstrated that although there are diverse communities of microorganisms in the well during all tested phases, bacteria of the iron cycle dominate the populations during extended periods of non-pumping.

CONCLUSIONS AND RECOMMENDATIONS

This initial set of laboratory experiments leads to several preliminary conclusions about short term effects of strongly oxidizing conditions on mineral transformations and arsenic release. Exposure of St Peter sample to high chlorine solution significantly enhances sulfide oxidation (by a factor of 2) compared with oxidation by 8 mg/L O_2 in water. The patterns of As and sulfate release in the 8 mg/L O_2 water experiment suggest that As is released as a dissolved phase and is not removed by adsorption to Fe oxides over the course of the 24-hour experiment. As concentrations remained elevated under these moderately oxidizing conditions compared to those under high-chlorine treatment. This may be due to a difference in the type of Fe oxide that forms under each condition or in the rate of oxide formation.

The patterns of As, Fe and sulfate in the high chlorine experiment indicate cycling of iron and arsenic in the system. Rapid precipitation of Fe oxides followed the initial release of dissolved As and Fe, and the As released during sulfide oxidation subsequently adsorbed to the Fe oxide precipitate, effectively removing As from solution. Increases in Fe and As in solution later in the 24-hour experiment is attributed to deflocculation of the Fe oxides. In previous studies, Fe oxides have been shown to release nanoparticles to solution (Tadanier et al. 2005). These nanoparticles can easily pass through 0.2 micron filters, and their formation is significant because they can be suspended in well water. If the nanoparticles remain in association with aquifer solids, they are effectively sequestered under oxidizing conditions.

The field experiments reported on here support the conclusion that microbially facilitated reduction of arsenic-bearing iron (hydr)oxides contributes low, but regulatory significant, concentrations of arsenic to well water. Water with a longer residence time in the well tends to have higher concentrations of arsenic, iron and other trace metals such as nickel and zinc. Strongly reducing geochemical conditions develop in the well during periods of non-use. The development of lower quality well water in stagnant well water correlates with growth of DIRB and DARB microorganisms in the well.

Water quality at the test well improved under pumping conditions without chlorine treatment, and under pumping following i*n situ* disinfection with low-dose chlorine, as currently recommended by the DNR for arsenic-impacted regions of the State. Chlorine treatment caused oxidizing conditions in the well bore for less than one day, but there were no apparent detrimental affects to water quality. This finding is consistent with earlier work at this site by Sonzogni et al. (2004), which demonstrated that high-dose chlorination had no detrimental effect in this setting. In fact, effective well disinfection may reduce arsenic in well water in settings where the arsenic is a result of biogeochemical reactions that occur within the well.

Here, where the St. Peter aquifer is under confined conditions and the source of arsenic is likely reduction of Fe(hydr)oxides, short-term imposition of oxidizing conditions is unlikely to lead to arsenic release because sulfides are not the source of aqueous arsenic. In fact, effective chlorination may limit arsenic release by reducing the numbers of iron-reducing and other anaerobic bacteria. The low-dose chlorination treatment at the test well reduced the numbers of all microorganisms tested, but the populations recovered within three weeks. This suggests that either fresh formation water re-inoculated the well or that biofilm and scale in the well harbored some microbes from the disinfectant.

Taken together, this set of laboratory and field experiments do not provide scientific evidence for recommendation of low-dose well chlorination in all arsenic-impacted areas of Wisconsin. Presumably, in areas such as southeast Wisconsin, where the source of aqueous arsenic is reductive dissolution of Fe (hydr)oxides (Root et al. 2005), imposing strongly oxidizing conditions over short time periods is unlikely to exacerbate arsenic release because the source is not sulfide minerals. In this setting, high-dose chlorination may be preferable because it may be more effective in ridding the well of pathogenic and nuisance bacteria.

In northeast Wisconsin, the St. Peter sandstone aquifer contains arsenic-bearing sulfide minerals and arsenic-bearing iron oxides. The complexity and variability in arsenic geochemistry and aquifer mineralogy in this region preclude a single preferred method for well disinfection. Where the aquifer is under confined conditions, well water has very low DO, and aqueous arsenic is relatively low (about a few tens of µg/L), the source of arsenic is more likely attributable to reduction of iron hydr(oxides). Under these conditions at the test well, effectively ridding the well of Fe-reducing bacteria (though routine pumping or *in situ* chlorination) improved well water quality. In earlier work, Sonzogni et al. (2004) demonstrated that high-dose chlorination had no detrimental effect in this setting.

Where water levels in wells completed in the St. Peter aquifer suggest unconfined conditions (that is, where static water levels are close to the elevation of the top of the formation), well water is generally oxygenated, and where aqueous arsenic concentrations are relatively high, the source of aqueous arsenic is likely oxidation of arsenic-bearing sulfide minerals. The current guidelines for low-dose chlorination are appropriate in this setting. The laboratory experiments reported on here demonstrate that strongly oxidizing conditions imposed under high-dose chlorine treatment can increase the rate of sulfide oxidation. However, the experiments also indicated that strongly oxidizing conditions favor the formation of iron oxides and lead to complex cycling of iron and arsenic. These experiments were limited in nature and do not provide conclusive evidence of long-term geochemical impacts to water quality from high-dose chlorination.

REFERENCES

Battaglia-Brunet, F. et al., 2006. A simple biogeochemical process removing arsenic from a mine drainage water. Geomicrobiology J., 23: 201-211.

Bruneel, O., Duran, R., Casiot, C., Elbaz-Poulichet, F. and Personne, J.-C., 2006. Diversity of microorganisms in Fe-As-rich acid mine drainage waters of Carnoules, France. Applied and Environmental Microbiology, 72(1): 551-556

Gotkowitz, M. B., M. S. Schreiber, J.A. Simo, 2004. Effects of water use on arsenic release to well water in a confined aquifer. *Ground Water* 42(4): 568-575.

Hoeft, S.E., Kulp, T.R., Stolz, J.F., Hollibaugh, J.T. and Oremland, R.S., 2004. Dissimilatory arsenate reduction with sulfide as electron donor: experiments with Mono Lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate reducer. Appl Environ Microbiol., 70(5): 2741-2747.

Holmes, D., Finneran, K., O'Neil, R. and Lovley, D., 2002. Enrichment of Geobacteraceae Associated with Stimulation of Dissimilatory Metal Reduction in Uranium-Contaminated Aquifer Sediments. Appl. Environ. Microbiol.

Holmes, D.E., Bond, D.R. and Lovley, D.R., 2004. Electron Transfer by Desulfobulbus propionicus to Fe(III) and Graphite Electrodes. Appl Environ Microbiol., 70 (2):1234-7.(2): 1234-1237.

Hungate, R.E., 1969. A roll tube method for cultivation of strict anaerobes. Methods Microbiol., 3B: 117-132.

Kelly, W.R., T.R. Holm, S.D. Wilson, and G.S. Roadcap. 2005. Arsenic in glacial aquifers: sources and geochemical controls. *Ground Water* 43, no. 4: 500-510.

Le, X. C., S. Yalcin, and M. Ma (2000). Speciation of submicrogram per liter levels of arsenic in water: On-site species separation integrated with sample collection. *Environmental Science and Technology* 34: 3094-3100.

Nordstrom, D.K., 2002. Public health - Worldwide occurrences of arsenic in ground water. Science, 296 (5576): 2143-2145.

Ohnuki, T. et al., 2004. Mechanisms of arsenic immobilization in a biomat from mine discharge water. Chemical Geology, 212: 279-290.

Root, T., Bahr, J.M. and Gotkowitz, M.B., 2005. Controls on arsenic Concentrations in Groundwater near Lake Geneva, Wisconsin. In: P.A. O'Day, D. Vlassopoulos, X. Meng and L.G. Benning (Editors), Advances in Arsenic Research. American Chemical Society, Washington, D.C., pp. 161-174.

Schreiber, M. E., J. A. Simo, and P.G. Freiberg, 2000. Stratigraphic and geochemical controls on naturally occurring arsenic in groundwater, eastern Wisconsin, USA." Hydrogeology Journal **8**: 161-176.

Schreiber, M.E., M.B. Gotkowitz, J.A. Simo and P.G. Freiberg, 2003, Mechanisms of Arsenic Release to Groundwater from Naturally Occurring Sources, Eastern Wisconsin in Welch, A. and K. Stollenwerk eds., Arsenic in Groundwater, Boston: Kluwer Academic Publishers: pp. 488.

Seiler, R.L., 2006. Mobilization of lead and other trace elements following shock chlorination of wells. Science of The Total Environment, 367(2-3): 757-768.

Shelobolina, E.S. et al., 2007. Geobacter pickeringii sp. nov., Geobacter argillaceus sp. nov. and Pelosinus fermentans gen. nov., sp. nov., isolated from subsurface kaolin lenses. IJSEM, 56: 126-135.

Simo, J.A., Freiberg, P.G. and Schreiber, M.E., 1997. Stratigrpahic and geochemical controls on the mobilization and transport of naturally occurring arsenic in groundwater: implications for water supply protection in northeastern Wisconsin., Water Resources Center, University of Wisconsin - Madison, WRC GRR 97-05, Madison, Wisconsin.

Sonzogni, W.C., A. Clary, G. Bowman, J. Standridge, D. Johnson, and M.Gotkowitz. 2004. Importance of Disinfection on Arsenic Release from Wells Final Report to the Wisconsin DNR Groundwater Management Practice Monitoring Program, DNR Project #172.

Stolz, J.F., Basu, P., Santini, J.M. and Oremland, R.S., 2006. Arsenic and selenium in microbial metabolism. Annu. Rev. Microbiol., 60: 107-130

Tadanier CJ, Schreiber ME, Roller JW. 2005. Arsenic mobilization through microbially-mediated reduction of iron-(hydr)oxide nanoparticles. Environmental Science and Technology 39(9): 3061-3068.

Taylor, S.W., Lange, C.R. and Lesold, E.A.L., 1997. Biofouling of contaminated Ground-water recovery wells: characterization of microorganisms. Ground Water, 35(6): 973-980.

Thomas, M.A. 2003. Arsenic in Midwestern glacial deposits - occurrence and relation to selected hydrogeologic and geochemical factors. USGS WRIR 03-4228.

Warner, K.L. 2001. Arsenic in glacial drift aquifers and the implication for drinking water - Lower Illinois River Basin. *Ground Water* 39, no. 3:433-442.

Welch, A.H., Westjohn, D.B., Helsel, D.R. and Wanty, R.B., 2000. Arsenic Occurrence in Ground Water of the United States: Occurrence and Geochemistry. Ground Water, 38(4): 589-604.

Wisconsin Department of Natural Resources, 1999. Sulfur Bacteria Problems in Wells PUB-DG-005 99REV.

Wisconsin Department of Natural Resources, 2002. Well Chlorination in Arsenic Sensitive Areas: Too much of a good thing? PUB-DG-069 2002.

Wisconsin Department of Natural Resources, 2005. Iron Bacteria Problems in Wells PUB-DG-004 2005.

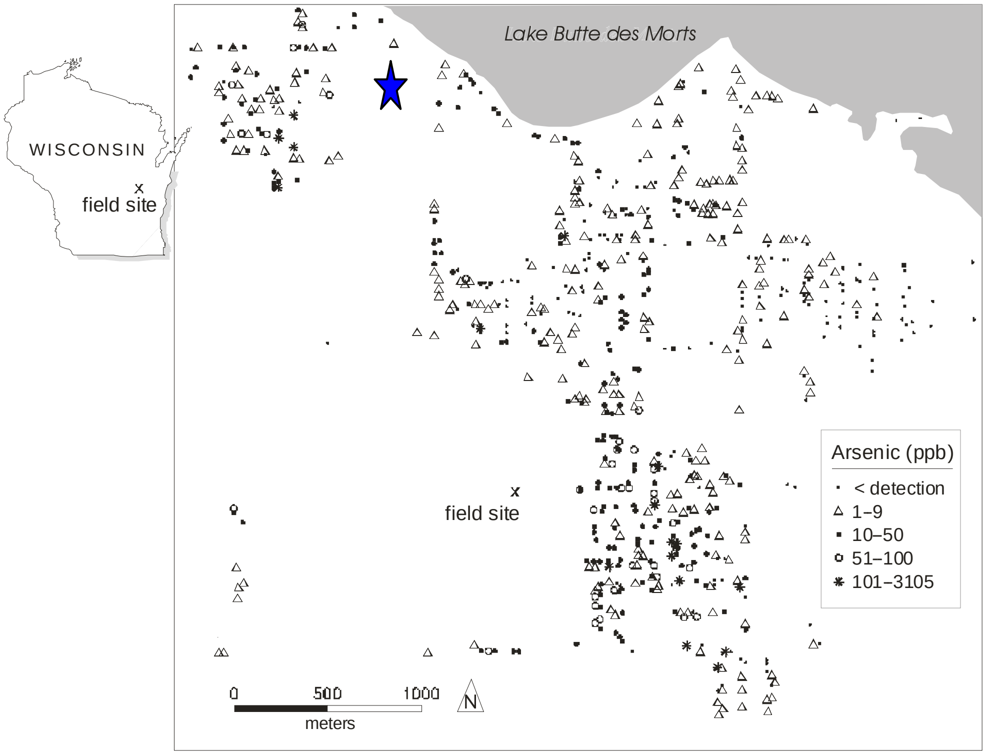


Figure 1. Location of field site and concentrations of arsenic in well water in near-by wells. Blue star shows location of Michael's quarry on Leonard Road (LM site).

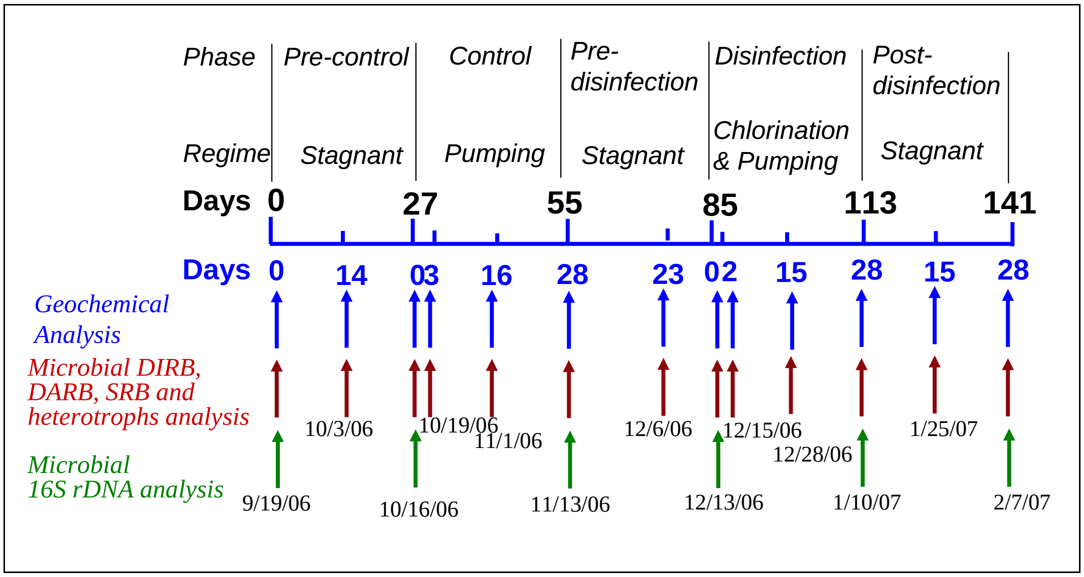


Figure 2. Field experimental design and sample collection schedule

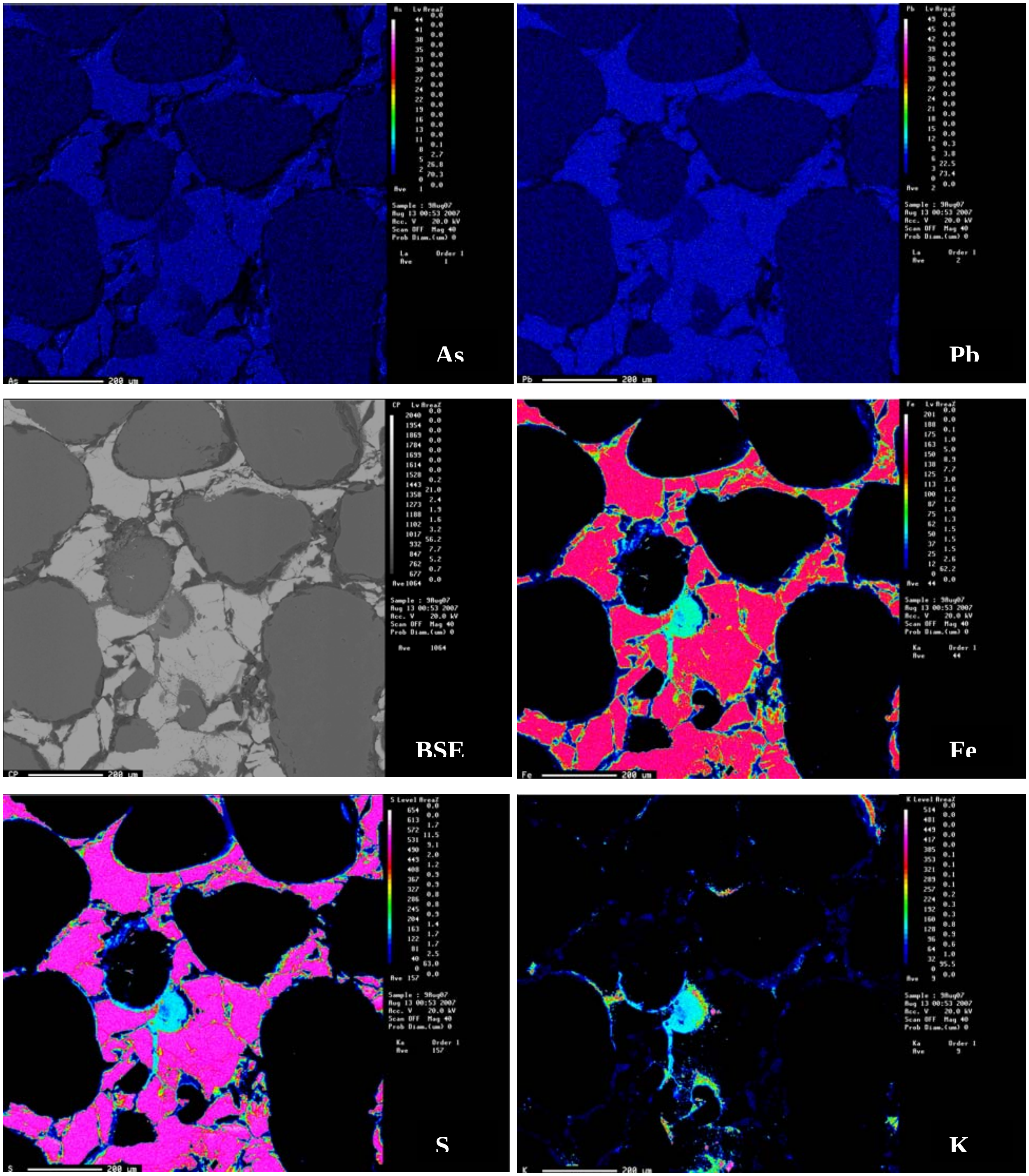


Figure 3. Pre-reacted thin section of Skunk Hill Quarry material.
Color scale shown on right of each image shows concentrations of each element: As, Pb, S, Fe and K. Figure on left side of middle row is the back scattered electron (BSE) image.

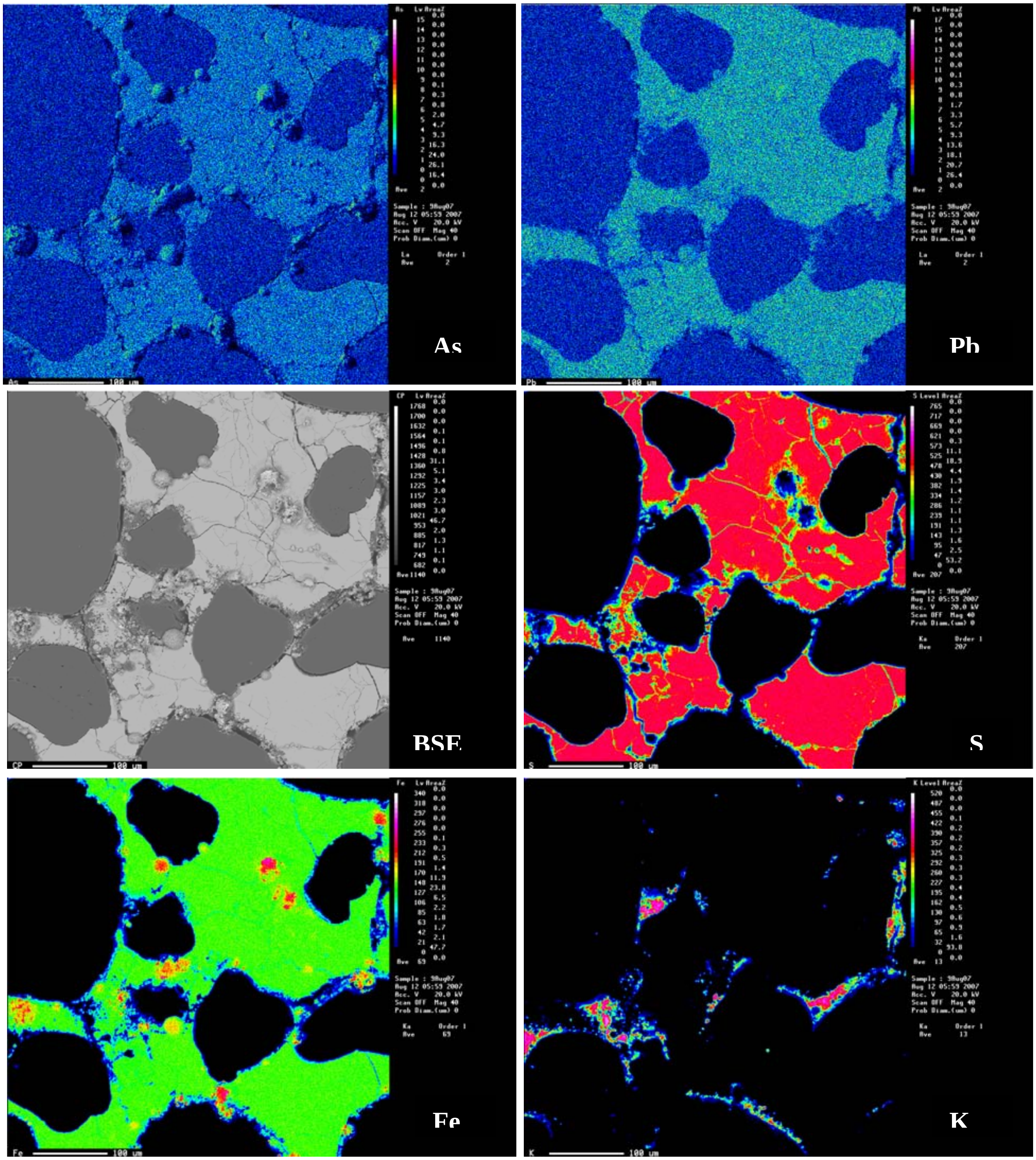


Figure 4. Post-reacted (exposure to 1200 mg/L free chlorine for 24 hours) thin section of Skunk Hill Quarry material.

Color scale shown on right of each image shows concentrations of each element: As, Pb, S, Fe and K. Figure on left side of middle row is the back scattered electron (BSE) image.

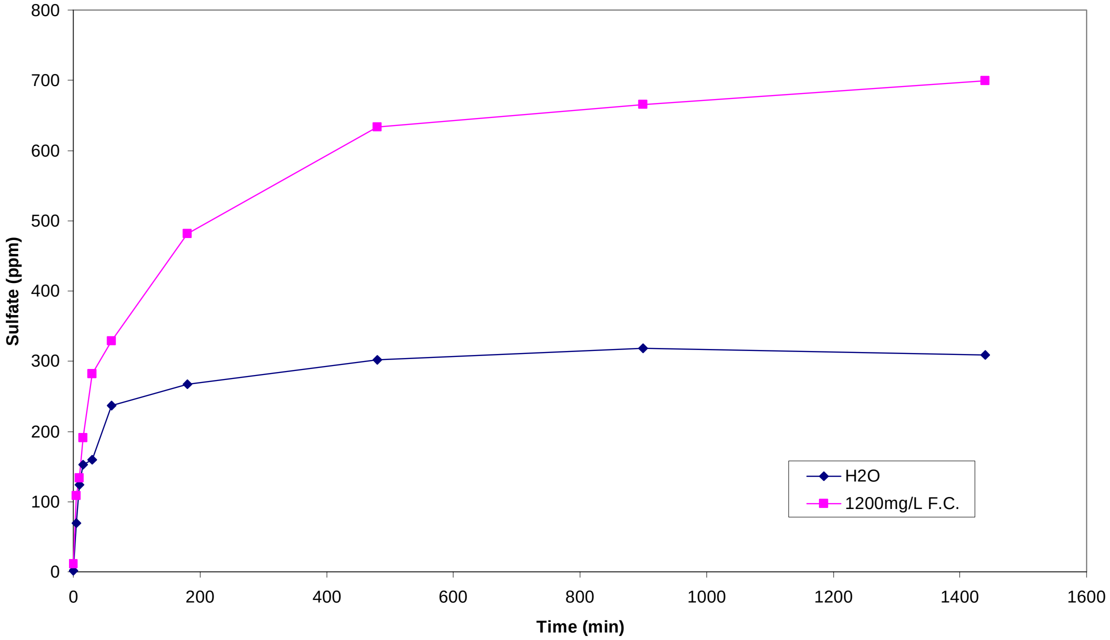


Figure 5. Filtered (0.2 micron) sulfate concentrations in solution after 24 hour exposure to LM sample: nanopure water and 1200 mg/L free chlorine.

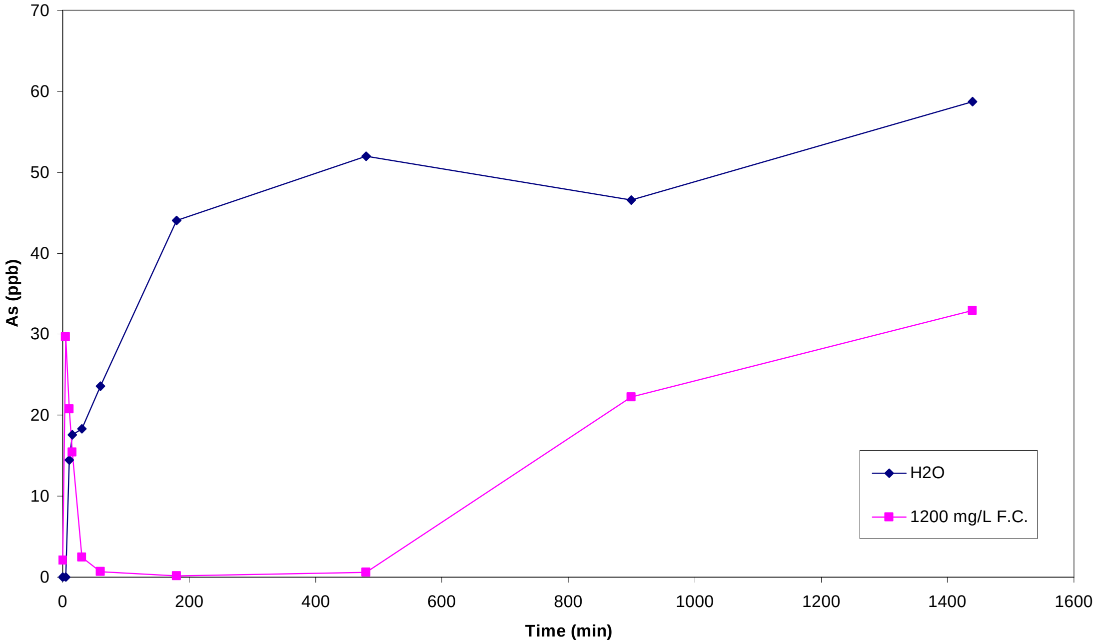


Figure 6. Filtered (0.2 micron) arsenic concentrations in solution after 24 hour exposure to LM sample: nanopure water and 1200 mg/L free chlorine.

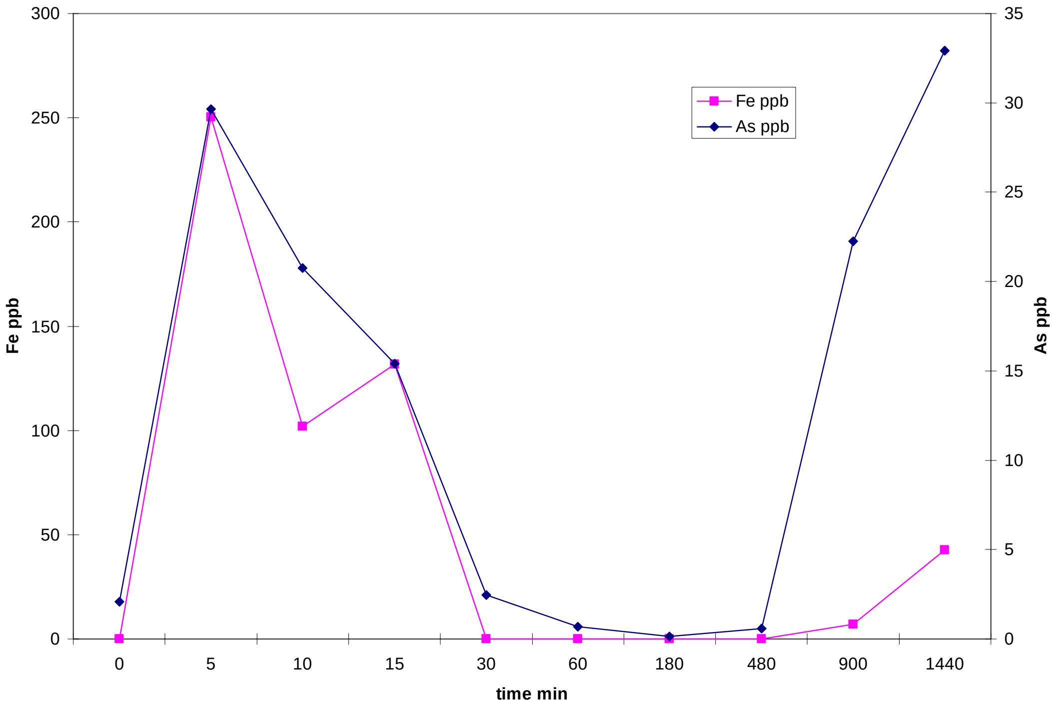


Figure 7. Filtered (0.2 micron) arsenic and iron concentrations in solution after 24 hour exposure to LM sample: 1200 mg/L free chlorine.

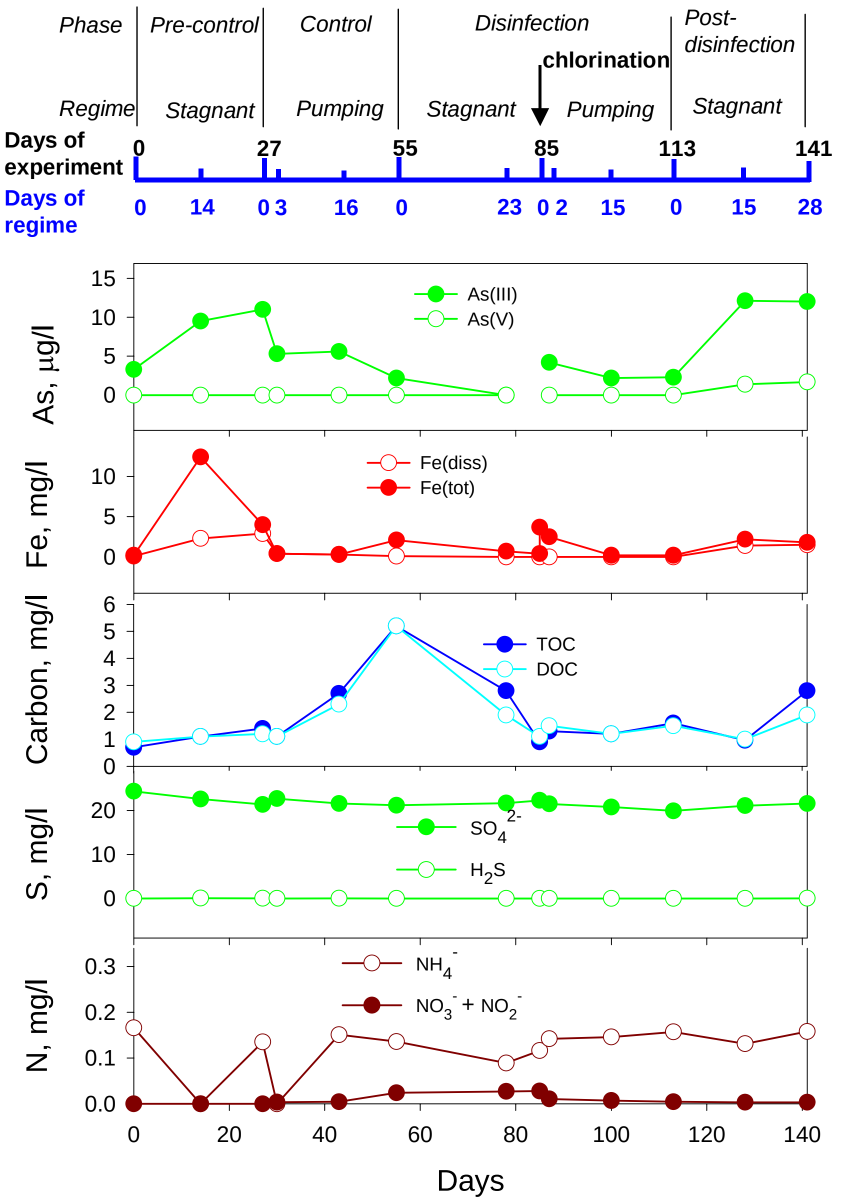
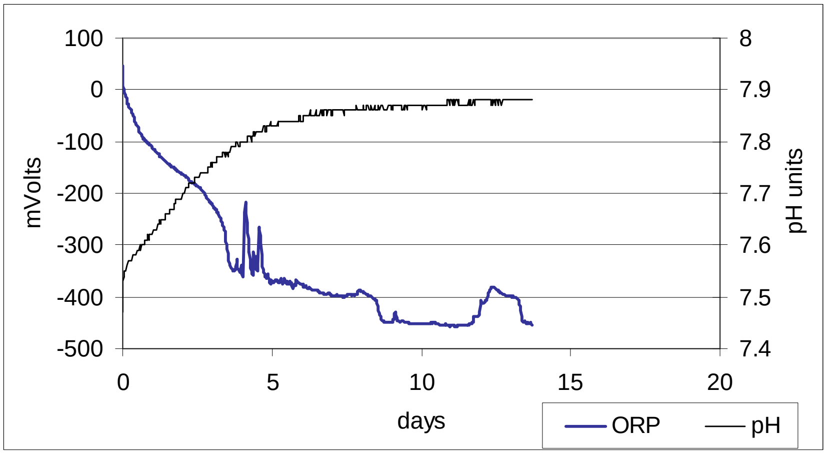


Figure 8. Water chemistry in test well.



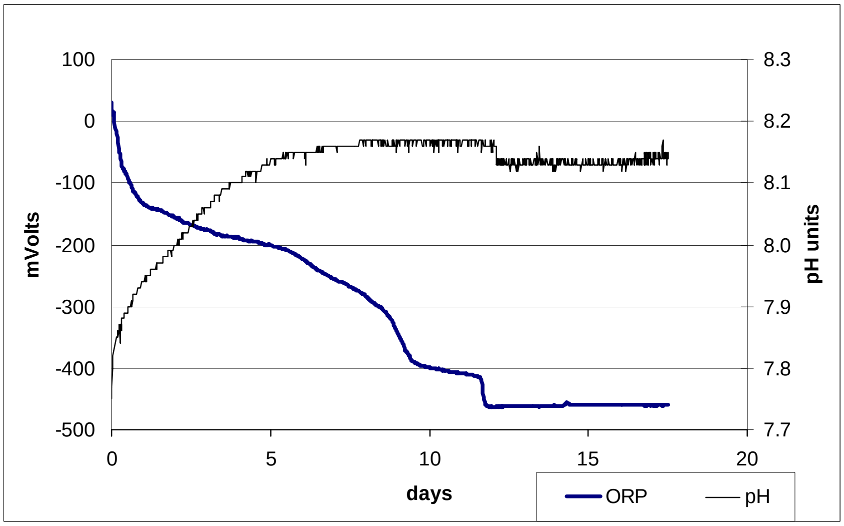


Figure 9. Figure 9ORP and pH of borehole water during pre-control stagnant phase (top) and pre-chlorination stagnant phase (bottom).

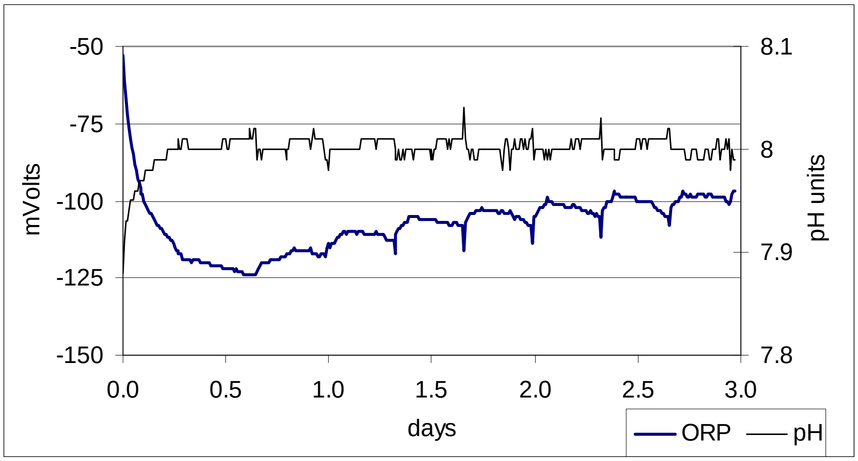


Figure 10. Figure 10ORP and pH of well water during control pumping phase.

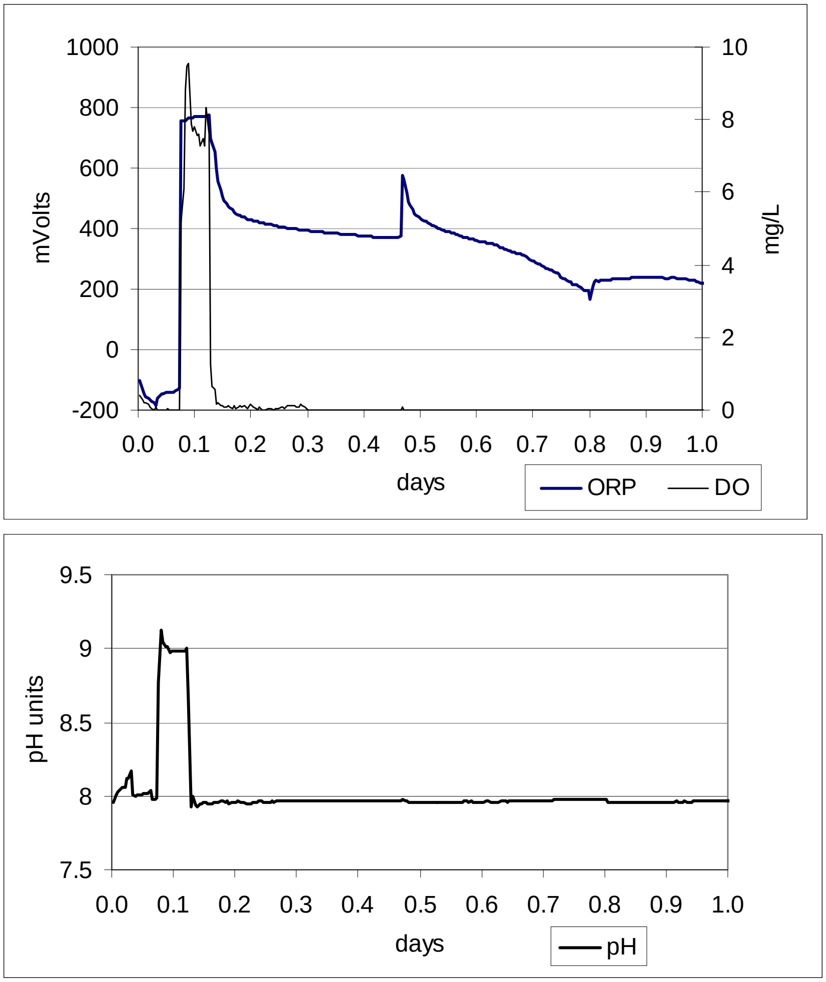


Figure 11. ORP and DO (top) and pH (bottom) in well water at time of chlorination. Treatment solution was added to the well at time = 0.07 day. Purging of treatment solution was completed at 0.13 day.

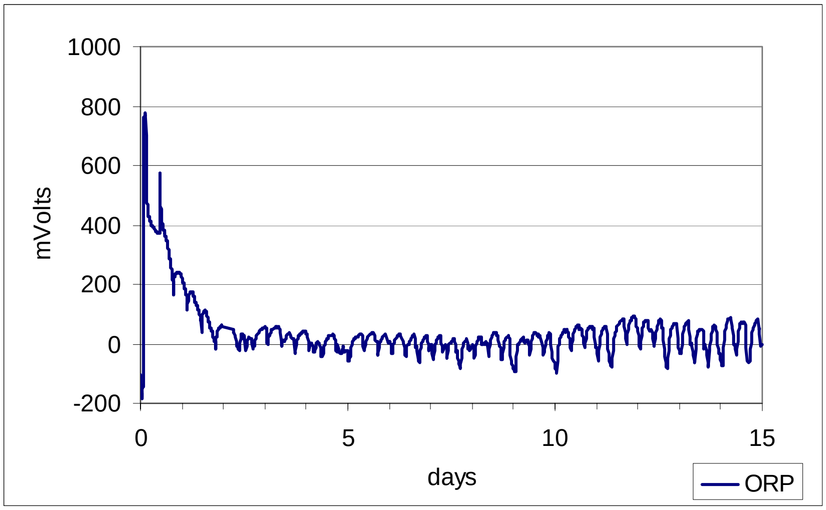


Figure 12. ORP in well water during chlorination and subsequent routine pumping.

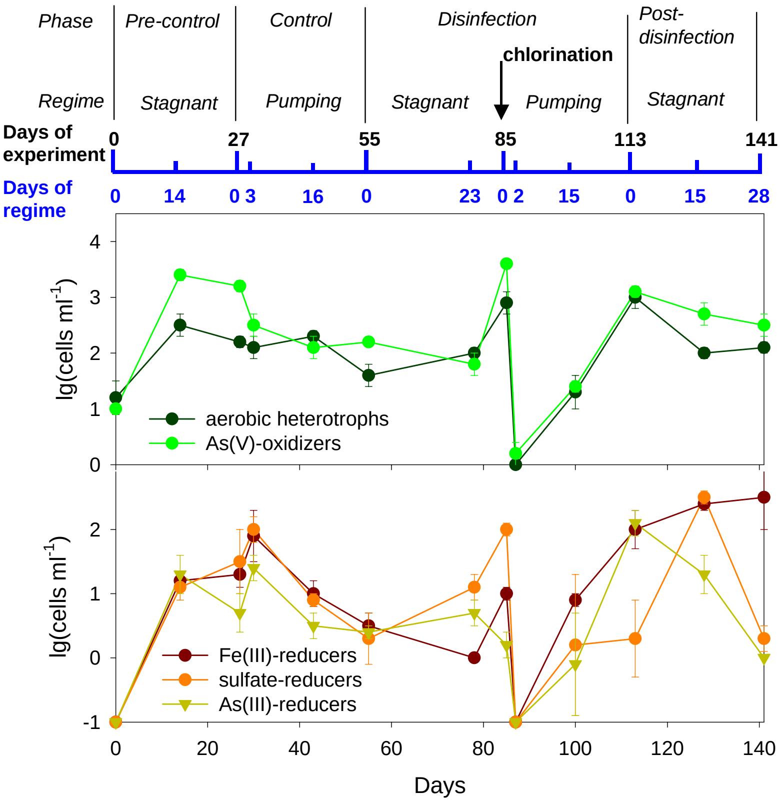


Figure 13. Results of microbial community analysis using culturing-dependent methods.

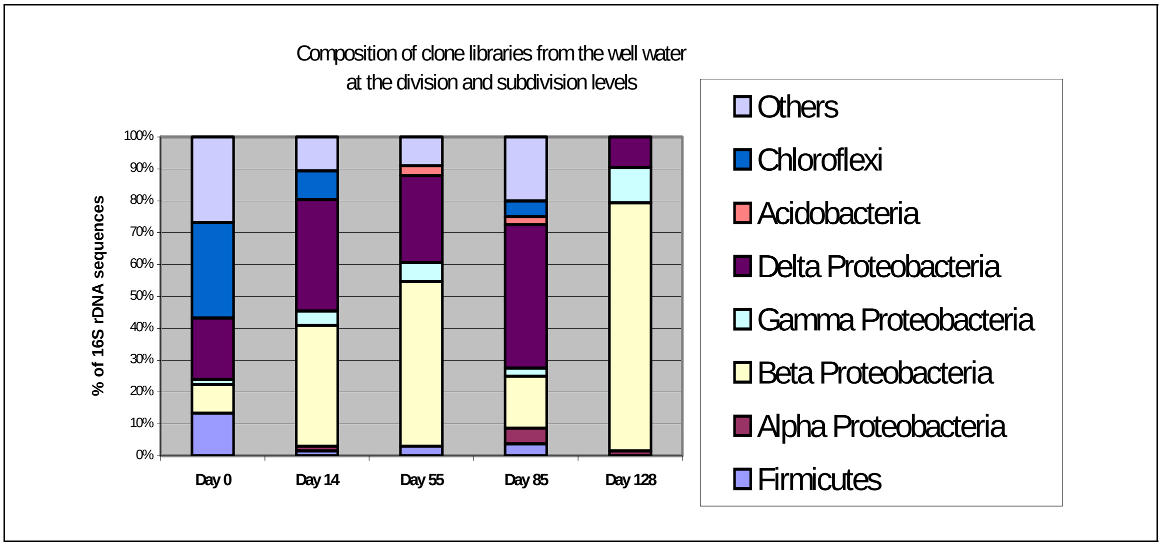


Figure 14. Composition of clone libraries from culture-independent analyses.

Table 1. Unreacted pyrite chemistry from SH thin section (weight %) quantified using WDS

	Min	Max	Average
Fe	45.22	47.21	46.51711
Ni	0	0.147	0.014275
As	0	0.134	0.026121
Co	0	0.093	0.040805
S	52.92	55.11	53.96302
Cu	0	0.259	0.018248
Pb	0	1.42	0.199376
Total	99.012	102.171	100.7788

Table 2. Reacted pyrite chemistry from SH thin section (weight %) quantified using WDS

	Min	Max	Average
Fe	46.15	47.57	46.8942
Ni	0	0.022	0.005043
As	0	0.054	0.014841
Co	0	0.089	0.03771
S	52.08	53.93	53.14188
Cu	0	0.053	0.00671
Pb	0	0.345	0.130957
Total	99.018	100.981	100.2313

Table 3. Water quality results

Phase	**Well status and number of days**	**Date**	**As^{3+}** µg/L	**As, diss** µg/L	**As, total** µg/L	**Fe, diss** mg/L	**Fe, total** mg/L	**Mn, diss** µg/L	**Mn, total** µg/L
Pre-control	purged	9/19/2006	3.3	3.0	< 5	0.1	0.2	50	49
Pre-control	stagnant, 14 d	10/3/2006	9.5	10.1	*14	2.3	12.4	123	144
Pre-control	stagnant, 27 d	10/16/2006	11.0	9.2	*11	2.9	4.0	95	96
Control	pumping, 3 d	10/19/2006	5.3	6.0	< 5	0.4	0.4	52	53
Control	pumping, 16 d	11/1/2006	5.6	5.2	< 5	0.3	0.3	48	47
Control	pumping, 28 d	11/13/2006	2.2	2.6	< 5	0.1	2.1	44	52
Pre-disinfection	stagnant, 23 d	12/6/2006	< 1	1.0	< 5	< 0.1	0.7	57	56
Pre-disinfection	stagnant, 30 d	12/13/2006	--	--	< 5	< 0.1	0.4	69	64
Disinfection	disinfection purge	12/13/2006	--	--	< 5	--	3.7	--	76
Disinfection	pumping, 2 d	12/15/2006	4.2	4.8	< 5	< 0.1	2.5	44	63
Disinfection	pumping, 15 d	12/28/2006	2.2	3.0	< 5	< 0.1	0.2	47	46
Disinfection	pumping, 28 d	1/10/2007	2.3	1.8	<5	< 0.1	*0.2	48	47
Post-disinfection	stagnant, 15 d	1/25/2007	12.1	13.6	14	1.4	2.2	48	49
Post-disinfection	stagnant, 28 d	2/7/2007	12	13.7	15	1.5	1.8	44	44

diss = dissolved; * indicates result is between limit of detection and limit of quantification; -- not analyzed

Table 4. Additional water quality results

Phase	Well status and number of days	Date	Zn, diss µg/L	Zn, total µg/L	Al, diss µg/L	Al, total µg/L	Cu, diss µg/L	Cu, total µg/L	Ni, diss µg/L	Ni, total µg/L
Pre-control	purged	9/19/2006	< 1	17	4.0	13.0	5	26	< 1	1
Pre-control	stagnant, 14 d	10/3/2006	7	29	4.0	14.0	< 2	43	3	4
Pre-control	stagnant, 27 d	10/16/2006	3	21	< 3	6.0	2	14	4	4
Control	pumping, 3 d	10/19/2006	4	6	< 3	4.0	< 2	5	1	1
Control	pumping, 16 d	11/1/2006	3	5	9	12.0	< 2	5	< 1	2
Control	pumping, 28 d	11/13/2006	1	4	6	20.0	< 2	14	1	1
Pre-disinfection	stagnant, 23 d	12/6/2006	sc	sc	6	10.0	sc	sc	sc	sc
Pre-disinfection	stagnant, 30 d	12/13/2006	sc	sc	5	9.0	sc	sc	sc	sc
Disinfection	Cl treatment purge	12/13/2006	--	sc	--	23	--	sc	--	sc
Disinfection	pumping, 2 d	12/15/2006	14	20	5	18	< 2	54	< 1	5
Disinfection	pumping, 15 d	12/28/2006	8	18	5	14	< 2	6	< 1	< 1
Disinfection	pumping, 28 d	1/10/2007	*1	3	6	15	< 2	6	< 1	< 1
Post-disinfection	stagnant, 15 d	1/25/2007	16	21	8	10	< 2	16	4	4
Post-disinfection	stagnant, 28 d	2/7/2007	17	22	6	9	< 2	19	3	4

diss = dissolved; * indicates result is between limit of detection and limit of quantification; -- not analyzed; sc sample contaminated

Table 5. Organic carbon and major ions

Phase	Well status and number of days	Date	TOC mg/L	DOC mg/L	SO_4^{2-} mg/L	Mg mg/L	Ca mg/L
Pre-control	purged	9/19/2006	0.7	0.9	24.4	41.2	37.8
Pre-control	stagnant, 14 d	10/3/2006	1.1	1.1	22.6	40.6	37.6
Pre-control	stagnant, 27 d	10/16/2006	1.4	1.2	21.4	40.1	37.6
Control	pumping, 3 d	10/19/2006	1.1	1.1	22.7	40.7	39.9
Control	pumping, 16 d	11/1/2006	2.7	2.3	21.6	40.2	39.9
Control	pumping, 28 d	11/13/2006	5.2	5.2	21.2	42.1	40.4
Pre-disinfection	stagnant, 23 d	12/6/2006	2.8	1.9	21.7	41.0	41.9
Pre-disinfection	stagnant, 30 d	12/13/2006	0.9	1.1	22.3	38.3	35.8
Pre-disinfection	disinfection purge	12/13/2006	--	--	--	37.3	35.1
Disinfection	pumping, 2 d	12/15/2006	1.3	1.5	21.5	37.9	37
Disinfection	pumping, 15 d	12/28/2006	1.2	1.2	20.8	41.3	39.6
Disinfection	pumping, 28 d	1/10/2007	1.6	1.5	19.9	41.4	40.4
Post-disinfection	stagnant, 15 d	1/25/2007	0.96	1.0	21.1	39.8	39.4
Post-disinfection	stagnant, 28 d	2/7/2007	2.8	1.9	21.6	41.0	41.4

-- not analyzed

Table 6. Additional trace metal results

Phase	Well status and number of days	Date	Cd, diss µg/L	Cd, total µg/L	Cr,diss µg/L	Cr, total µg/L	Co, diss µg/L	Co, total µg/L	Pb, diss µg/L	Pb, total µg/L	V, diss µg/L	V, total µg/L
Pre-control	purged	9/19/2006	< 0.5	< 0.5	< 1	< 1	5	< 1	<3	<3	< 1	< 1
Pre-control	stagnant, 14 d	10/3/2006	< 0.5	< 0.5	< 1	3	< 1	< 1	<3	*3	< 1	< 1
Pre-control	stagnant, 27 d	10/16/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	*4	<3	< 1	< 1
Control	pumping, 3 d	10/19/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	*3	<3	< 1	< 1
Control	pumping, 16 d	11/1/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	*4	<3	< 1	< 1
Control	pumping, 28 d	11/13/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	<3	< 1	< 1
Pre-disinfection	stagnant, 23 d	12/6/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	sc	< 1	< 1
Pre-disinfection	stagnant, 30 d	12/13/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	sc	< 1	< 1
Disinfection	disinfection purge	12/13/2006	--	< 0.5	--	4	--	< 1	--	sc	--	< 1
Disinfection	pumping, 2 d	12/15/2006	< 0.5	< 0.5	< 1	7	< 1	< 1	<3	<3	< 1	< 1
Disinfection	pumping, 15 d	12/28/2006	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	<3	< 1	< 1
Disinfection	pumping, 28 d	1/10/2007	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	<3	< 1	< 1
Post-disinfection	stagnant, 15 d	1/25/2007	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	<3	< 1	< 1
Post-disinfection	stagnant, 28 d	2/7/2007	< 0.5	< 0.5	< 1	< 1	< 1	< 1	<3	<3	< 1	< 1

diss = dissolved; * indicates result is between limit of detection and limit of quantification; -- not analyzed; sc sample contaminated

Table 7. Results of low-level analysis for ammonia and nitrate

Phase	Well status and number of days	Date	ammonia µg/L	FB ammonia µg/L	nitrate+nitrite µg/L	FB nitrate+nitrite µg/L
Pre-control	purged	9/19/2006	**166	**< 3.1	** < 3.1	** < 3.1
Pre-control	stagnant, 14 d	10/3/2006	< 3.1	< 3.1	< 3.1	< 3.1
Pre-control	stagnant, 27 d	10/16/2006	135	< 3.1	< 3.1	< 3.1
Control	pumping, 3 d	10/19/2006	< 3.1	< 3.1	*3.3	3.3
Control	pumping, 16 d	11/1/2006	**151	**6.2	4.2	16.1
Control	pumping, 28 d	11/13/2006	136	3.1	24	8.0
Pre-disinfection	stagnant, 23 d	12/6/2006	89	4.6	27	3.8
Pre-disinfection	stagnant, 30 d	12/13/2006	****116	****8.24	****27.8	****5.41
Disinfection	disinfection purge	12/13/2006	--	--	--	--
Disinfection	pumping, 2 d	12/15/2006	****142	*3.8	****10.4	*4.65
Disinfection	pumping, 15 d	12/28/2006	***'*146	****5.04	****7.06	****5.03
Disinfection	pumping, 28 d	1/10/2007	157	< 3.1	*4.3	*4.72
Post-disinfection	stagnant, 15 d	1/25/2007	131	< 3.1	< 3.1	*4.88
Post-disinfection	stagnant, 28 d	2/7/2007	***158	*4.65	< 3.1	*4.28

*** indicates lab reagent blank exceeded 2 µg/L ****indicates quality control samples exceeded controls

Appendix 1: Probe Data

Appendix 1. EMPA Analyses on pre- and post-reacted Skunk Hill thin sections

Analyzed by Nicole West

Results presented as Weight %

Sample #	Fe	Ni	As	Co	S	Cu	Pb	Total
Pre-reacted								
1	46.51	0	0.029	0.048	54.28	0.006	0.181	101.053
2	46.08	0	0.022	0	54.09	0	0.043	100.235
3	47.05	0	0.021	0.043	54.12	0	0.112	101.346
4	46.71	0	0.04	0	54.23	0.013	0.076	101.069
5	46.74	0	0.031	0.079	54.1	0.011	0.051	101.011
6	46.99	0	0.02	0.045	54.38	0.009	0.136	101.579
7	46.89	0	0.016	0.027	55.11	0	0.128	102.171
8	47.18	0.009	0.043	0.03	54.28	0.014	0.105	101.661
9	46.56	0	0.014	0	54.02	0	0.165	100.759
10	46.77	0	0.011	0.028	54.34	0.008	0.15	101.307
11	46.72	0	0.02	0.013	53.96	0.005	0.1	100.818
12	44.96	0	0.039	0	53.16	0	0.124	98.283
13	46.77	0	0.011	0	54.36	0.005	0.162	101.308
14	46.64	0	0.033	0.036	54.23	0	0.147	101.085
15	46.85	0	0.017	0.073	53.95	0	0.083	100.973
16	46.42	0.007	0.02	0.071	54.25	0	0.167	100.936
17	46.86	0	0.036	0.03	53.85	0	0.134	100.91
18	47.09	0	0.011	0.058	54.2	0.02	0.119	101.498
19	46.92	0	0.033	0.064	54.23	0	0.154	101.401
20	47.06	0	0.031	0.031	53.96	0.01	0.175	101.267
21	46.64	0.026	0.092	0.04	54.01	0.009	0.514	101.331
22	46.83	0.017	0.054	0.084	54.13	0	0.234	101.35
23	46.14	0.006	0.086	0.03	53.71	0.009	0.406	100.387
24	46.55	0.015	0.046	0.064	54.07	0.009	0.169	100.922
25	46.25	0.017	0.07	0.006	54.12	0	0.164	100.627
26	46.72	0.011	0.08	0.03	53.78	0	0.253	100.874
27	45.84	0.01	0.006	0.039	54.05	0	0.224	100.168
28	46.6	0.014	0.029	0.049	54.24	0	0.184	101.116
29	46.82	0.013	0.017	0.054	54.17	0.019	0.114	101.206
30	46.9	0	0.016	0.049	54.32	0.006	0.119	101.411
31	46.86	0.01	0.048	0.039	53.97	0	0.269	101.196
32	46.94	0	0.018	0.019	54.07	0	0.173	101.221
33	46.18	0	0.008	0.042	54.02	0	0.131	100.38
34	46.8	0.007	0.023	0.064	54.23	0.015	0.131	101.27
35	46.76	0.005	0.042	0.065	54.4	0.017	0.157	101.447
36	46.59	0.008	0	0.018	54.16	0.01	0.208	100.994
37	46.45	0.006	0.024	0.079	53.89	0	0.178	100.626
38	46.95	0	0	0.033	54.14	0	0.104	101.226
39	45.82	0.01	0.027	0.016	52.92	0.008	1.42	100.221
40	46.79	0.012	0.024	0.045	54.16	0	0.235	101.266
41	46.26	0	0.033	0.064	53.71	0	0.584	100.651
42	46.34	0	0.028	0.034	54.27	0.007	0.211	100.89
43	46.93	0	0.011	0.052	54.31	0	0.204	101.507
44	46.43	0.017	0.023	0.022	54.46	0	0.198	101.151
45	46.95	0	0.019	0	54.42	0	0.019	101.407
46	47.09	0	0.017	0.013	54.47	0.005	0.213	101.809
47	46.35	0	0.036	0.015	54.44	0.011	0.112	100.964
48	46.83	0	0.021	0.047	54.62	0	0.051	101.569

Sample #	Fe	Ni	As	Co	S	Cu	Pb	Total
49	46.55	0.006	0	0.042	54.18	0	0.086	100.863
50	46.38	0.036	0.005	0.019	54.4	0.089	0.3	101.229
51	46.7	0	0.029	0.05	54.19	0.011	0.262	101.242
52	46.58	0.013	0.023	0.037	53.94	0.02	0.261	100.874
53	46.06	0.014	0	0.03	53.94	0.027	0.165	100.235
54	46.43	0.013	0.018	0.025	54.02	0.01	0.217	100.733
55	46.76	0.018	0.032	0.024	53.92	0.055	0.217	101.025
56	46.66	0.009	0.042	0.067	53.93	0.007	0.31	101.025
57	46.83	0	0.01	0.05	53.77	0.006	0.127	100.793
58	45.22	0	0.029	0.045	54.97	0.02	0.204	100.487
59	45.75	0.147	0.134	0.041	53.11	0.058	1.02	100.26
60	46.07	0.034	0.04	0.01	54.05	0.009	0.497	100.711
61	46.27	0	0.014	0.027	53.77	0.019	0.416	100.516
62	46.58	0.024	0.031	0.043	53.71	0.007	0.495	100.89
63	46.46	0.015	0.014	0.083	53.73	0	0.086	100.388
64	46.76	0	0.01	0.041	54.01	0.008	0.157	100.986
65	46.78	0	0.019	0.027	54.29	0.01	0.051	101.177
66	46.99	0	0	0.043	54.25	0.006	0.037	101.326
67	47.19	0.021	0.013	0.053	54.23	0.006	0.107	101.62
68	46.95	0.006	0.035	0.016	54.31	0	0.047	101.364
69	46.76	0	0	0.038	53.88	0.012	0.294	100.985
70	46.97	0	0.041	0.037	54.02	0	0.112	101.18
71	46.81	0.03	0.006	0.018	53.86	0.056	0.158	100.937
72	46.48	0.024	0	0.044	54.43	0.058	0.144	101.181
73	46.68	0.021	0.011	0.027	54.01	0.052	0.141	100.941
74	47.21	0.012	0.031	0	54.16	0.06	0	101.473
75	46.77	0.026	0.006	0.038	54.21	0.062	0.299	101.412
76	45.7	0	0.008	0.089	53.62	0.013	0.085	99.515
77	46.71	0.022	0.039	0.022	54.07	0.023	0.175	101.06
78	46.47	0.046	0.017	0.019	53.6	0.098	0.378	100.627
79	46.07	0.073	0.027	0.05	53.65	0.031	0.385	100.286
80	46.58	0.078	0.025	0.032	53.46	0.071	0.335	100.581
81	46.64	0.083	0.041	0.047	53.72	0.093	0.304	100.928
82	46.51	0.055	0.026	0.064	53.84	0.054	0.419	100.967
83	45.84	0	0.075	0.074	53.72	0.012	0.327	100.049
84	46.73	0.01	0.069	0.018	53.72	0.011	0.275	100.832
85	46.51	0	0.024	0.049	53.95	0	0.07	100.603
86	45.34	0.04	0.106	0.053	53.6	0	0.339	99.479
87	46.17	0	0.029	0.016	54.22	0.008	0.176	100.619
88	46.93	0	0.054	0.043	54.36	0	0.186	101.573
89	46.39	0	0.102	0.04	53.49	0.009	0.169	100.199
90	46.35	0	0.025	0.05	53.92	0.006	0.106	100.457
91	46.7	0	0.026	0.04	53.94	0	0.045	100.751
92	46.76	0.019	0.013	0.019	54.19	0	0.14	101.14
93	46.41	0.01	0	0.061	54.23	0.005	0.131	100.846
94	46.92	0	0.025	0.041	54.22	0	0.135	101.342
95	46.35	0.023	0.016	0.027	54.09	0.009	0.149	100.663
96	46.71	0.005	0.018	0.022	54.01	0	0.026	100.79
97	46.93	0.009	0.033	0.031	54.31	0.01	0.111	101.433
98	46.18	0	0.029	0.019	54.1	0	0.11	100.438
99	46.41	0.009	0	0.059	54.03	0.008	0.047	100.563
100	47.19	0	0.02	0.052	54.01	0	0.078	101.349
101	46.34	0.007	0.013	0.031	53.95	0.007	0.183	100.53

Sample #	Fe	Ni	As	Co	S	Cu	Pb	Total	
102	46.67	0	0	0.031	53.67	0	0.073	100.444	
103	46.97	0.005	0.024	0.044	54.08	0	0.172	101.296	
104	46.51	0.026	0	0.068	53.96	0	0.027	100.591	
105	46.73	0	0	0.05	54	0	0.134	100.914	
106	45.94	0.007	0	0.025	53.4	0	0.14	99.512	
107	46.17	0.03	0.048	0.062	54.11	0.025	0.26	100.706	
108	46.97	0	0.015	0.046	54	0.009	0.128	101.168	
109	46.89	0	0.023	0.04	53.84	0	0.131	100.923	
110	46.34	0	0.02	0.016	53.82	0.01	0.211	100.417	
111	46.35	0.01	0.022	0.055	53.91	0.011	0.069	100.427	
112	46.7	0.009	0.029	0.04	53.95	0.012	0.126	100.865	
113	46.65	0.008	0.023	0.066	53.91	0.007	0.105	100.77	
114	46.71	0.007	0.017	0.03	53.83	0	0.18	100.773	
115	3.67	0	0	0	10.34	0	51.73	65.74	off grain
116	45.88	0	0	0.071	53.26	0.013	0.197	99.421	
117	46.29	0	0.026	0.04	53.61	0.013	0.155	100.135	
118	46.78	0	0.008	0.028	53.94	0	0.131	100.887	
119	46.37	0.008	0.04	0.022	53.13	0	0.157	99.727	
120	45.81	0.013	0.019	0.037	54.1	0.009	0.083	100.071	
121	46.58	0.011	0.023	0.025	53.8	0	0.267	100.706	
122	46.64	0.009	0	0.047	53.65	0	0.094	100.441	
123	46.59	0.007	0.025	0.065	53.98	0	0.12	100.786	
124	46.65	0	0	0.015	54.02	0	0.15	100.835	
125	45.59	0.012	0.011	0.053	53.88	0	0.118	99.665	
126	46.23	0.02	0.014	0.063	53.78	0.006	0.111	100.225	
127	46.89	0.009	0.034	0.053	54	0.02	0.046	101.052	
128	46.62	0.021	0.035	0.044	53.94	0.013	0.18	100.852	
129	46.47	0	0.04	0.032	53.77	0.008	0.201	100.52	
130	46.79	0	0	0.06	53.65	0	0.205	100.706	
131	47.2	0.007	0.009	0.022	53.83	0	0.131	101.199	
132	46.96	0	0.03	0.05	53.88	0.006	0.111	101.037	
133	45.51	0	0.032	0.082	53.44	0.005	0.105	99.174	
134	45.74	0.056	0.026	0.059	53.64	0.104	0.198	99.823	
135	46.41	0.057	0.024	0.043	53.27	0.05	0.276	100.13	
136	46.15	0.029	0.051	0.057	53.77	0.02	0.268	100.344	
137	45.63	0.075	0.035	0.054	54	0.091	0.379	100.264	
138	46.21	0.065	0.027	0.075	54.05	0.109	0.444	100.98	
139	45.98	0.009	0.063	0.007	53.5	0.044	0.182	99.785	
140	45.64	0.119	0.035	0.073	53.57	0.173	0.358	99.968	
141	46.75	0	0.025	0.028	53.58	0.005	0.299	100.686	
142	46.25	0.033	0.025	0.054	53.33	0.036	0.25	99.979	
143	46.2	0	0.038	0.04	53.7	0.02	0.335	100.332	
144	45.54	0.037	0.037	0.069	53.47	0.026	0.187	99.367	
145	46.46	0.019	0.033	0.062	53.96	0.011	0.096	100.64	
146	45.57	0.031	0	0.012	53.28	0	0.12	99.012	
147	46.63	0.045	0	0.03	54.03	0.078	0.239	101.051	
148	46.22	0.094	0.022	0.093	53.6	0.259	0.258	100.546	
149	46.54	0.031	0.016	0.052	53.7	0.03	0.314	100.682	
150	46.29	0.018	0.02	0.018	53.75	0.071	0.336	100.502	
151	46.48	0.012	0.038	0.041	53.64	0.063	0.389	100.663	
Maximum	47.21	0.147	0.134	0.093	55.11	0.259	51.73	102.171	
Minimum	3.67	0	0	0	10.34	0	0	65.74	
Average	46.22305	0.014086	0.026033	0.040265	53.66881	0.018007	0.540139	100.5303	

Sample #	Fe	Ni	As	Co	S	Cu	Pb	Total	
Sigma	3.512223	0.023081	0.021714	0.020864	3.564947	0.033633	4.196681	2.908891	
Post-reacted									
152	46.42	0.01	0.017	0.04	53.65	0.01	0.194	100.34	
153	46.84	0	0.019	0.019	53.32	0	0.067	100.264	
154	45.83	0.017	0.01	0.035	45.58	0.048	0.132	91.652	off grain
155	46.61	0.022	0.005	0.054	53.23	0.032	0.176	100.129	
156	46.85	0.022	0.022	0	53.34	0.045	0.097	100.377	
157	46.47	0.016	0.015	0.049	53.29	0.053	0.2	100.095	
158	46.72	0	0.028	0.032	53.12	0.025	0.212	100.137	
159	46.75	0.015	0	0.059	53.1	0.03	0.15	100.103	
160	49.31	0.013	0.035	0.075	30.86	0.009	0.154	80.457	off grain
161	46.69	0	0	0.039	52.9	0	0.1	99.729	
162	46.82	0	0.028	0.069	53.2	0	0.061	100.179	
163	46.91	0.012	0	0.019	53.4	0	0.02	100.36	
164	47.23	0.007	0	0.034	53.58	0	0.012	100.863	
165	46.89	0	0.011	0	53.5	0	0.119	100.521	
166	46.15	0	0.007	0.049	53.39	0	0.061	99.657	
167	47	0	0.011	0.015	53.15	0.007	0.049	100.232	
168	46.38	0.008	0.041	0.037	53.79	0	0.212	100.468	
169	46.65	0.018	0.012	0.054	53.31	0	0.115	100.159	
170	46.76	0.005	0	0.057	53.64	0.012	0.025	100.499	
171	46.77	0	0.031	0	53.54	0.007	0.059	100.407	
172	46.97	0	0.026	0.046	53.57	0.011	0.255	100.878	
173	46.57	0	0	0.045	53.61	0	0.113	100.337	
174	46.21	0	0.006	0	49.92	0	0.171	96.307	off grain
175	44.04	0.012	0.036	0	49.89	0	0.151	94.13	off grain
176	46.8	0	0	0.048	53.93	0	0.138	100.916	
177	47.03	0.005	0.019	0.054	53.5	0	0.177	100.785	
178	46.98	0	0.023	0.026	53.39	0	0.213	100.632	
179	46.31	0	0.026	0.026	53.67	0	0	100.032	
180	47.2	0	0.027	0.017	53.45	0	0.107	100.801	
181	46.73	0.013	0.011	0.079	51.5	0.009	0.12	98.461	
182	47.57	0.011	0.015	0.048	53.14	0.014	0.066	100.865	
183	47.5	0.007	0	0.052	52.81	0	0.123	100.492	
184	47.16	0	0.011	0.082	52.79	0	0.149	100.192	
185	47.1	0.008	0.033	0.051	53.14	0	0.161	100.493	
186	47.02	0.013	0.015	0.063	53.41	0.013	0.159	100.693	
187	47	0.007	0	0.054	52.88	0	0.179	100.119	
188	47.16	0.013	0.023	0.035	52.82	0	0.087	100.138	
189	46.51	0	0.021	0.019	52.69	0	0.158	99.398	
190	47.38	0	0	0	52.4	0.006	0.201	99.987	
191	46.98	0	0.02	0.009	52.43	0.007	0.096	99.543	
192	46.87	0	0.011	0.031	52.32	0	0.141	99.373	
193	46.97	0	0	0.04	52.33	0.006	0.105	99.45	
194	47.44	0.009	0.034	0.054	52.78	0.007	0.172	100.495	
195	46.86	0	0.006	0.009	52.78	0.009	0.162	99.826	
196	46.67	0	0.013	0.014	52.76	0	0.185	99.642	
197	46.85	0	0.007	0.029	52.84	0.009	0.034	99.769	
198	47.21	0.013	0.023	0.052	52.08	0.006	0.112	99.496	
199	46.61	0	0	0.077	52.18	0	0.019	98.885	
200	46.81	0	0.018	0	52.65	0.009	0.101	99.589	
201	46.35	0	0	0.035	52.59	0.01	0.032	99.018	

Sample #	Fe	Ni	As	Co	S	Cu	Pb	Total
202	46.71	0	0.028	0.022	53.33	0	0.06	100.149
203	46.93	0	0.023	0.052	53.41	0.014	0.075	100.505
204	47.19	0.009	0.027	0.052	52.71	0	0.161	100.148
205	46.93	0	0	0.045	52.58	0	0.139	99.694
206	47.45	0	0.006	0.051	53.4	0.006	0.068	100.981
207	47.04	0	0	0	53.15	0	0.081	100.271
208	47.04	0	0.015	0	52.85	0	0.033	99.938
209	47.09	0	0.026	0.062	53.38	0.006	0.019	100.582
210	46.25	0.006	0.054	0.034	53.28	0	0.163	99.788
211	46.82	0.013	0.013	0.034	53.52	0	0.085	100.485
212	47.12	0	0.022	0.043	53.65	0	0.099	100.934
213	46.44	0.006	0.014	0.049	52.91	0.012	0.155	99.587
214	45.51	0.071	0	0.017	51.93	0.033	0.228	97.789
215	47.1	0.013	0	0	53.43	0	0.02	100.563
216	47.19	0	0.014	0.055	53.4	0.018	0.141	100.818
217	46.82	0	0.023	0.046	53.05	0	0.153	100.091
218	46.99	0.008	0.007	0.048	52.86	0.017	0.269	100.199
219	47.19	0.012	0.009	0.048	53.02	0.01	0.152	100.441
220	46.92	0.009	0.007	0.051	53.11	0.006	0.345	100.448
221	46.44	0.015	0	0.089	53.17	0	0.257	99.97
222	47	0.017	0.017	0.049	53.29	0.011	0.214	100.598
223	46.86	0.006	0.01	0.052	53.48	0.019	0.075	100.502
224	46.77	0.013	0.013	0.057	53.26	0.008	0.319	100.439
225	46.78	0	0.02	0.032	53.09	0	0.189	100.111
226	47.1	0	0.023	0.046	53.07	0.008	0.177	100.424
227	47.35	0	0.035	0.02	53.18	0	0.232	100.817
Maximum	49.31	0.071	0.054	0.089	53.93	0.053	0.345	100.981
Minimum	44.04	0	0	0	30.86	0	0	80.457
Average	46.84132	0.006237	0.014763	0.037961	52.61382	0.007395	0.131724	99.65318
Sigma	0.562215	0.009937	0.012104	0.022222	2.753865	0.011507	0.072936	2.622973