Microbial Arsenic Release from Historic Gold Mine Tailings, Nova Scotia, Canada

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Abstract

The oxidation state of arsenic is an important consideration in the remediation planning for arsenic contaminated tailings, frequently associated with gold mines. As(III) is more toxic and mobile than As(V) and arsenate can be reduced by bacteria. This study focuses on the microbial arsenic release from two abandoned Nova Scotia tailings sites. DNA fragment analysis showed that the microbial ecology varied across the two compositionally diverse sites. Microbial diversity was related to the concentrations of arsenic and iron, which implies the presence of metal reducing bacteria. Known metal reducers belonging to the *Geobacter* and *Schewanella* families were identified through gene cloning. Anaerobic microcosms quantified the activity of these bacteria. High calcium tailings showed the most arsenic release, while hardpan areas showed dramatic iron reduction. The presence of reducing bacteria can therefore affect remediation planning, since the use of tailings covers or liming techniques could increase the activity and diversity of metal reducing bacteria.

Résumé

L'état d'oxydation de l'arsenic est un facteur important à considérer lors de la restauration de parcs à résidus miniers associés aux mines d'or. L'arsenic réduit (As(III)) est plus toxique et soluble que l'arsenic oxydé (As(V)) et l'arséniate peut être réduit par les bactéries. La présente étude porte sur la solubilisation de l'arsenic dans des résidus de mine d'or de la Nouvelle Ecosse. L'analyse de fragments d'ADN a démontré que la diversité microbienne varie entre les deux sites étudiés. La diversité a été corrélée aux concentrations de As et Fe, ce qui suggère la présence d'activité microbienne dans les résidus. Les bactéries appartenant aux familles *Geobacter* et *Schewanella* ont été identifiées. L'utilisation de microcosmes anaérobiques a quantifié l'activité de ces bactéries. Les résidus riches en calcium avaient la plus grande solubilisation de As alors que les résidus de la couche cimentée (hardpan) ont montré des évidences de réduction microbienne du Fe. La présence de bactéries réductrices peut potentiellement affecter les stratégies de restauration des sites miniers, tout particulièrement si des membranes ou l'addition de chaux est envisagée car ces stratégies créeront des conditions propices pour la prolifération de bactéries capables de réduire le fer et l'arsenic.

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List of Acronyms

ARM	Arsenic Resistant Microogranisms
BIOS	Biogenic Iron Oxides
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
CAREG	Centre for Advanced Research in Environmental Genomics
DARP	Dissimilatory Arsenic Reducers
DIC/DOC	Dissolved Inorganic/Organic Carbon
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide triphosphate
DO	Dissolved Oxygen
GCL	Geosynthetic Clay Liner
GD	Goldenville
ICP-ES	Inductively Coupled Plasma Emission Spectroscopy
MG	Montague
NCBI	National Center for Biotechnology Information
NMDS	Non-Metric Multidimensional Scaling
ORP	Oxidation Reduction Potential
OTU	Operational Taxanomic Unit
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PHREEQC	pH, REDOX, Equilibrium, Programming Language
ppm	Parts Per Million
RDP	Ribosomal Database Project
RNA	Ribonucleic Acid
SI	Saturtaion Index
TIC/TOC	Total Inorganic/Organic Carbon
T-RFLP	Terminal Restriction Fragment Length Polymorphism
wt%	Weight Percent
XRD	X-Ray Diffraction

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Introduction

Background:

Gold mining in Nova Scotia:

Although Nova Scotia is no longer known for gold production, from the mid-1800s to the mid-1900s, the province produced over 27 tonnes of gold from 65 official mining districts (Dale and Freedman, 1982; Wong et al., 1999). Most of these mines were concentrated in the Halifax and Guysborough counties along the eastern shore (Fig. 1), the largest being the Goldenville district near Sherbrooke (Dale and Freedman, 1982). With an average ore grade of 1.6 oz Au/ton, these mining districts also produced over 3 000 000 tonnes of tailings (Mills, 1997; Goodwin et al., 2008).

Gold occurred in quartz veins within the Halifax and Goldenville meta-turbidite groups of the Meguma terrane (Kontak et al. 1990). These veins ranged from <1cm to >6m wide with strikes as long as 11km (Bierlein et al., 2004). Importantly gold mineralization was associated with arsenopyrite (AsFeS) mineralization in the quartz veins and host rocks (Bierlein et al., 2004).

Once the ores were mined, they were processed at a stamp mill where they were crushed to silt-sand sized grains (Fig. 2) (Dale and Freedman, 1982; Mills, 1997). Gold was extracted through mercury amalgamation until the 1880s when the process switched to cyanidation (Goodwin et al., 2008).

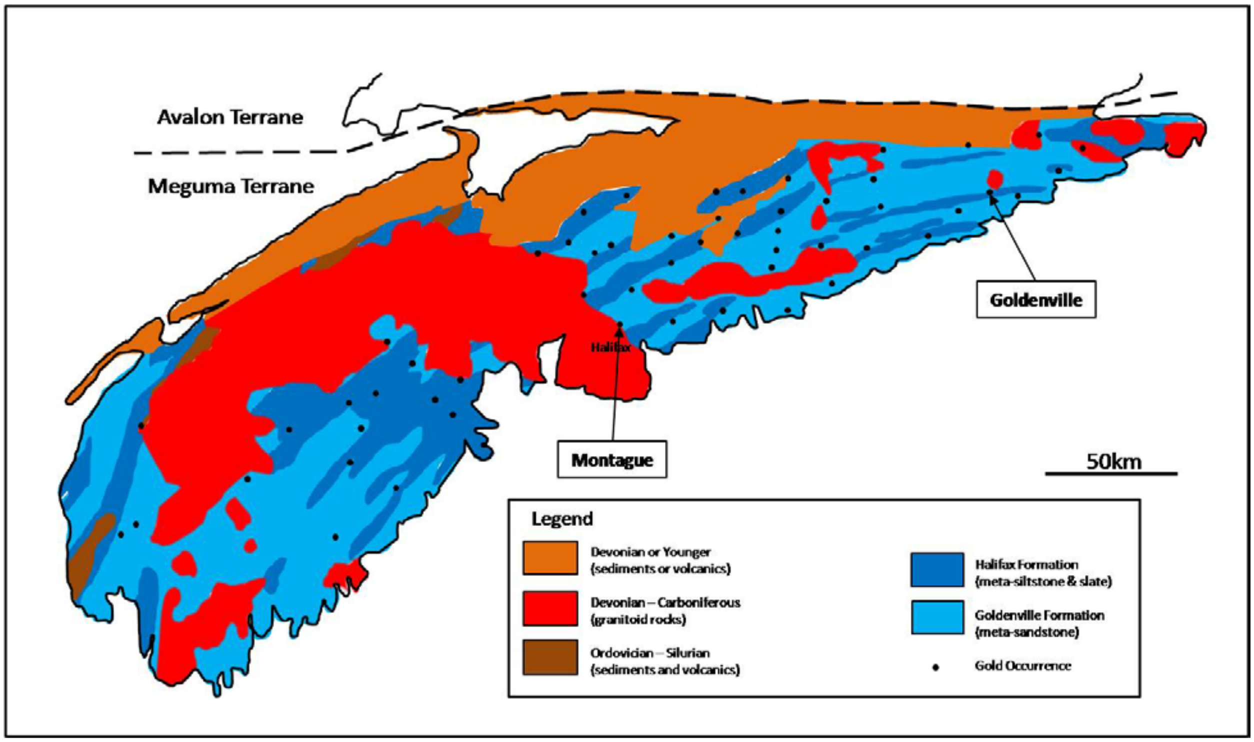

Figure 1: Major historic gold mining districts in Nova Scotia and regional geology. Study sites labelled: Montague, Goldenville (modified from Bierlein et al., 2004).

A major economic concern in gold mining during the early Nova Scotian gold mines was the need for adequate, proximal fresh water (Wong et al., 1999). This meant that most processing sites were located near streams, rivers or wetlands, which also meant that tailings were deposited near or in fresh water bodies or wetlands (Wong et al., 1999). Tailings from the processing sites contained high concentrations of mercury (Hg) and cyanide from the processing techniques, arsenic (As) and iron (Fe) from the host rocks and other potentially hazardous metals and chemicals from processing or host rocks (Goodwin et al., 2008).

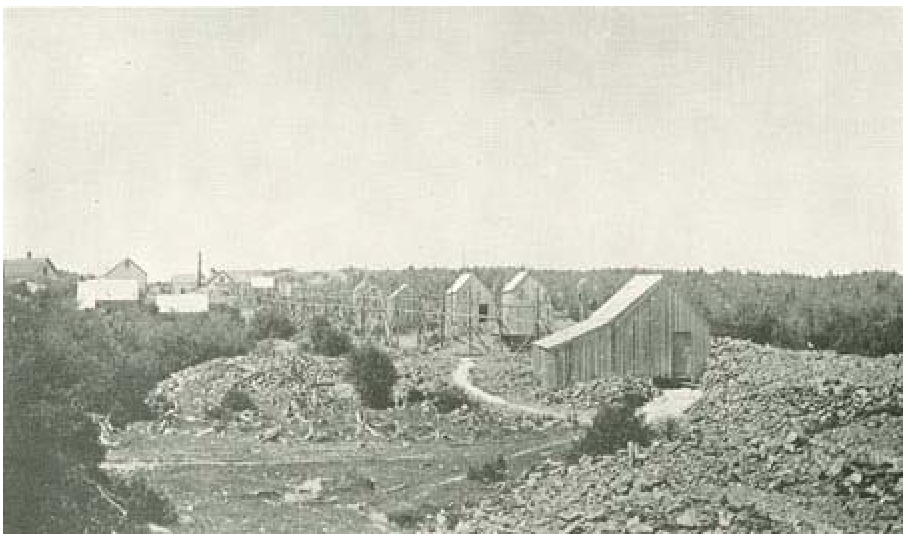

Figure 2: Historic photograph of surface operations in the Montague gold mining district, 1912. The photo shows line of mine shafts along the quartz vein and processing facilities in the background. Published by the Dept. of Mines, Canada.

The primary health and environmental concern for these tailings sites is their high arsenic concentrations. There was little to no environmental regulation of gold mines through most of the gold production period in Nova Scotia, which resulted in many highly contaminated abandoned sites, with some tailings sites having arsenic concentrations as high as 31 weight percent (wt%, Goodwin et al., 2008).

This study focuses on two such tailings sites in Nova Scotia: Goldenville and Montague (near Cole Harbour). Details on these sites can be found in the site description section (Pg. 15). Both sites have been identified as targets for remediation by the provincial government (Goodwin et al., 2008; Walker et al., 2009).

Arsenic Toxicity:

The toxic effects of arsenic have been known and exploited since antiquity: arsenic trioxide (As_2O_3) was a popular poison used by and on royalty in the past, earning the regal nickname “The King of Poisons,” (Henke, 2009a). The toxic effects of arsenic were further exploited in recent history through the use of arsenic based pesticides and preservatives (Henke, 2009a).

Human exposure to arsenic can occur via inhalation, dermal absorption or ingestion of food, water or soil (Henke, 2009a). Potential health effects include: cardiovascular disease, diabetes and cancer of the lungs, skin or bladder (Henke, 2009a). Recognition in the 19th and 20th centuries of widespread arsenic contamination and its deleterious health effects led to regulations being put into place on arsenic wastes and emissions (Henke, 2009a). The current Canadian guideline concentration for arsenic for human health concerns is just 12ppm in soils (Meunier et al., 2010), which is 4 orders of magnitude lower than some of the concentrations encountered at the Montague and Goldenville tailings sites.

The oxidation state of arsenic has a great effect on its toxicity. The two most common species of arsenic are the oxidized form, arsenate (As(V)) and the less oxidized form, arsenite (As(III)) (Henke, 2009a; Oremland and Stolz, 2003). Arsenic also exists in a 1, 0, -1, and -3, oxidation state. Since the vast majority of arsenic occurs either as As(III) or As(V), these are referred to here as the oxidized (As(V)) and reduced (As(III)) forms. Both of these forms are toxic, but in different ways. Arsenate is an analog of phosphate and can be pumped into cells by phosphate pumps (Oremland and Stolz, 2003). Once in the cell, arsenate disrupts the cell's energy production system by impeding oxidative phosphorylation (Oremland and Stolz, 2003). Arsenite is far more toxic than arsenate (Henke, 2009b). Arsenite binds to the sulyhydryl groups on proteins inhibiting their function and can disrupt respiratory function (Oremland and Stolz, 2003). In addition, unlike arsenate, arsenite does not bind to common mineral groups making it more hydrogeologically mobile (Meunier et al., 2010). This means that the oxidation state of arsenic is an important concern in devising effective remediation programs for contaminated sites, such as those studied here.

Microbial Reduction of Arsenic:

The oxidation state of arsenic in tailings is often affected by the activity of bacterial communities (Héry et al., 2008). Bacteria have the ability to reduce arsenic from its As(V) state to the more toxic and mobile As(III) state. It is important to consider both the abundance and activity of these bacteria when evaluating the cycling and release of arsenic from a system. There are two distinct types of arsenic reducers in the prokaryote world: the arsenic resistant microbes (ARMs) and the dissimilatory arsenic reducing prokaryotes (DARPs) (Oremland et al., 2005).

The ARMs reduce arsenate to arsenite in order to rid their cells of the toxic arsenate (Oremland and Stolz, 2003). When arsenate enters the cell via phosphate pumps, ARMs use the *ars* pathway to first reduce the arsenate to arsenite, and then pump it out of the cell through arsenite pumps (Fig. 3). It is vital to the microbe that the arsenate is first reduced prior to being pumped out, as an arsenate pump would also pump out the phosphate the cell requires for oxidative phosphorylation (Oremland and Stolz, 2003). The microbe also relies on the increased mobility of the reduced arsenite to move the toxin away from itself (Oremland et al., 2005).

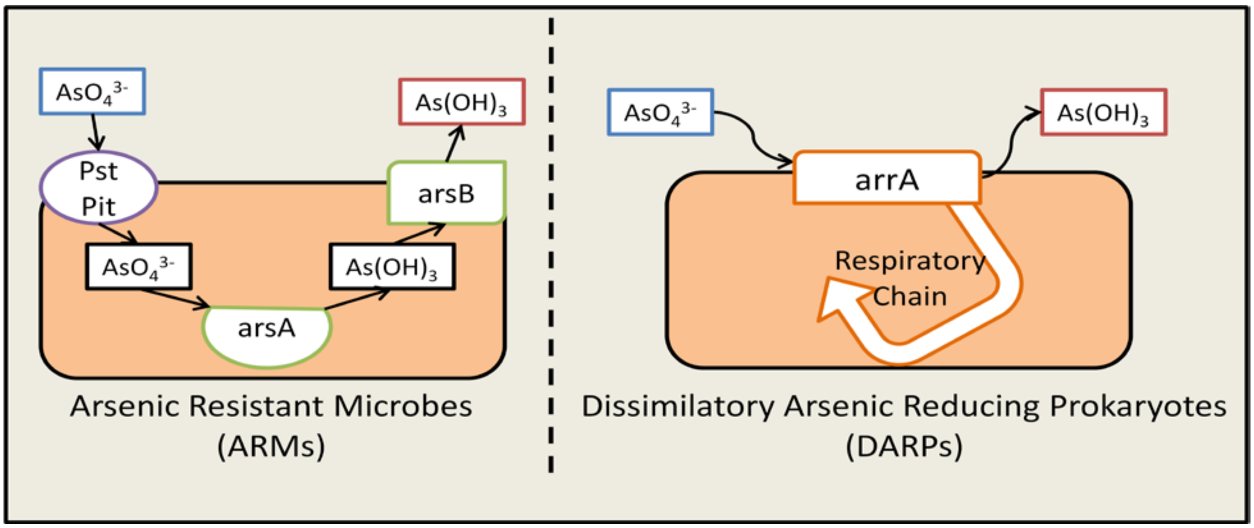

Figure 3: Mechanisms of microbial arsenic reduction in arsenic resistant microbes (ARMs, left) and dissimilatory arsenic reducing prokaryotes (right). (Modified from Silver and Phung, 2005)

DARPs, on the other hand, use As(V) as a terminal electron acceptor in their respiration pathway; this process reduces the arsenic to its more toxic form (Fig. 3). This reaction in exothermic and the cell gains 135mV of energy through this process (Oremland and Stolz, 2003). This process is achieved through the *arr* operon (Héry et al., 2008).

Microbial transformation of arsenic in the environment

Worldwide, the number of people at risk from arsenic contaminated groundwater is thought to be in the millions (Islam et al., 2004). One of the most well known and well studied sites where natural arsenic contamination occurred is in the West Bengal region of Bangladesh (Islam et al., 2004). These authors used microcosms and molecular methods similar to those in this study to determine the role of metal reducing bacteria in the mobilization of arsenic in aquifers in the West Bengal region. Subsurface sediments were mixed with simulated groundwater and incubated to track the reduction of arsenic and iron over time. Initially, pore-water arsenic was dominated by the As(V) species, but after 24 days, the concentration of As(III) in the porewaters rose to the equivalent of 24µM (Islam et al., 2004). Using 16S PCR based techniques; the authors were able to show that the bacterial communities in the samples were diverse, and sequencing identified many bacteria known to be capable of metal reduction, including 11% of the species belonging to the *Geobacter* family (Islam et al., 2004).

Arsenic reducers have previously been found in gold mine tailings sites. Macy et al. (1996) described a dissimilatory arsenic reducer in gold mine wastewaters from the Ballarat Goldfields in Australia. These bacteria were able to use As(V) as an electron acceptor when acetate was present as an electron donor (Macy et al., 1996). More recently, Anderson and Cook (2004) studied 100-year old mine tailings from the Barewood mine in Central Otago, New Zealand. These tailings are similar in age and origin to those in Nova Scotia, but these authors failed to identify any arsenic reducers in their investigation; however, the Anderson and Cook (2004) study was limited to arsenic resistant microorganisms (ARMs).

Importance of Iron Reduction:

In anaerobic environments, several bacteria are known to couple the oxidation of simple organic compounds to the reduction of sulfur, iron, and other metals. The microbial reduction of Fe(III) to Fe(II) in iron-arsenic minerals can liberate arsenic from the mineral, mobilizing it into the environment (Héry et al., 2008; Henke 2009b). If the mineral is an iron-arsenate mineral, As(V) can also be reduced to As(III) under reducing conditions and become more mobile and toxic (Héry et al., 2008).

Additionally, many bacteria are able to reduce more than one metal, allowing them to thrive in many conditions (Henke, 2009b). Sometimes, the ability to reduce iron is paired with the ability to reduce arsenic, including some members of the *Geobacter* family such as *G. uraniireducens* (Héry et al., 2008), which means that the presence of iron reducing bacteria in a sample may imply the presence of arsenic reducing bacteria in the same sample as both abilities can exist in a single species or population.

Islam et al. (2005) showed mobilization of arsenic can be triggered by the activity of iron reducing bacteria. This mobilization is through the reductive dissolution of Fe-As minerals and compounds in arsenic contaminated sediments in West Bengal by members of the *Geobacter* family (Islam et al., 2005). As these arsenic contaminated sediments are similar to the sediment-like arsenic rich tailings in this study, this process may be occurring at both the Montague and Goldenville sites.

Effect of Site Remediation on Bacterial Populations:

The Montague and Goldenville mine sites have been identified as important sites for remediation by the provincial government (Goodwin et al., 2008; Walker et al., 2009). Understanding the microbial ecology of the tailings is vital to an effective remediation plan, as demonstrated in previous studies. Lee et al. (2009) showed that the activity of arsenic reducers at two Korean gold mine tailings sites can increase when a carbon source is introduced. Macur et al. (2001) showed that the abundance of many arsenic reducing bacteria increases in response to an increase in pH by liming. The rise in pH from to 4 to 8 resulted in a 5 fold increase in the total arsenic released from the sediments (Macur et al., 2001). These studies show that proceeding with a remediation plan without assessing the effect on the microbial ecology could actually increased the amount of arsenic mobilized and release from the tailings sites in Nova Scotia.

Molecular Microbiology:

Determining the composition of a bacterial community was, until recently, reliant on culture based microbiology techniques. In these techniques, bacteria were grown on media and subsequently identified (Röling and Head, 2005). These methods carried with them an essential flaw: not all bacteria grow in growth media (Röling and Head, 2005). This culture bias is magnified when working with bacteria that have complex environmental requirements, such as arsenic reducing bacteria.

The introduction of the polymerase chain reaction (PCR) in the 1980's revolutionized microbiology by allowing researchers to quickly amplify (replicate) the DNA of an organism in the laboratory (Mullis, 1994). PCR simulates the cells' own DNA replication mechanism. PCR is also targeted, allowing the choice of specific parts of the genome to be amplified (Röling and Head, 2005). The result of PCR is countless copies of a gene of interest from very little starting material. Comparing the resultant amplified genes can determine the diversity of a community and analyzing the sequences of these genes allows for the identification of specific bacteria at the species level.

PCR based culture-independent techniques gave rise to the field of molecular microbiology; now, after 25 years of use, these techniques are central to modern microbiology (Mullis 1994; Röling and Head, 2005). There are now several kits and/or published procedures for DNA extraction, amplification, cloning and sequencing for almost any type of sample.

In microbial ecology, the most oft targeted gene is the bacterial 16s rRNA gene (Röling and Head, 2005). This gene codes for a ribosomal subunit and is common to all bacteria. By amplifying this gene from an environmental sample, one can compare all the bacteria within the sample. As a result, there is an abundance of information about the 16s sequences of all bacteria, making it a perfect target for sequencing as a multitude of species can be identified from existing databases.

However, when looking for specific taxa of bacteria, choosing a different gene target can be more useful than the 16S gene. Some taxa show little to no heterogeneity in their 16S sequences, meaning this approach may not resolve the diversity and richness of the bacterial taxa. In these instances, it is best to find a gene shared within the taxa of interest to serve as the target PCR.

For the two groups of arsenic reducing bacteria (DARPs and ARMs), a gene specific to the arsenic reducing mechanism serves as an ideal target for resolving the presence, richness and diversity of arsenic reducers in a sample. In the case of DARPs, the *arrA* gene has been shown to be a good indicator of dissimilarity arsenic reduction in a sample (Malarsarn et al., 2004). This gene codes for a reductase which allows for the respiratory reduction of As(V) to As(III) (Malarsarn et al., 2004) In the case of ARMs, the *arsC* gene can be used to indicate the presence of resistant arsenic reduction in a sample (Sun et al., 2004). This gene codes for the arsenate reductase enzyme active in ARMs (Sun et al., 2004).

Targeted PCR approaches allow for quick, easy identification of arsenic reducing bacteria within a sample. Meanwhile, 16s analysis provides information on bacterial community richness and evenness. The more laborious cloning and sequencing will identify the specific arsenic reducers active in the samples.

PCR Inhibition:

Many studies have used PCR based molecular microbiology to identify arsenic reducers in contaminated samples. Although PCR is a sophisticated technique that is widely used, it is by no means perfect. PCR remains a sensitive reaction which must be optimized for every new sample type analyzed. Varying concentrations of components by as little as 0.1mM can be the difference between success and failure. This can be a particular problem when dealing with samples containing known PCR inhibitors such as calcium and heavy metals.

Opel et al. (2010) evaluated the mechanisms of calcium PCR inhibition. Calcium was found to reduce the efficiency of the amplification by competing with magnesium, thereby inhibiting the Taq polymerase used in the reaction (Opel et al., 2010). This inhibitory effect may be important in the high calcium end-members studied at the Montague and Goldenville tailings.

Heavy metals, including arsenic, are also known PCR inhibitors. Arsenic in its arsenate state can substitute for phosphate in DNA (Wolfe-Simon et al. 2009). It is possible that this substitution could reduce PCR efficiency. Although at least one bacterium can remain viable when substituting arsenate for phosphate, it is unlikely that PCR can remain efficient when there is the possibility of arsenate substitution. In this study the inhibitory effect of arsenic is important since concentrations of the contaminant are extremely high.

Bacterial activity and microcosm experiments:

Although PCR based techniques are ideal in identifying bacteria in a sample, they cannot quantify the activity of these bacteria. Microcosm experiments mimicking natural conditions can help differentiate between abiotic and biotic processes, and provide information about reaction rates. In this study, replicating the conditions of the tailings from Montague and Goldenville in a closed system allows for the tracking of arsenic reduction over time and identification of the key bacteria involved through the use of PCR based techniques.

Islam et al. (2004) performed a similar microcosm experiment using arsenic contaminated sediment from West Bengal aquifers. The authors tracked both iron and arsenic speciation over time to identify the potential role of microbes in the mobilization of arsenic (Islam et al., 2004). It was found that under anaerobic conditions, with an adequate carbon source, metal reducing bacteria play an important role in the release of mobile arsenic from the sediments (Islam et al., 2004). Iron reduction took place prior to arsenic reduction in these experiments (Islam et al., 2004). Further studies showed that the reductive dissolution of Fe-As compounds by iron reducing bacteria can contribute to the release of arsenic from these systems (Islam et al., 2005).

Objectives:

The main purpose of this study is to evaluate the microbial release of arsenic from two tailings sites in Nova Scotia: Montague and Goldenville. Understanding the microbial ecology at these sites is vital to designing an appropriate remediation plan for these contaminated areas. The first objective is to determine the microbial population diversity (with respect to Fe and As reducers) in selected mineralogical end members representative of the 2 sites, namely Hardpan, "Typical", High Ca:As, and Saturated. The second objective is to investigate the rate of release of Fe and As from the same gold mine tailings in microcosm experiments mimicking the tailings aqueous chemical conditions and determine the change of microbial population diversity as the microcosms evolve over time.

Mine tailings rich in iron and arsenic can be prime habitats for metal reducing bacteria (Lee et al., 2009). It is expected that both iron and arsenic reducers will be found at both the Montague and Goldenville sites. However, it is unlikely that the diversity and abundance of these bacteria will be constant throughout these compositionally diverse sites. Instead, the microbial ecology is expected to vary between tailings end-members. Likely the bacteria identified in the tailings will not be the same for each end-member. Some end-members, like the saturated end-member, may be more ideal environments for the growth of metal reducers.

Similar studies of arsenic contaminated sediments have shown that activity of metal reducing bacteria can trigger mobilization of toxic arsenite: As(III) (Islam et al., 2004; Lee et al., 2001). It is expected that microcosm experiments conducted with these historic tailings will show evidence of microbial arsenic reduction and release under anaerobic conditions. Further, it is expected that this reduction can happen in a short time frame (less than 20 days), in keeping with similar results of microcosm experiments (Islam et al., 2004; Lee et al., 2001).

The release of mobile arsenic from the tailings could be due to either the activity of arsenic reducing microorganisms, or iron reducing microorganisms. Islam et al. (2004), and Héry et al. (2008) have demonstrated that the reductive dissolution of Fe-As minerals by iron reducing microorganisms can lead to the release of arsenic into the environment. Also, the activity of arsenic reducers can directly reduce and release As(III) from these compounds (Héry et al., 2008). It is expected that the activity of both iron and arsenic reducing microorganisms in the tailings samples will lead to a substantial release of arsenic from the tailings.

This microbial trigger of arsenic release is important to remediation efforts. Given the wide geochemical and mineralogical heterogeneity at these sites, it is expected that both the microbial community composition and activity of metal reducers will vary between the four end-members. The objective here is to assess the microbial ecology and activity of each end-member to inform future remediation efforts of the importance of microbiology in the release of arsenic from these contaminated sites. This is important as it is known that many common mining processes and remediation efforts (tailings covers, liming, etc.) can create ideal environments for the growth of metal reducing bacteria in tailings (Lee et al., 2009).

Site Descriptions:

Mineralogy and Geochemistry of the End-members:

The arsenic found in the tailings comes from the arsenopyrite found in the host rocks (Goodwin et al., 2008). Gold mines are known to have high concentrations of arsenic, commonly up to 100 times the crustal average (Haffert and Craw, 2008). Arsenopyrite is not stable at the surface, and alteration and oxidation following ore processing have created a complex geochemical environment at the Montague and Goldenville tailings (Henke, 2009b; Walker et al., 2009).

There are many intermediate steps in the oxidation of aresnopyrite which give rise to an abundance of secondary arsenic minerals (Henke, 2009b). The major secondary arsenic minerals at the tailings are scorodite ($FeAsO_4 \cdot 2H_2O$) and hydrous ferric arsenate (HFA; Walker et al., 2009). However, the mineralogy at both sites varies widely, sometimes changing dramatically over just a few meters. The development of secondary arsenic minerals is influenced by weathering and mining history, pH and concentrations of other metals, such as iron and mercury (Walker et al., 2009).

To simplify the complex geochemistry and mineralogy of the sites, four characteristic end-members have been identified, all of which occur at both sites (Table 1). The main minerals found in all end-members are quartz, muscovite, clinochlore and albite (Jamieson et al., 2006); these end-members vary in their arsenic secondary mineralogy and REDOX conditions.

Table 1: Summary of the geochemical and mineralogical characteristics of tailings end-members from Montague and Goldenville, Nova Scotia.

	End-Member	Colour	Description	Main Arsenic Minerals	Map References
1	Hard-Pan	Green-Yellow	Impermeable, cemented	Scorodite, Fe-Arsenates	MG: 3 GD: 2, (1)
2	“Typical”	Red- to Orange-	Oxidized to un-oxidized, sand-	Scorodite, HFA, HFO	MG: 1, 2 GD: 3, 4, 5, 6
3	High Ca:As	Grey to Brown	≥1.5 Ca/As, sand-sized	Yukonite, Pharmacosiderite	MG: 5, 6 GD: 7
4	Permanently Saturated	Dark Brown to	Un-oxidized, water saturated	Arsenopyrite, HFA	MG: 4 GD: 8, 9

The hard-pan (1) end-member is characterized by hard, impermeable layers of cemented tailings (Table 1). The hard-pan is green-red to orange and looks like a coarse grained sandstone. However, the grains in the hard-pan are scorodite and iron arsenates, which are cemented by gypsum and goethite (Walker et al., 2009). Hard-pans commonly form in tailings areas following repeated wet-dry cycles (Henke, 2009b). These features are important because they affect drainage, and keep the underlying layers un-oxidized (Henke, 2009b).

The “Typical” (2) end-member is so named because it is the most common end-member: it constitutes the bulk of the tailings at both sites (Table 1). “Typical” tailings are typically sandy, red- to orange-brown and un-cemented. These tailings are not homogenous laterally or at depth, but are characterized by lower calcium concentrations, a lack of cementation, and are either oxidized or have been oxidized in the past. Arsenic occurs as scorodite, HFA and within hydrous iron oxides (HFO) (Walker et al., 2009).

The High Ca/As (3) end-member, is characterized by having a Ca/As ratio of ≥1.5 (Table 1, Kavalench, 2010). Like the "typical" tailings, this end-member is sandy, un-cemented and varies in color. The greater concentration of calcium changes the arsenic secondary mineralogy; in this end-member arsenic is found in yukonite ($Ca_7Fe_{11}(AsO_4)_9O_{10}$:24.3$H_2O$) and pharmacosiderite ($KFe_4(AsO_4)_3(OH)_4$:6-7$H_2O$, Walker et al., 2009). Importantly, this end-member has been identified as having the most bioaccessible arsenic of all end member samples (Meunier et al., 2010).

The final Saturated (4) end-member is distinguished by being permanently water saturated (Table 1). The permanently saturated end-member is found in swampy areas at both sites and is characterized by being water saturated and under reducing conditions. These tailings are darker than the "typical" tailings ranging from dark-brown to black. Arsenic occurs in its un-oxidized arsenopyrite state (Walker et al., 2009). There is more vegetation at these locations and a higher amount of organic matter in the tailings. Beyond these distinctions, the saturated tailings are similar to the "Typical" end-member.

1: HARDPAN

- Hard, impermeable layers of cemented tailings
- Map locations: MG: 3, GD: 1, 2
- Scorodite and iron arsenates cemented by gypsum and goethite

Concentration (ppm)	Arsenic	Iron
Average	114 025	107 700
Maximum	271 700	250 100

2: "TYPICAL"

- Most common end-member, constitutes bulk of tailings
- Map locations: MG: 1, 2 , GD:3-6
- Un-cemented, oxidized, sand sized scorodite, hydrous iron oxides

Concentration (ppm)	Arsenic	Iron
Average	3260	22 900
Maximum	16 300	73 300

3: HIGH Ca:As

- Similar to "Typical" but with
Ca:As > 1.5, higher bioaccessibility
- Map locations: MG: 4, GD: 7
- Ca-As 2o minerals: yukonite, pharmacosiderite

Concentration (ppm)	Arsenic	Iron
Average	2585	35 000
Maximum	3200	51 600

4: SATURATED

- Swampy, high organic matter, under reducing conditions
- Map locations: MG: 5, 6, GD: 8, 9
- Arsenopyrite, Hydrous Iron Arsenates

Concentration (ppm)	Arsenic	Iron
Average	6850	29 900
Maximum	30 200	55 700

Figure 4: Photographs, descriptions, and basic geochemistry of the tailings end-members.

Montague:

The Montague tailings site is located near the small community of Montague Gold Mines, a suburb located about 6km north-east of Dartmouth, Nova Scotia (Fig. 1). Gold mining operations commenced following the discovery of the ore in 1862 and operated until 1927 (Mills, 1997). The district produced almost 70 000 ounces of gold, making it the 5th largest producer in the province (Goodwin et al., 2008; Utting et al., 2009). Most of the gold was mined from a single quartz vein and processed at the nearby Boyd's Crusher, a 10-stamp mill (Figures 5 and 6, Goodwin et al., 2008; Utting et al., 2009). Tailings were deposited into low lying swampy areas along Mitchell Brook (Fig. 5, Goodwin et al. 2008). Over the course of the mining operations, approximately 132 000 tons of host rocks were processed (Goodwin et al., 2008).

Today, the tailings form a large, flat, sandy area which has become a popular spot among local ATV and motorbike enthusiasts (Goodwin et al., 2008). Arsenic concentrations at Montague range from about 2000ppm in the "typical" tailings, up to 80 000ppm in the hardpan (Table 2). The tailings extend into the swamp surrounding Mitchell Brooke and up to the forested area nearer to the community (Fig. 6).

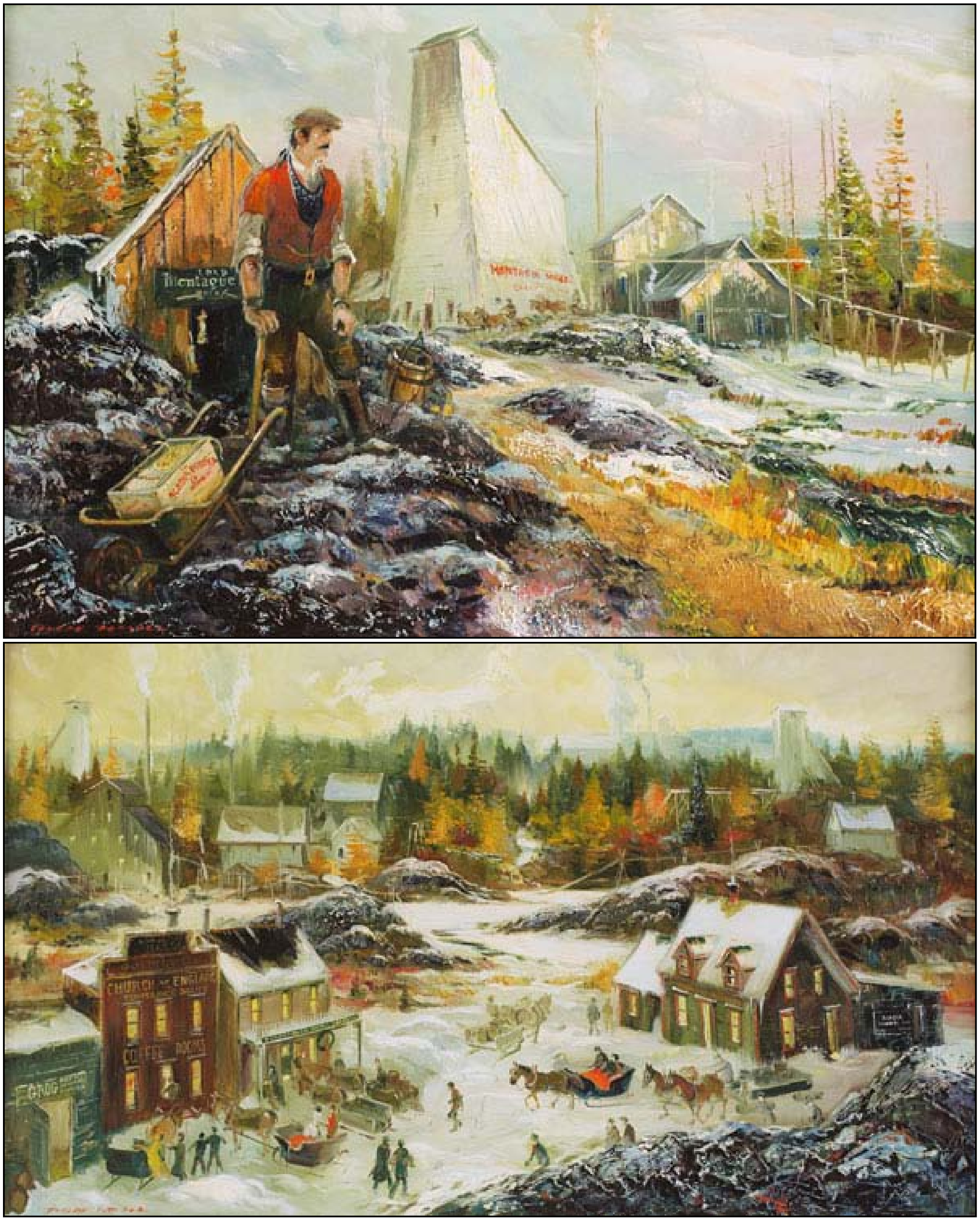

Figure 5: Two historic paintings by Joseph Purcell showing gold mine sites in actions. Top: Montague district, late 1800s, showing a rudimentary mine shaft in the foreground and processing facilities in the background. Tailings would have been slurried into the swampy area on the right. Bottom: Goldenville, early 1860's, showing bustling town in foreground and intensive mining and processing facilities in background. (Image: Nova Scotia Archives).

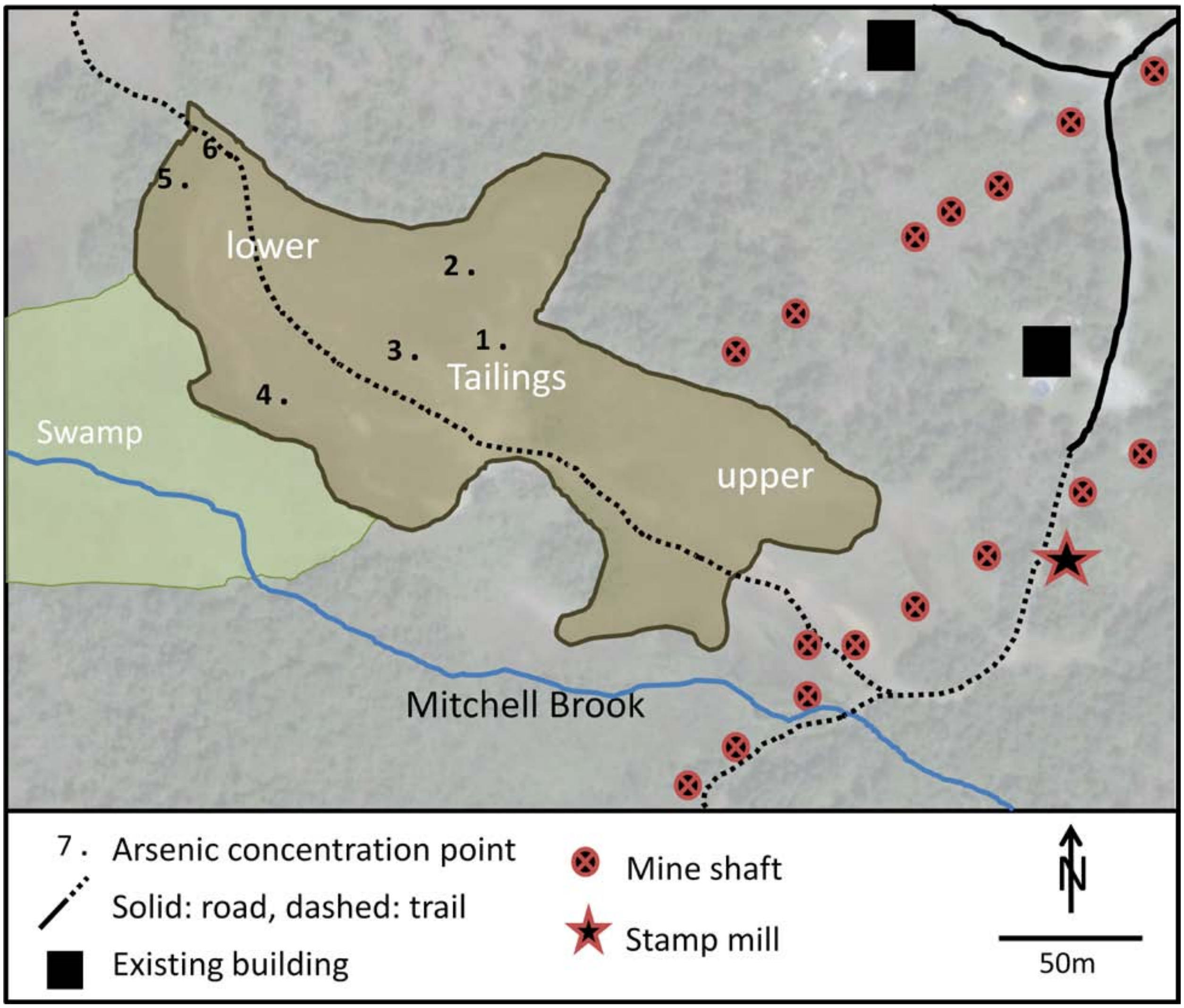

Figure 6: Map of Montague tailings site, near Dartmouth, Nova Scotia and location of the samples (Table 2, Figure 4). (Modified from Goodwin et al., 2008; background image © Google 2011)

Table 2: Average chemical composition of various tailings samples from the Montague and Goldenville sites (Maximum values are in brackets).

Site	Sample	As (ppm)	Fe (ppm)	Ca (ppm)	End-Member
MG	1	2715.3 (4323.9)	38 220 (73 300)	3950 (6300)	"Typical"
MG	2	5728.2 (16 300)	31 050 (36 800)	6300 (9300)	"Typical"
MG	3	70 750 (79 800)	56 100 (90 100)	3850 (16800)	Hardpan
MG	4	11 400 (30 200)	40 800 (55 700)	3400 (4100)	Permanently Saturated
MG	5	2418 (2754)	36 300 (48 600)	3100 (3400)	High Ca/As
MG	6	2136 (2236)	38 900 (51 600)	4300 (6100)	High Ca/As
GD	1	157 300 (271 700)	159 300 (250 100)	>100 (100)	Hardpan/Mill Concentrate
GD	2	62 300 (131 700)	68 143 (148 400)	180 (300)	Hardpan
GD	3	2876 (4052)	18 600 (24 000)	1200 (2800)	"Typical"
GD	4	2228 (4425)	26 000 (31 600)	1400 (1900)	"Typical"
GD	5	2444 (8154)	25 000 (30 500)	2800 (8600)	"Typical"
GD	6	3564 (11 100)	22 000 (28 800)	4200 (10 000)	"Typical"
GD	7	1860 (3197)	29 900 (39 500)	6900 (11 400)	High Ca/As
GD	8	7472 (13 300)	26 300 (32 200)	2200 (2700)	Permanently Saturated
GD	9	1698 (2816)	22 600 (33 300)	1600 (1900)	Permanently Saturated

Goldenville:

Goldenville was the largest of the gold mining districts in Nova Scotia, producing over 200 000 ounces of gold from 1863 to 1927 (Mills, 1997; Wong et al., 1999). Gold was mined from 105 named quartz veins (and many more un-named), by over 19 companies (Smith et al., 2005). Several stamp mills operated in the area and the tailings were deposited into swampy areas surrounding the Gegogan Brook (Figs. 5 and7; Smith et al., 2005). The brook transported some of the tailings downstream creating a tailings field of about 2km^2 (Wong et al., 1999).

Similar to Montague, the Goldenville site is popular for off-road vehicles and was home to an annual 4X4 rally, now cancelled due to health concerns (Smith et al., 2005). The tailings are estimated to contain a total of 120 000kg of arsenic, at concentrations of 1000 to 10 000µg/g (Wong et al., 1999).

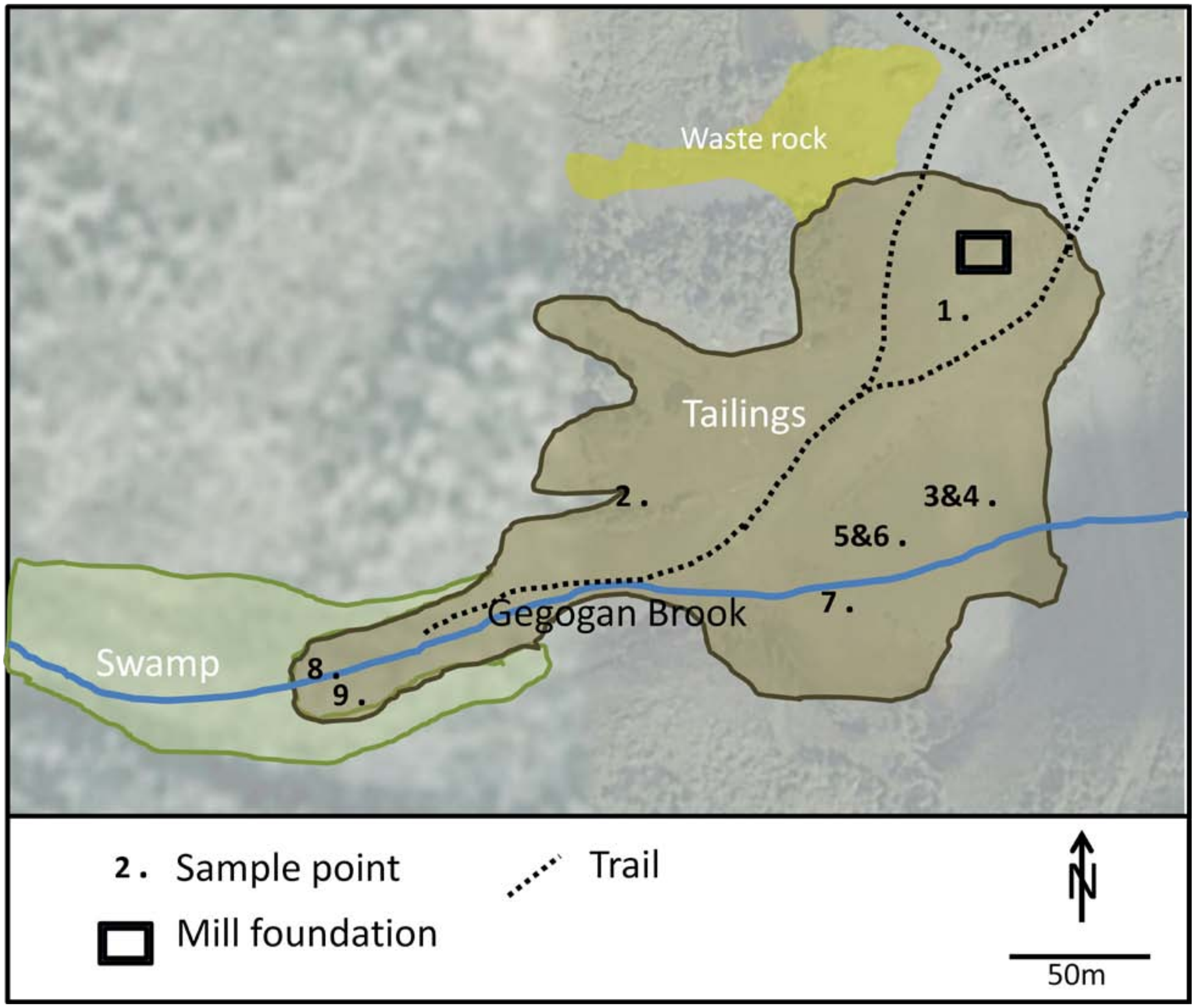

Figure 7: Map of Goldenville tailings, Sherbrooke, Nova Scotia. (Modified from Smith et al., 2005, background image © Google 2010)

2

Methods

Field Work:

Tailings samples for microbial analyses were collected in the summer of 2009. Material was taken from depth at each sampling site (Fig. 6, 7) using an ethanol sterilised scoopula. The tailings samples were taken at depths where water saturation occurred in order to collect sub-oxic to anoxic sediments. Tailings were placed in 15 or 50 ml sterile centrifuge tubes. The tubes were kept on ice in the field and placed at -20^{o} C as soon as possible. Samples were transported to Ottawa on dry ice where they were stored at -80^{o} C.

Large samples of each tailings end-member used for the microcosm experiments were collected during the summer of 2010. These samples were taken at ~30-60 cm depth, near the water table. Microcosm samples were double bagged in heavy duty zip-lock bags and air inside the bags was pushed out by hand. Samples were stored on ice and transported back to Ottawa where they were stored in a cold room at 4^{o} C.

Accompanying surface water samples for the microcosm experiments were collected from onsite streams in 2L Nalgene bottles. Bottles were filled completely and capped while under water with no head space. Water samples were stored on ice and transported back to Ottawa, where they were placed in a cold room at 4^{o} C. Some physico-chemical characteristics of the water were measured on site with an YSI handheld multi-parameter instrument (556MPS), i.e.: pH, temperature, ORP, specific conductance, dissolved oxygen (DO) and alkalinity.

Molecular Microbiology:

DNA Extraction:

DNA was extracted from approximately 1g of tailings samples using the UltraClean Soil DNA Isolation Kit (MoBIO #12800) following the manufacturer's directions with one exception: steps 15 and 16 were repeated. Extracted DNA was stored at -20^{o} C until amplification.

General Bacterial Gene Amplification: 16S

DNA samples were amplified using the Polymerase Chain Reaction (PCR) using universal primers 8f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). These primers amplify most of the 1500bp length of the 16C gene, common to all prokaryotes (Table 3).

Table 3: Primers used for amplification of 16S bacterial genes, arrA arsenic reductase genes, and arsC arsenic reductase genes.

Name	Sequence (5' – 3')	Length (bp)	Tm (°C)	%GC	Length amplified (bp)
8f	AGA GTT TGA TCC TGG CTC AG	20	51	50%	~1484
1492r	TAC GGY TAC CTT GTT ACG T	22	49	41%	
ArrAfwd	AAG GTG TAT GGA ATA AAG CGT TTg tbg ghg ayt t	34	63	35%	~160-200
ArrArev	CCT GTG ATT TCA GGT GCC cay tyv ggn gt	29	63	45%	
amlt-42-f	TCG CGT AAT ACG ATG GAG AT	20	54	50%	~350
amlt-376-r	ACT TTC TCG CCG TCT TCC TT	20	54	50%	
smrc-42-f	TCA CGC AAT ACC CTT GAA ATG ATC	24	59	41%	~350
smrc-376-r	ACC TTT TCA CCG TCC TCT TTC GT	23	59	47%	

Several different reactions were attempted with total volumes of 25 or 50 µl, mixed in 0.2 ml tubes. 10X PCR buffers used were Invitrogen 10X PCR Buffer, NEB Standard and NEB Crimson PCR Buffer, paired with Invitrogen *Taq* Polymerase (10342-052), Standard *Taq* Polymerase (NEB, M0273S) and Crimson *Taq* Polymerase (NEB, M0325S) respectively. The $MgCl_2$ concentration in the reactions was usually 0.5 mM, but some reactions used up to 2.0 mM final concentration. Template DNA was diluted 1, 10 or 100X for PCR amplification. Some reactions were run with Q solution (QIAGEN, part of PCR Core Kit 201223) and/or Dimethyl Sulfoxide (DMSO).

These reactions were incubated on an Eppendorf Epigradient Mastercycler or BioRad thermal cycler under the following conditions: an initial denaturing step at 94° C for 4 minutes, 35 cycles of: 94° C for 30 seconds (melting), 57° C for 30 seconds (annealing), 72° C for 1 minute (extension), with a 10 minute final extension at 72° C. PCR products were visualized on a 1% agarose gel with a 1 kilobase (kb) ladder.

Taxa Specific Gene Amplification: *arrA, arsC*:

Primers specific to Dissimalatory Arsenic Reducing Prokaryotes (DARPs) were used to quantify the diversity and abundance of these bacteria in each sample. The arsenic reductase gene (*arrA*) found in DARPs was amplified using degenerate ArrAfwd (5'-AAG GTG TAT GGA ATA AAG CGT TTg tbg gbg ayt t-3') and ArrArev (5'-CCT TGT ATT TCA GGT GCC cay tyv ggn gt-3') primers (Malasam et al. 2004). Reaction recipes varied as described above for 16S amplification. PCR conditions were as follows with a concentration of each primer of 0.5 µM: incubation at 95° C for 10 minutes, followed by 40 cycles of 95° C for 15 seconds, 50° C for 40 seconds, and 72° C for one minute PCR products were visualized on a 1% agarose gel using a 100bp ladder.

An additional set of primers was required to quantify the diversity and abundance of Arsenic Resistant Microbes (ARMs). Sun et al. (2004) designed a set of four primers to target the arsC gene, which is active in resistant arsenic reduction: amlt-42-f: TCG CGT AAT ACG CTG GAG AT, amlt-376-r: ACT TTC TCG CCG TCT TCC TT, smrc-42-f: TCA CGC AAT ACC CTT GAA ATG ATC, and smrc-376-r: ACC TTT TCA CCG TCC TCT TTC GT. Two sets of primers were used to ensure amplification of all the diverse sequences for the arsC gene found among ARMs (Sun et al. 2004). Again, various PCR recipes were used as described above. Thermocycler conditions were as follows: incubation at 95° C for 3 minutes, followed by 40 cycles of 95° C for 15 seconds, 60° C for 15 seconds, and 72° C for 15 seconds (Sun et al. 2004). PCR products were visualized on a 1% agarose gel using a 100bp ladder.

Terminal Restriction Fragment Length Polymorphism (T-RFLP):

Select samples representing all geochemical end members at both sites were chosen for DNA fingerprinting by Terminal Restriction Fragment Length Polymorphism (T-RFLP). 16S PCR was performed using the above protocol; however, a 6-FAM dyed forward primer was used. Amplified 16S DNA was digested using the MspI enzyme (Fermantas). A reaction including the PCR product, enzyme, buffer Tango and sterile water was incubated at 37^{o} C for 1-3 hours. Digested DNA was visualized on a 2% agarose gel. Successfully digested samples were sent to the University of Guelph Laboratory Services for fluorescent fragment analysis. Fragments were run on a 3730 DNA sequencer (Applied Biosystems Inc., Fredmont, California) with a 1200bp standard.

Peak information was normalised for the area under each peak. The normalised abundance data used to calculate ecological indices of diversity and evenness were calculated from the results using the diversity add-in for Microsoft Excel. Non-metric Multidimensional Scaling (NMDS) and Cluster Dendrograms were created using the vegan add-in to R statistical software. Principal Component Analysis (PCA) was used to compare these ecological indices with geochemical and physical measures.

Cloning and Sequencing

16S PCR and purification

16S PCR products without fluorescently dyed primers were used for gene cloning and sequencing. PCR products were visualised on a 1% agarose gel. The ~1500bp band of each successfully amplified sample was excised using sterile razors and forceps. The bands were purified using the QIAquick Gel Purification Kite (28706). DNA concentrations were calculated using a Thermo Scientific Nanodrop 2000 Spectrophotometer.

Ligation

Fresh PCR products (less than 24 hours old) were ligated with the pGEM-T plasmid vector (Promega), which contains genes for kanamycin and ampicilin resistance. Details of the ligation reaction can be found in the Promega pGEM-T handbook (Promega Corp., 2010).

Transformation

After ligation, the plasmid was transformed into chemically competent *Escherichia coli* cells provided by Phillip Pelletier (CAREG, University of Ottawa). Cells were grown for 1 hour at 37^{o} C in a shaker (200 rpm), and then spread on LB agar plates containing 50mg/ml kanamycin with IPTG and X-Gal for blue/white selection of clone colonies. Plates were incubated at 37^{o} C for 16 – 24 hours.

Only *E. coli* with successfully ligated and transformed plasmids grow white on the antibiotic medium. Non-ligated colonies appear blue. The white colonies were used for subsequent analysis.

Isolation of Plasmid

Colonies were analyzed following a similar protocol to Pellerin (2008). White colonies were picked off the LB media plates using sterile toothpicks and transferred to a microtube, or plate containing 100 µl of sterile water. The tubes or plates were micro-waved for 45 seconds at high power (800 W) to lyse the *E. coli* cells. The tubes or plates were then left on ice for 10 minutes and spun for 20 minutes at ~400 rpm at 4^{o} C. 50 µl of the supernatant was sub-sampled and spun down again. 2 µl of the supernatant from the second spin was used for subsequent M13 PCR analysis.

M13 PCR

The M13 reaction contained 0.2 µl dNTP, 4 µl NEB standard PCR Buffer (M0273S), 0.2 µl of the M13 forward (5' – GTA AAA CGA CGG C – 3') and reverse (5' – CAG GAA ACA GCT A – 3') primers (Table 3), 0.1 µl NEB standard Taq polymerase, 0.2 µl 25 mM $MgCl_2$ with sterile H_2O to make the volume up to 25 µl. The reactions were processed in an Eppendorf Epigradient Mastercycler or BioRad thermal cycler under the following conditions: initial denaturation at 94^{o} C for 5 minutes, followed by 30 cycles of 94^{o} C for 45 seconds, 54^{o} C for 45 sec and 72^{o} C for 2 minutes with a final extension step of 72^{o} C for 10 minutes.

Purification and Concentration

M13 PCR products were purified using the Qiagen MINelute PCR purification kit (28006). DNA concentrations were quantified for each reaction using a Thermo Scientific Nanodrop 2000 Spectrophotometer. Samples with a concentration in excess of 10 ng/µl were used for subsequent analysis.

Sequencing

At least 10 µl of each successful sample was submitted to Philip Pelletier for DNA sequencing at the University of Ottawa Centre for Advanced Research in Environmental

Genomics (http://www.careg.uottawa.ca/home.htm). Samples were sequenced using the 8f primer on a CEQ™ 8000 Genetic Analysis System.

Construction of Phylogenetic Trees

Sequences were aligned using the ClustalW program in BioEdit. Terminal, non-aligning regions were deleted from sequences.

Trimmed, aligned sequences were analyzed for most probable matches using the Ribosomal Database Project (RDB, Cole et al., 2008) SeqMatch program (http://rdp.cme.msu.edu/index.jsp). The most probable matches were chosen by selecting the highest similarity scores for each sequence.

A phylogenetic tree was created using the Tree Builder program on RBD. *Aquifex pyrophilus* was selected as an out-group. The tree used all experimental sequences and their closest matches found using SeqMatch (Cole et al., 2008). The resulting tree was saved as a MEGA compatible file and the tree was further analyzed and edited using MEGA.

Identification of Species

Identification of the closest matching bacteria to experimental sequences was performed using the SeqMetch program RBD, and using the BLAST program on the NCBI website. Closest matching species were looked-up in the NCBI database to determine their function and metal-reducing capacity.

Microcosms:

Surface water samples used in the microcosms were autoclaved and deoxygenated with N_2 for four hours soon after returning to the laboratory. Deoxygenated samples were stored at 4^oC until use. Prior to the microcosm setup, surface waters were taken out of the fridge and boiled and deoxygenated with N_2 for one hour and then moved into an anaerobic chamber.

Microcosm setup

Each microcosm consisted of 166 g of tailings mixed with 333 ml of autoclaved, deoxygenated surface water. The experiments were not amended with any carbon source. Microcosms were mixed in 500 ml square Pyrex bottles, adding the sediment first, then the water. Four bottles were made for each of the 5 samples: one of each end-member from Goldenville, one of the “Typical” end-member from Montague. Prior to adding surface water, half of the microcosms (with tailings) were autoclaved to sterilize, creating duplicate controls for each sample. Microcosms were run in duplicate: two experimental setups along with two controls. Results are averaged between these two values.

Sampling

The microcosms were run for 35 days. Sub-samples were taken from the bottles approximately every 5 days through the course of the experiment. Prior to sub-sampling, the bottles were shaken to re-suspend the sediments and about 20 ml of material was poured out into a sterile 50 ml centrifuge tube. About 9 ml was filtered through a 0.22 µm filter into a sterile 15 ml centrifuge tube for soluble metal concentration analysis. This material was acidified with 12 M HCl after removal from the anaerobic chamber. A sub-sample (i.e., 2.6 ml) of this filtered material was passed through a SepPak cartridge into a new sterile 15 ml centrifuge tube for arsenic speciation analysis. Approximately 2 ml of the filtered, SepPak'ed sample was recovered. This material was acidified with HCl after removal from the anaerobic chamber.

Fe(II) Concentration determination

During sampling, 0.5 ml of the slurried material (unfiltered) was pipetted into a sterile 15 ml centrifuge tube containing 4.5 ml 0.5 M HCl (Trace Metal Grade). The acid was allowed to digest the sample for at least one hour prior to analysis. 50 µl of either the HCl digested (Total) sample, or the filtered (Soluble) sample was added to 2450 µl of ferrozine reagent. Absorbance at 562 nm wavelength was immediately taken using a BioChrom UltroSpec 1100 pro spectrophotometer. Samples with extremely high Fe(II) concentrations were diluted 2X in order to fit within the calibration curve using standards of 0.0, 0.1, 0.5, 1.0, and 2.0 mg/kg Fe(II). This method is subsequently referred to as the ferrozine method.

Inductively Coupled Plasma Emission Spectroscopy (ICP-ES):

The soluble and total metal concentrations of the sub-samples were analyzed using inductively coupled plasma emission spectroscopy (ICP-ES), at the University of Ottawa Geochemistry Laboratory (http://www.earth.uottawa.ca/geochemistry-laboratory.html). Concentrations of soluble and total Al, As, Ca, Fe, K, Mg, Mn, Na, S, and Si were analyzed. The samples passed through the SepPak cartridge were also analyzed to determine soluble arsenic speciation.

A ratio of soluble metal concentration of total metal concentration (e.g., Soluble Fe / Total Fe) was calculated to track the release of metals into solution over the course of the microcosms.

Arsenic speciation

Soluble arsenic speciation was determined by ICP analysis of arsenic concentration from both the filtered sample and the sample passed through the SepPak cartridge. The cartridge separates the two arsenic species allowing for the determination of the As(III) and As(V) concentrations.

X-Ray Diffraction (XRD):

To analyse the effects of autoclaving on the mineralogy of the tailings end-members, two samples of each end-member were analyzed using X-Ray Diffraction (XRD) in the Department of Physics at the University of Ottawa (http://www.physics.uottawa.ca/services.html). One of each pair of samples was autoclaved prior to analysis to investigate the effect of the sterilization process. Samples were analyzed using a 40kV radiation at a wavelength of 1.541 Å. The scan ranged from 5 – 70^{o} at a scan speed of 1^{o}/min and a 0.02^{o} scan step.

Additional samples of remaining sediments were taken at the end of the 35 day experiments from the Goldenville Hardpan and Saturated end members and the Montague "typical" end member for XRD analysis. Samples were analyzed as above.

Carbon Concentration

40 ml duplicate subsamples of the Goldenville surface water used in the microcosms experiments was analyzed for dissolved organic carbon (DIC), dissolved inorganic carbon (DIC), total organic carbon (TOC), and total inorganic carbon (TIC). The samples for DIC/DOC were syringe filtered through a sterile 0.45 µm filter. Samples were analyzed at the G.G. Hatch laboratory in the Department of Earth Sciences at the University of Ottawa (http://www.isotope.uottawa.ca/welcome.html) on an OI Analytical "TIC-TOC" Analyser Model 1030 normalised to internal standards (St.-Jean, 2003).

Additional samples of remaining sediments were taken at the end of the 35 day experiments from the Goldenville Hardpan and Saturated end members and the Montague “typical” end-member for DOC/DIC measurements by the same method as above.

Geochemical Modelling

Saturation Indices (SI) for common and important minerals potentially forming in the microcosms were calculated using PHREEQC Interactive using the wateQ4f database (USGS, 2010).

3

Results

Gene specific PCR:

PCR specific to the *arrA* and *arsC* genes proved difficult to optimize. No single reaction recipe worked for all samples from both sites, and several issues with both contamination and failure to amplify occurred. In both cases, only one complete gel was created in which there was neither contamination nor a failure to amplify the positive control (Figs. 8 and 9). Accordingly, this approach was quickly abandoned in favour of more successful methods.

In the case of the *arrA* gene amplification, positive bands (~150-200bp) were seen for several samples from the Goldenville site (Fig. 7). From location 1 (Fig. 7), a band was seen for the samples taken at 10 cm and 25 cm depths (Fig. 8). For location 2 (Fig. 7), a band was seen for the samples taken at 20 cm and 60 cm depths (Fig. 8). A faint band was visible for location 4 at a depth of 5 cm, but this band does not appear well in the figure. The bands in each sample were not of the same length: at location 1 the bands were ~150bp, and at location 2 ~200bp (Fig. 8).

The *arsC* gene amplification revealed positive bands (~350bp) for samples from the Goldenville site, at location 1 only (Fig. 9). Signals for the ARM gene were found at the surface, at 10 cm and at 25 cm depth. In all cases the band was of the same length (~350bp).

The gene specific PCR approach was abandoned shortly after these two working gels were made. Frustrations with the optimization of the PCR recipe forced a different approach.

Figure 8: Amplification of the *arrA* arsenic reductase gene specific to DARPs.

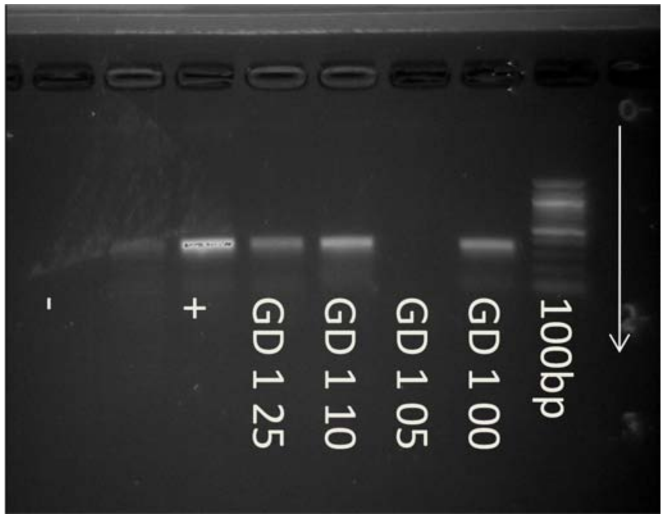

Figure 9: Amplification of the *arsC* arsenic reductase gene specific to ARMs.

T-RFLP

A total of 11 samples were analysed using Terminal Restriction Length Polymorphism (T-RFLP) for this study. These samples came from 3 locations at the Montague site (1, 3, 4) and 4 locations at the Goldenville site (2, 5, 7, 8; Figs. 6 and 7). Samples from the Montague site represent 3 of the 4 end-members: 1: Typical, 3: Hardpan, and 4: Permanently Saturated; while the samples from Goldenville represent all 4 end-members: 2: Hardpan, 5: Typical, 7: High Ca:As, 8: Permanently Saturated. Samples analysed by T-RFLP were taken from various depths in the tailings from 0 cm (surface) to 60 cm.

Forty-four unique Operational Taxanomic Units (OTUs) were identified in the samples with between 1 and 11 OTUs present in each sample (Fig. 10). The relative abundances of each OTU were used to calculate diversity and evenness in each sample (Appendix). Richness varied from 0 (1 OTU present) to 2.25. Evenness was near 1 in all samples except for MG1-40 cm which had an evenness of 1, having only 1 OTU present.

The richness (#OTUs) and diversity were strongly correlated to total arsenic, lead, and sulphur concentrations with weaker correlations to total iron, soluble iron, soluble arsenate and organic carbon (%) (Table 4).

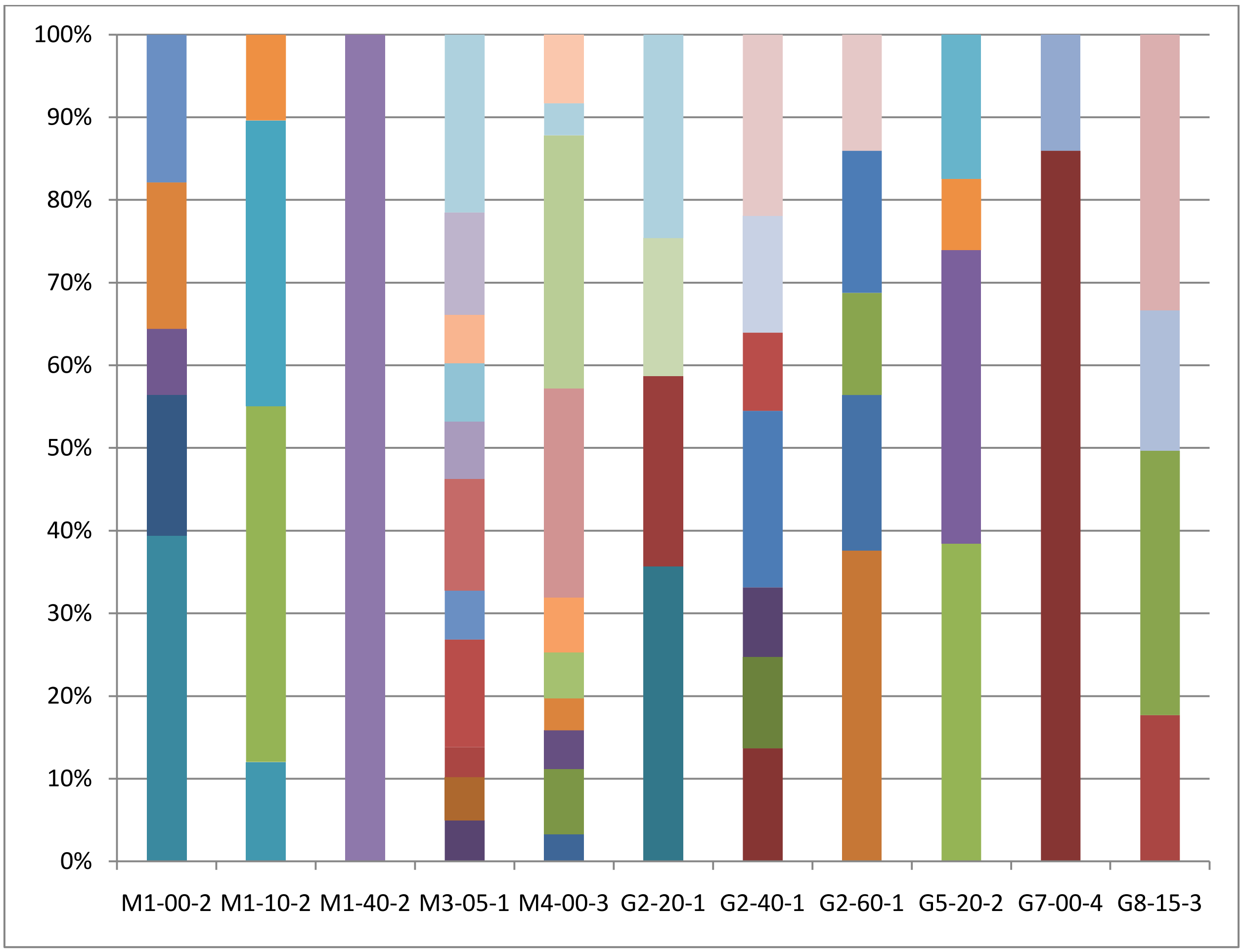

Figure 10: Bar graph showing diversity and relative abundance of bacterial communities as indicated by T-RFLP. Vertical axis indicates the relative abundance of each OTU represented by a separate coloured block.

Table 4: Linear regression analysis of T-RFLP data with geochemistry of tailings.

	Total As			Soluble As			Soluble As(III)			Soluble As(V)		
	R^2	F	p	R^2	F	p	R^2	F	p	R^2	F	p
#OTU	0.33	**4.35**	**0.00**	0.02	0.19	0.63	0.02	0.15	0.38	0.21	**2.43**	0.86
Richness	0.20	**2.31**	**0.00**	0.06	0.62	0.95	0.00	0.01	0.68	0.23	**2.75**	0.65
Evenness	0.04	0.39	**0.00**	0.09	0.92	0.77	0.04	0.42	0.83	0.12	**1.28**	0.74
	Total Fe			**Soluble Fe**			**Total Pb**			**Total S**		
	R^2	F	p	R^2	F	p	R^2	F	p	R^2	F	p
#OTU	0.29	**3.63**	0.15	0.17	**1.84**	0.82	0.36	**5.03**	**0.00**	0.28	**3.53**	**0.00**
Richness	0.14	**1.44**	**0.03**	0.16	**1.75**	0.71	0.21	**2.34**	**0.00**	0.15	**1.63**	**0.00**
Evenness	**0.00**	0.04	**0.00**	0.08	0.79	0.81	0.03	0.27	**0.00**	0.01	0.13	**0.00**
	Depth			**pH**			**Total Ca**					
	R^2	F	p	R^2	F	p	R^2	F	p			
#OTU	0.06	0.61	**0.05**	0.02	0.21	**0.00**	0.08	0.79	**0.00**			
Richness	0.02	0.18	0.13	0.01	0.13	**0.00**	0.08	0.83	**0.00**			
Evenness	0.03	0.26	0.17	0.00	0.00	**0.02**	0.01	0.08	**0.00**			
	Total Carbon			**Organic Carbon (%)**			**Inorganic Carbon (%)**					
	R^2	F	p	R^2	F	p	R^2	F	p			
#OTU	0.03	0.29	**0.00**	0.01	0.05	**0.00**	0.08	0.82	**0.00**			
Richness	0.00	0.00	**0.00**	0.02	0.17	**0.00**	0.05	0.48	**0.00**			
Evenness	0.04	0.37	**0.00**	0.12	**1.28**	**0.00**	0.00	0.04	**0.00**			

Non-metric Multidimensional Scaling (NMDS) showed that variability in the microbial communities between the end-members was larger than the variability within end-members (Fig. 11). The highest variability within an end-member was seen in the typical tailings and the hard-pan. This similarity analysis is based on the relative abundances of the 44 unique OTUs identified in the samples.

Using permutational multivariate analysis of variance using distance matrices (ADONIS function in R), the similarities of the microbial communities in these 11 samples were found to be significantly related to depth (R^2=0.1293, p=0.0348) and soluble As(V) concentration (R^2=0.1262, p=0.08458). Other relationships are shown in Table 5.

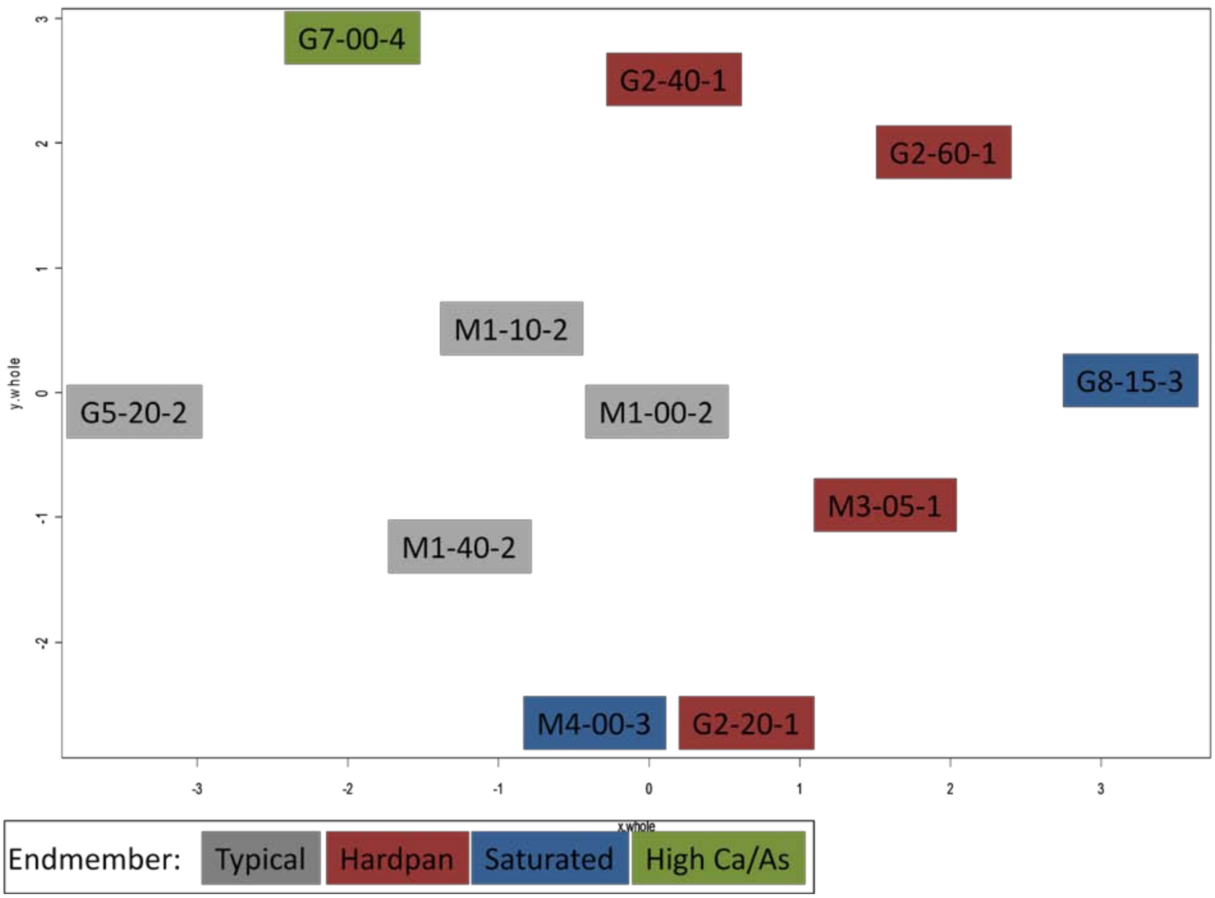

Figure 11: NMDS diagram showing similarities in microbial communities from different tailings end-members.

Table 5: Permutational multivariate analysis of variance of the similarities of microbial communities when grouped by various geochemical parameters.

Grouped by:	R^2	p
Depth	0.1209	0.1493
pH	0.1012	0.4279
Soluble As	0.1253	0.0597
Soluble Fe	0.1127	0.2189
Soluble As(III)	0.7879	1
Soluble As(V)	0.1198	0.1343
Total Carbon	0.1028	0.4279
Organic Carbon (%)	0.1114	0.1692
Inorganic Carbon (%)	0.0998	0.5323
Total As	0.0797	0.9701
Total Ca	0.1053	0.398
Total Fe	0.0787	0.9353
Total Pb	0.0829	0.9403
Total S	0.0851	0.8806

Cloning and Sequencing:

Cloning failed to produce large numbers of colonies as expected; however, several samples did yield clones for analysis (Table 6). Cloning was attempted on all end-members from both sites, but proved inefficient at the PCR, transformation and ligation steps.

After sorting out non-aligning sequences using BiotEdit, only the clones from locations 3 and 5 at Montague and 7 at Goldenville were used for phylogenetic analysis. When looking at clones from all these locations, 42% of clones belonged to the *Geobacteraceae* family, known metal reducing bacteria. Other clones belonged to the *Acidimicrobiaceae* (16%), *Bacteroidetes incertae sedis* (11%), *Methylobacteriaceae* (10%), *Acetobacteraceae* (5%) and *Schewanella* (5%) families (Fig 12). 10% of the clones were not able to be identified using the RDP SeqMatch program.

All of the clones from the *Geobacteraceae* family were found in the Goldenville location 7 sample from the High Ca:As end-member, where they accounted for 80% of the clones (Fig 12). The other 20% of these clones were the members of the *Bacteriodetes incertae sedis* family (Fig. 12).

All the members of the *Methylobacteriaceae* family were found in the Montague location 2 sample from the “Typical” tailings end-member, where they accounted for 67% of the clones (Fig. 12). The other 33% of clones were from the *Acidimicrobiaceae* family (Fig. 12).

The High Ca:As from the Montague site (M5) contained the *Proteobacterium Schewanella putrefaciens*, as well as members of the *actinobacter* group, which were also found in the typical tailings sample from Montague (Fig. 12).

A phylogenetic tree of these clones was created using the RDP Tree Builder program (Fig. 12). Various known and described species from each of the identified families were used to group the clones into their taxanomic locations. *Aquifex pyrophilus* was used as an out-group to root the tree.

Table 6: Number of clones recovered for each tailings end-member from both sites.

Site	(Location #)	End-Member	Number of clones
Montague	(3)	Hardpan	7
		Typical	X
	(5)	Saturated	10
		High Ca:As	X
Goldenville		Hardpan	X
	(5)	Typical	7
	(8)	Saturated	1
	(7)	High Ca:As	12

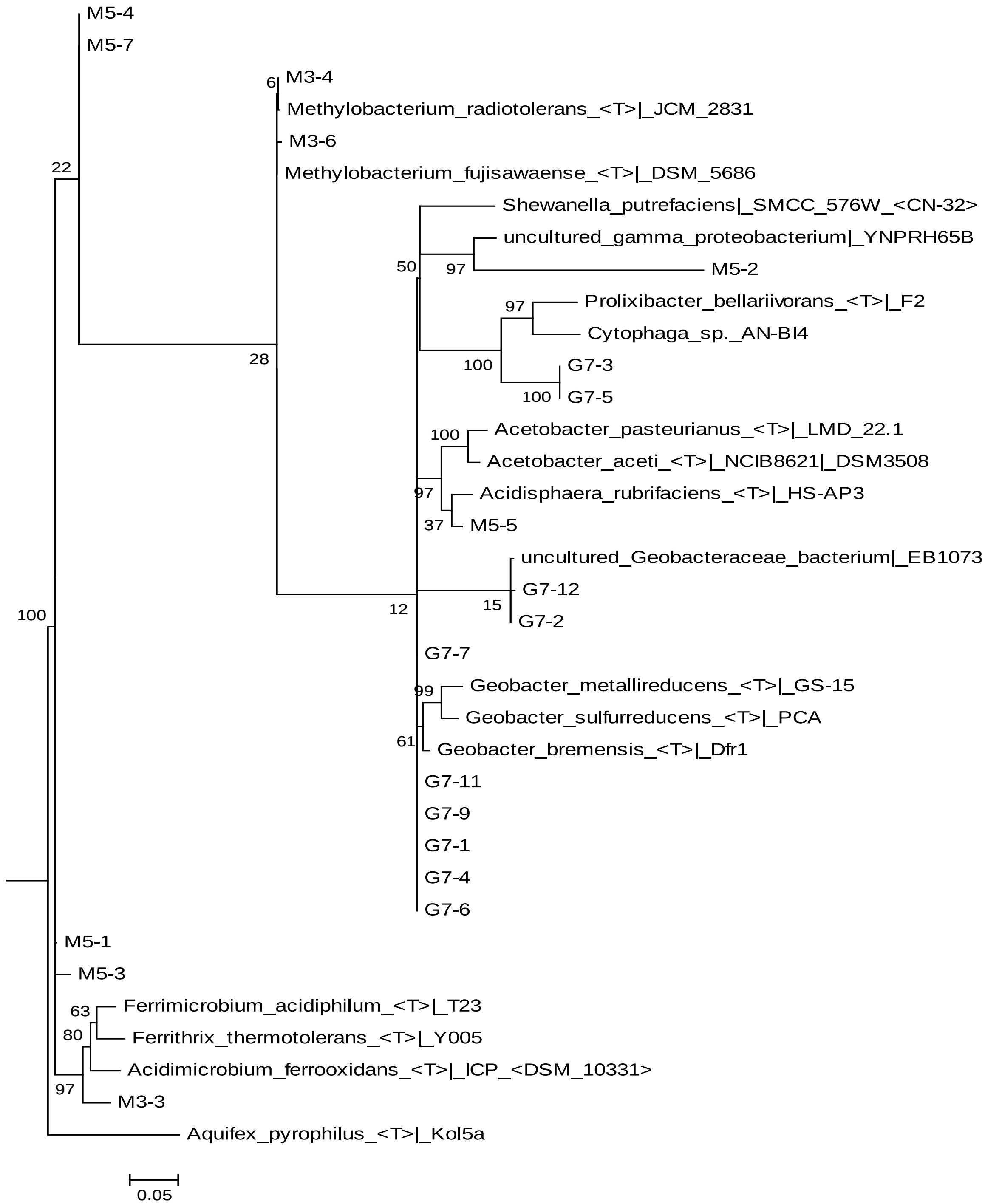

Figure 12: Phylogenetic tree of bacteria cloned from tailings samples compared to closest known bacteria species. *Aquifex pyrophilus*is used as an outgroup. Numbers on nodes are bootstrap values based on 100 replicates. Scale bar indicates an estimate of the number of base changes per nucleotide sequence position.

Microcosms:

XRD

X-Ray Diffraction scans of tailings before and after autoclaving showed that little mineralogical changes occurred during the autoclaving (Fig. 13). In all samples, the main mineralogy of the tailings was quartz, mica minerals and feldspar (Fig. 13).

Carbon Concentration

The total organic carbon concentration for the Goldenville site water (used in all Goldenville microcosms) was 20.8 ppm , which is normal for wetlands and headland streams (Wetzel, 2001; Table 7). All of the organic carbon was dissolved, while 90% of the inorganic carbon was dissolved. The inorganic carbon concentrations (Total=1.0 ppm, Dissolved=0.9 ppm) were slightly lower than normal for wetlands and headland streams (Wetzel, 2001).

Table 7: Carbon concentrations in sample water used in microcosm construction.

TOC (ppm)	TIC (ppm)	DOC (ppm)	DIC (ppm)
20.8	1.0	20.8	0.9

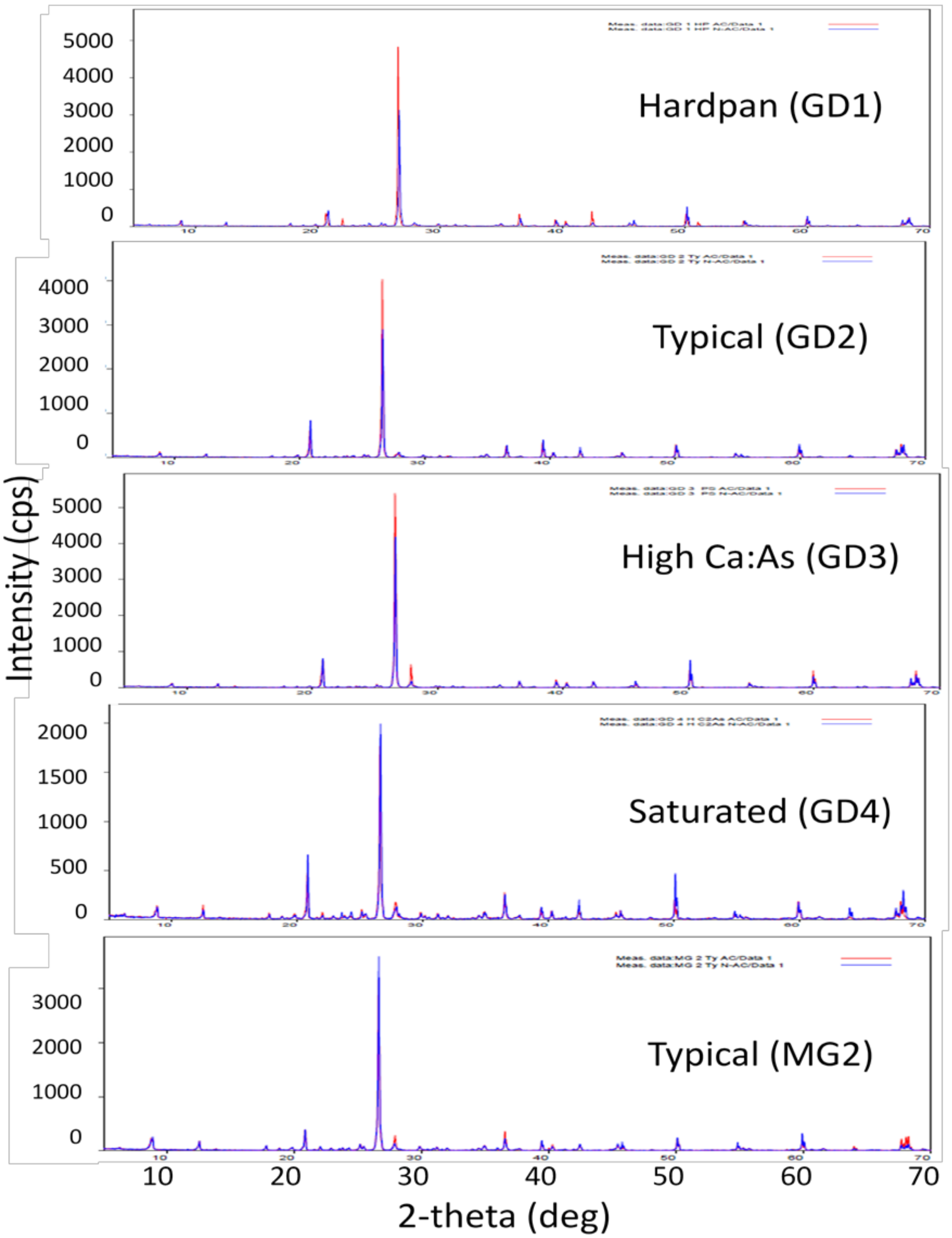

Figure 13: XRD spectra of tailings end-members prior to autoclaving (blue) and following autoclaving (red). Samples were analyzed using 40 kV radiation at a wavelength of 1.541 Å. The scan ranged from 5 – 70^{o} at a scan speed of 1^{o}/min and a 0.02^{o} scan step.

pH:

There was little change in pH throughout any of the microcosm experiments (Fig. 14). pH of microcosm waters remained neutral to sub-neutral in all end-members, except in the Hard-Pan end-member which was acidic (Fig. 14).

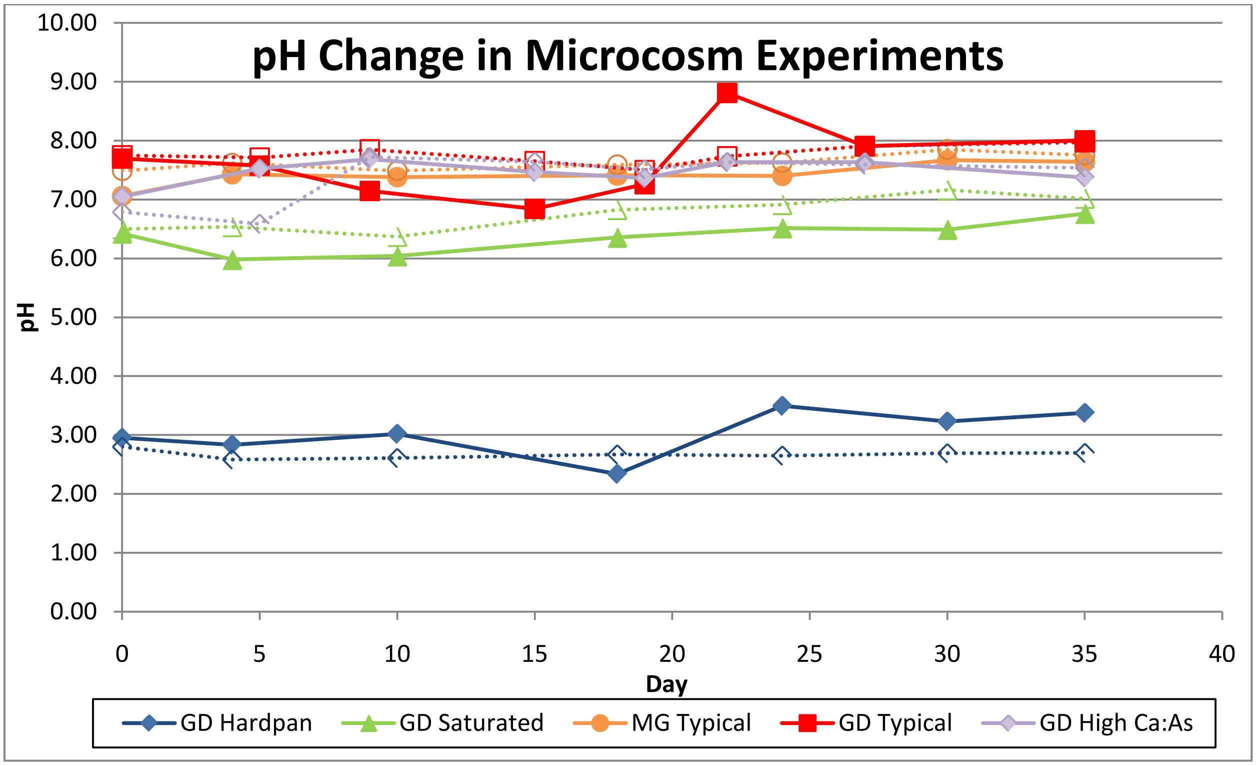

Figure 14: Changes in pH of micocosm waters over course of the experiments. Solid line represent experimental microcosms, while dotted lines indicate control microcosms.

Metal Concentrations

Using SepPak cartridges and the ferrozine assay, the speciation of both Fe and As were monitored through the course of the microcosms. The ratio of reduced metal to oxidised metal in the samples (e.g., Fe(II)/Fe(III)) is referred to as reduction, while the ratio of soluble metal to total metal (measured by ICP using 0.45µm filtered sample) is referred to as release.

Hardpan:

Metal concentrations in the Hardpan microcosms were highest amongst all experiments, with iron (Fe) and arsenic (As) concentrations as high as 4040 ppm and 3970 ppm respectively.

Total Fe(II) concentrations (i.e., soluble and particulate) in the experimental microcosm rose from 68.6 ppm to 4250 ppm over the 35 day experiment, while the control remained steady at an average of 158 ppm (Appendix, Fig 15). A similar pattern was seen in the concentrations of total soluble Fe and soluble Fe(II); the experimental microcosms saw an increase over the 35 days while the controls remained steady. The ratio of Total Fe(II) to Total Fe rose to 1 by day 25, while the proportion of soluble iron rose from near 0 to over 0.2 over the 35 days; in both cases, the control ratios remained steady near zero. Although the experimental Soluble As(III) : Total Soluble As ration rose over the 35 days to near 0.5, the control ration was steady near 0.5 throughout the experiment (Fig. 15).

There was also an increase in the proportion of soluble sulphur (sulphur release) over the first 10 days of the experiment. This increase in the soluble sulphur fraction was correlated significantly with the release of Fe (R^2=0.99, F=374, p<0.05), the increase in total Fe(II) (R^2=0.89, F=24.2, p<0.05), and the release of As (R^2=0.96, F=81.8, p<0.05, Table 8). Additionally, there was a smaller increase in soluble Si in the experimental system which was correlated to the increase in soluble Fe (R^2=0.96, F=261, p<0.05), and soluble As (R^2=0.90, F=97.9, p<0.05, Table 8).

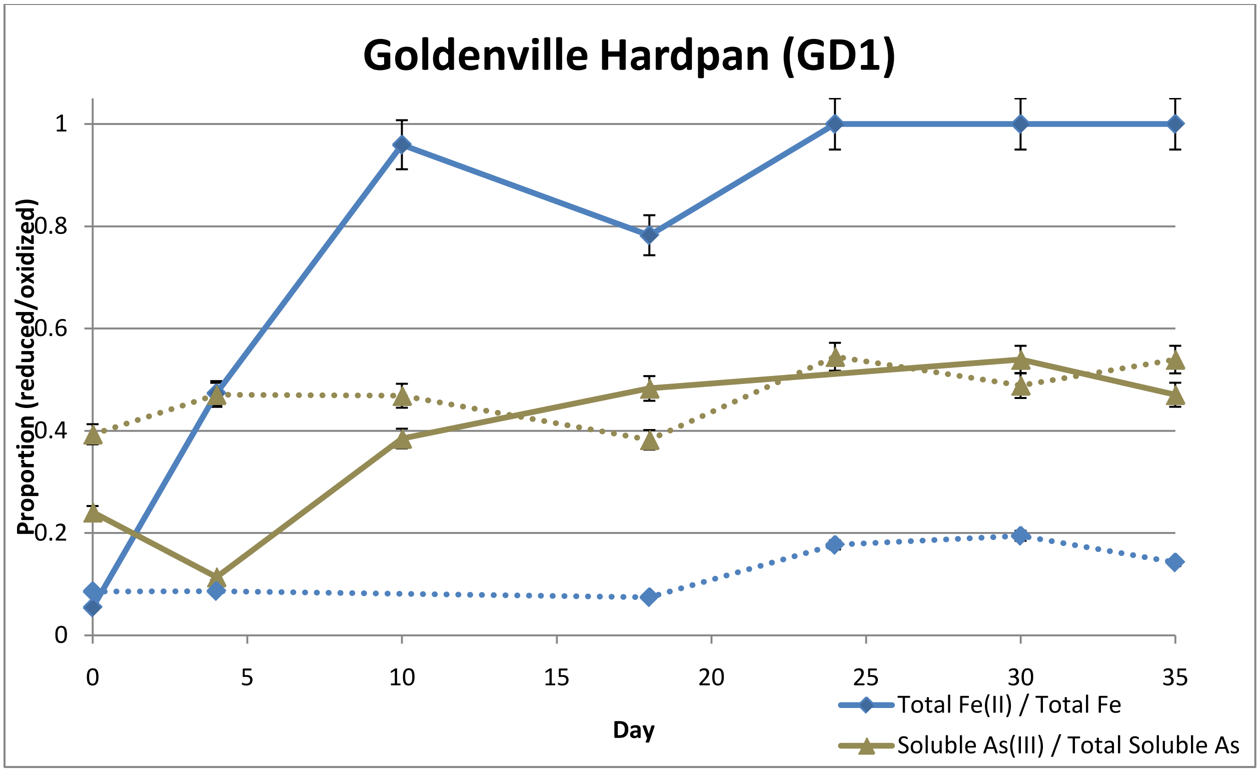

Figure 15: Changes in iron and arsenic speciation during the microcosm experiment for the Hardpan end-member, Goldenville (GD1). Changes in the control experiment are shown by the dotted line.

Table 8: Relationship of various geochemical changes during the microcosm experiment with the Hardpan end-member, Goldenville (GD1).

GD1	Soluble Fe(II)/ Total Soluble Fe	Total Fe(II)/ Total Fe	Soluble As(III)/ Total Soluble As	Soluble As/ Total As	Soluble Fe/ Total Fe	Soluble S/ Total S	Soluble Ca/ Total Ca	Soluble Si/ Total Si	Soluble Al/ Total Al	Soluble K/ Total K	Soluble Mg/ Total Mg	Soluble Mn/ Total Mn	Soluble Na/ Total Na
Soluble Fe(II)/ Total Soluble Fe	1.00												
Total Fe(II)/Total Fe	0.68	1.00											
Soluble As(III)/ Total Soluble As	0.07	0.22	1.00										
Soluble As/ Total As	0.32	0.62	0.32	1.00									
Soluble Fe/ Total Fe	0.38	0.71	0.30	**0.89**	1.00								
Soluble S/ Total S	0.68	**0.89**	0.09	**0.96**	**0.99**	1.00							
Soluble Ca/ Total Ca	0.26	0.16	0.03	0.01	0.02	0.57	1.00						
Soluble Si/ Total Si	0.33	0.68	0.22	**0.90**	**0.96**	**0.92**	0.00	1.00					
Soluble Al/ Total Al	0.14	0.33	0.25	0.28	0.38	0.65	0.30	0.29	1.00				
Soluble K/ Total K	0.21	0.28	0.03	0.11	0.13	0.12	0.36	0.11	0.35	1.00			
Soluble Mg/ Total Mg	0.03	0.02	0.00	0.01	0.00	0.54	0.44	0.00	0.57	0.39	1.00		
Soluble Mn/ Total Mn	0.16	0.06	0.01	0.03	0.00	0.28	0.62	0.02	0.36	0.38	**0.78**	1.00	
Soluble Na/ Total Na	0.32	0.22	0.04	0.02	0.08	0.34	0.71	0.02	0.30	0.23	0.38	0.47	1.00

Saturated:

There was very little change in the oxidation states of iron and arsenic throughout the course of the microcosm experiment with the saturated end-member (Fig. 16). The Fe(II):Total Fe and As(III):Total As ratios remained near 1 from day 0 to 35 (Fig. 16). There was no significant increase in the soluble Fe concentration or the reduced metal species. There was a small increase in the Soluble As : Total As ratio, from ~0.3 to ~0.4. Although there was little change, the concentrations of soluble Fe(II) and soluble As(III) were correlated (R^2=0.88, F=61.2, $p<0.05$, Table 9).

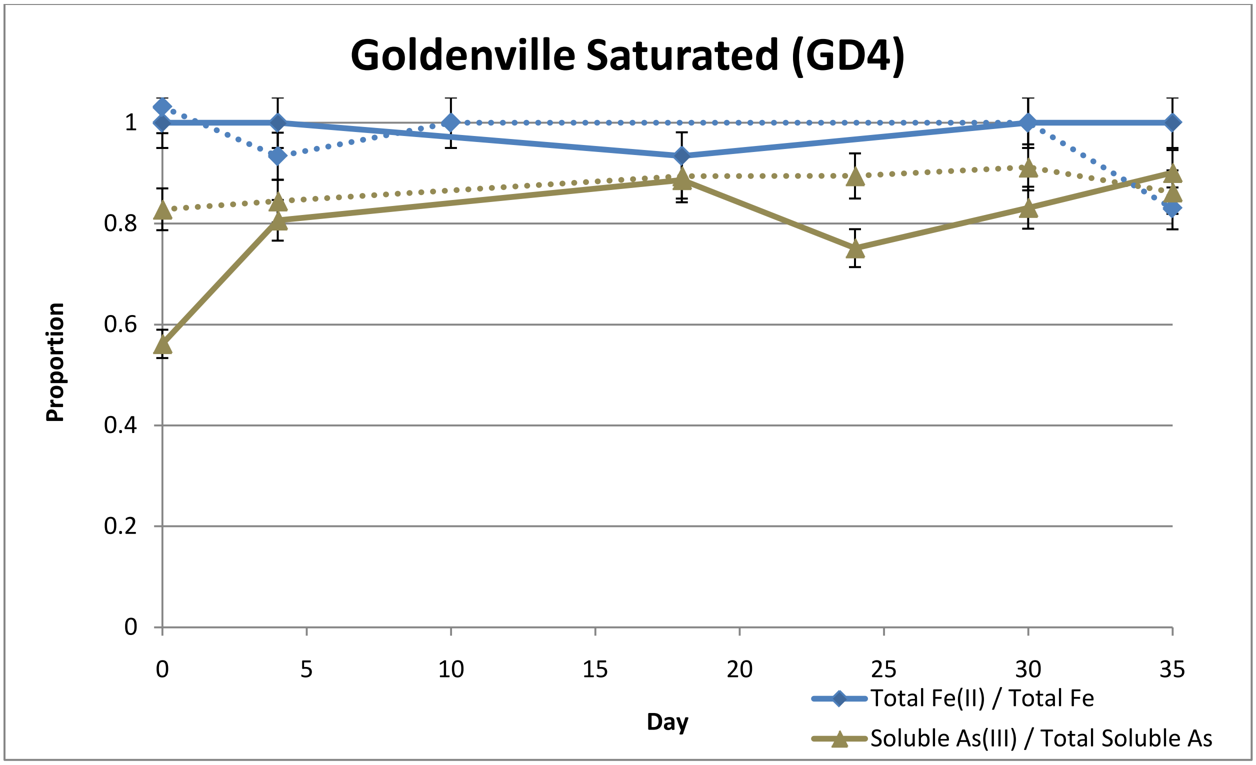

Figure 16: Changes in iron and arsenic speciation during the microcosm experiment for the Saturated end-member, Goldenville (GD4). Changes in the control experiment are shown by the dotted line.

Table 9: Relationship of various geochemical changes during the microcosm experiment with the Saturated end-member, Goldenville (GD4).

GD4	Soluble Fe(II)/ Total Soluble Fe	Total Fe(II)/ Total Fe	Soluble As(III)/ Total Soluble As	Soluble As/ Total As	Soluble Fe/ Total Fe	Soluble S/ Total S	Soluble Ca/ Total Ca	Soluble Si/ Total Si	Soluble Al/ Total Al	Soluble K/ Total K	Soluble Mg/ Total Mg	Soluble Mn/ Total Mn	Soluble Na/ Total Na
Soluble Fe(II)/ Total Soluble Fe	1.00												
Total Fe(II)/Total Fe	0.11	1.00											
Soluble As(III)/ Total Soluble As	**0.88**	0.08	1.00										
Soluble As/ Total As	0.18	0.01	0.40	1.00									
Soluble Fe/ Total Fe	0.07	0.01	0.15	0.59	1.00								
Soluble S/ Total S	0.26	0.06	0.35	0.15	0.06	1.00							
Soluble Ca/ Total Ca	0.00	0.02	0.00	0.25	0.59	0.18	1.00						
Soluble Si/ Total Si	0.20	0.12	0.13	0.03	0.36	0.58	0.66	1.00					
Soluble Al/ Total Al	0.18	0.06	0.16	0.01	0.16	0.71	0.29	**0.77**	1.00				
Soluble K/ Total K	0.29	0.11	0.55	0.17	0.00	0.68	0.29	0.52	0.43	1.00			
Soluble Mg/ Total Mg	0.01	0.02	0.03	0.27	**0.77**	0.27	0.66	0.69	0.50	0.10	1.00		
Soluble Mn/ Total Mn	0.06	0.09	0.02	0.13	0.56	0.44	0.70	**0.94**	0.69	0.31	**0.88**	1.00	
Soluble Na/ Total Na	0.00	0.24	0.02	0.09	0.10	0.01	0.42	0.25	0.02	0.20	0.10	0.22	1.00

High Ca:As:

The High Ca:As end-member experimental microcosms showed the most dramatic increase in the soluble As(III) to total soluble As ration (Fig 17). This value rose from below 0.2 to above 0.6 in the experimental system while dropping to near 0 in the control (Fig 17). This represents an increase from 0.02 ppm soluble As(III) at day 0 to 1.65 ppm at day 35. A similar pattern in the reduction of Fe was not determined as the iron concentration data became unreliable after day 20. However, the experimental system showed evidence of iron reduction not seen in the control (Fig. 17), and the soluble As concentration was correlated to soluble Fe (R^2=0.67, F=26.8, p<0.05, Table 10).

The calcium in the system was found more in the soluble fraction near the end of the 35 days than at the beginning (Appendix). This increase was weakly correlated to the increase in the soluble As fraction (R^2=0.46, F=11.2, p=0.09). Almost all the sulphur in the system was found in the soluble fraction, unlike most of the other experiments (Appendix).

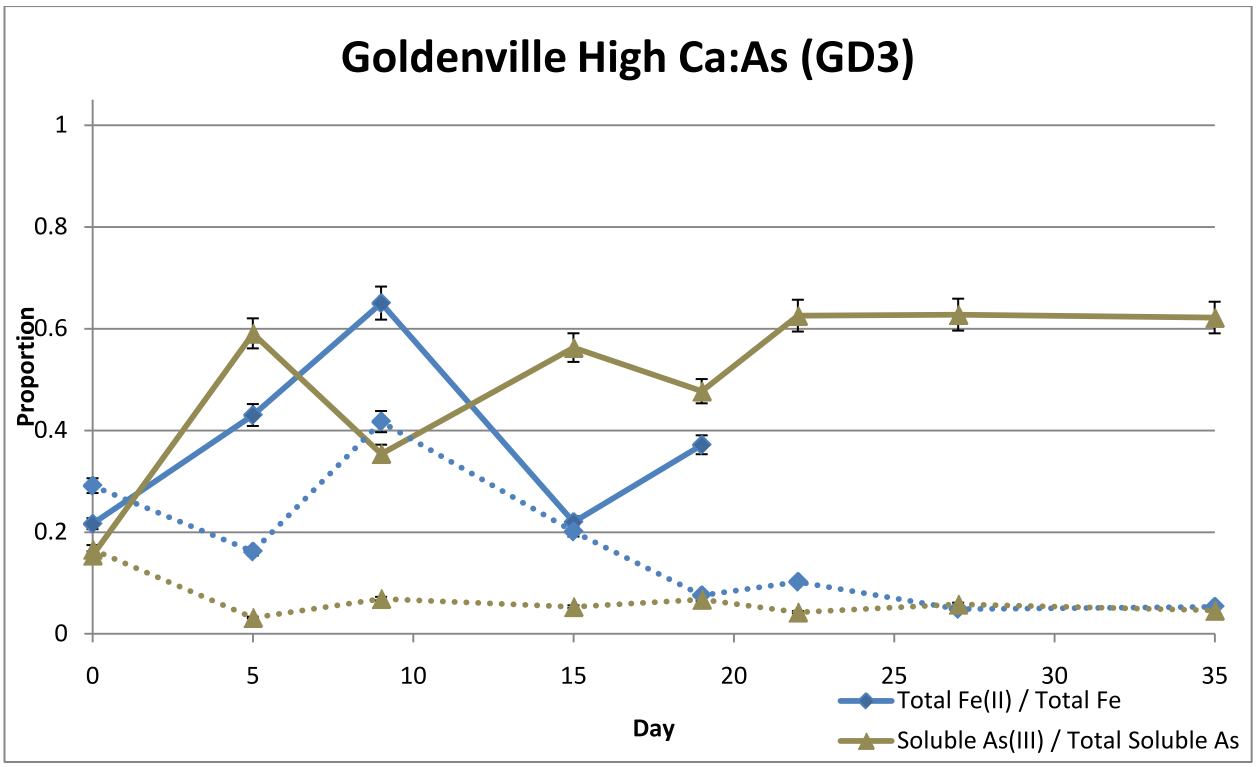

Figure 17: Changes in iron and arsenic speciation during the microcosm experiment for the High Ca:As end-member, Goldenville (GD3). Changes in the control experiment are shown by the dotted line.

Table 10: Relationship of various geochemical changes during the microcosm experiment with the High Ca:As end-member, Goldenville (GD3).

GD3	Soluble Fe(II)/ Total Soluble Fe	Total Fe(II)/ Total Fe	Soluble As(III)/ Total Soluble As	Soluble As/ Total As	Soluble Fe/ Total Fe	Soluble S/ Total S	Soluble Ca/ Total Ca	Soluble Si/ Total Si	Soluble Al/ Total Al	Soluble K/ Total K	Soluble Mg/ Total Mg	Soluble Mn/ Total Mn	Soluble Na/ Total Na
Soluble Fe(II)/ Total Soluble Fe	1.00												
Total Fe(II)/Total Fe	0.31	1.00											
Soluble As(III)/ Total Soluble As	0.40	0.37	1.00										
Soluble As/ Total As	0.11	0.43	0.00	1.00									
Soluble Fe/ Total Fe	0.01	0.07	0.17	0.67	1.00								
Soluble S/ Total S	0.00	0.08	0.13	0.06	0.01	1.00							
Soluble Ca/ Total Ca	0.14	0.55	0.02	0.46	0.17	0.04	1.00						
Soluble Si/ Total Si	0.02	0.00	0.14	0.18	0.45	0.02	0.00	1.00					
Soluble Al/ Total Al	0.01	0.06	0.18	0.67	**1.00**	0.01	0.16	0.45	1.00				
Soluble K/ Total K	0.17	0.42	0.00	0.52	0.25	0.03	**0.90**	0.00	0.25	1.00			
Soluble Mg/ Total Mg	0.32	0.54	0.06	0.60	0.40	0.03	0.62	0.08	0.40	0.54	1.00		
Soluble Mn/ Total Mn	0.08	0.28	0.04	0.67	0.61	0.04	0.70	0.08	0.61	**0.82**	0.59	1.00	
Soluble Na/ Total Na	0.08	0.05	0.00	0.00	0.06	0.00	0.12	0.01	0.07	0.04	0.03	0.00	1.00

“Typical” Tailings:

The Goldenville and Montague “Typical” tailings microcosms behaved similarly. In both systems, the Fe(III) : Total Fe ratio increased through the first half of the experiment, then decreased for the second half of the experiment, while the control systems did not rise above 0.6 and dropped to close to 0 for the second half of the experiment (Figs. 18 and 19). This trend was more pronounced in the Goldenville sample as the ratio began at ~0.2, while the Montague sample began ~0.8 (Figs. 18 and 19).

There was little release of other soluble metals in the systems, and there were no significant increases in soluble or reduced Fe concentrations in either typical sample. There was an increase in soluble As(III) in the Montague sample, a trend not seen in the Goldenville sample (Fig. 18 and 19). The soluble As(III) in the Montague experiment increased from 0.04 ppm at day 0 to 0.41 ppm at day 35, while the control remained under 0.10 ppm (Fig. 19). This trend was not seen in the Goldenville end-member where soluble As(III) concentration in both the experimental and control systems remained under 0.10ppm (Fig. 18).

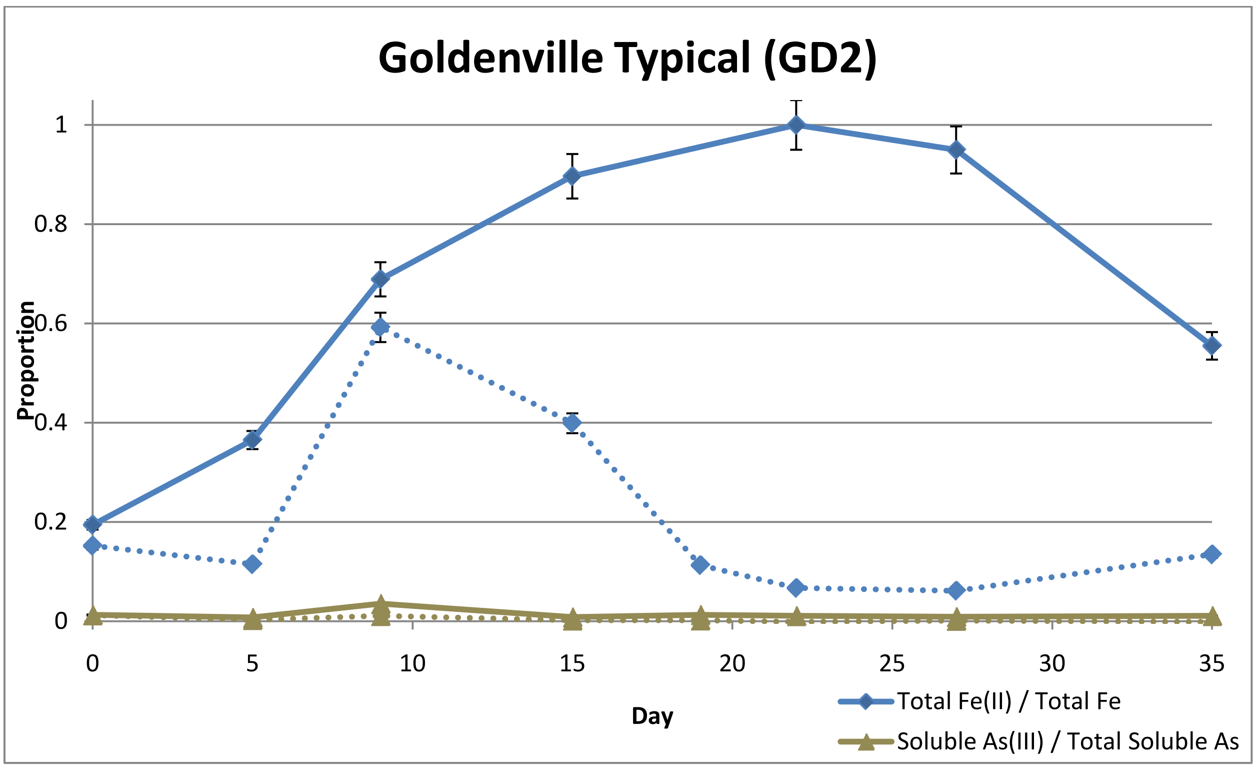

Figure 18: Changes in iron and arsenic speciation during the microcosm experiment for the "Typical" end-member, Goldenville (GD2). Changes in the control experiment are shown by the dotted line.

Table 11: Relationship of various geochemical changes during the microcosm experiment with the "Typical" end-member, Goldenville (GD2).

GD2	Soluble Fe(II)/ Total Soluble Fe	Total Fe(II)/ Total Fe	Soluble As(III)/ Total Soluble As	Soluble As/ Total As	Soluble Fe/ Total Fe	Soluble S/ Total S	Soluble Ca/ Total Ca	Soluble Si/ Total Si	Soluble Al/ Total Al	Soluble K/ Total K	Soluble Mg/ Total Mg	Soluble Mn/ Total Mn	Soluble Na/ Total Na
Soluble Fe(II)/ Total Soluble Fe	1.00												
Total Fe(II)/Total Fe	0.22	1.00											
Soluble As(III)/ Total Soluble As	0.02	0.01	1.00										
Soluble As/ Total As	0.07	0.09	0.05	1.00									
Soluble Fe/ Total Fe	0.05	0.00	0.08	**0.76**	1.00								
Soluble S/ Total S	0.04	0.09	0.02	0.30	0.09	1.00							
Soluble Ca/ Total Ca	0.00	0.44	0.01	0.51	0.10	0.30	1.00						
Soluble Si/ Total Si	0.20	0.04	0.05	0.68	**0.84**	0.11	0.05	1.00					
Soluble Al/ Total Al	0.03	0.00	0.08	**0.76**		0.09	0.12	**0.81**	1.00				
Soluble K/ Total K	0.02	0.29	0.02	0.60	0.21	0.45	**0.89**	0.13	0.22	1.00			
Soluble Mg/ Total Mg	0.00	0.11	0.09	**0.79**	**0.77**	0.20	0.38	0.49	**0.80**	0.54	1.00		
Soluble Mn/ Total Mn	0.01	0.25	0.04	**0.88**	0.50	0.40	**0.76**	0.38	0.53	**0.86**	**0.78**	1.00	
Soluble Na/ Total Na	0.01	0.05	0.00	0.00	0.06	0.00	0.14	0.08	0.06	0.12	0.00	0.01	1.00

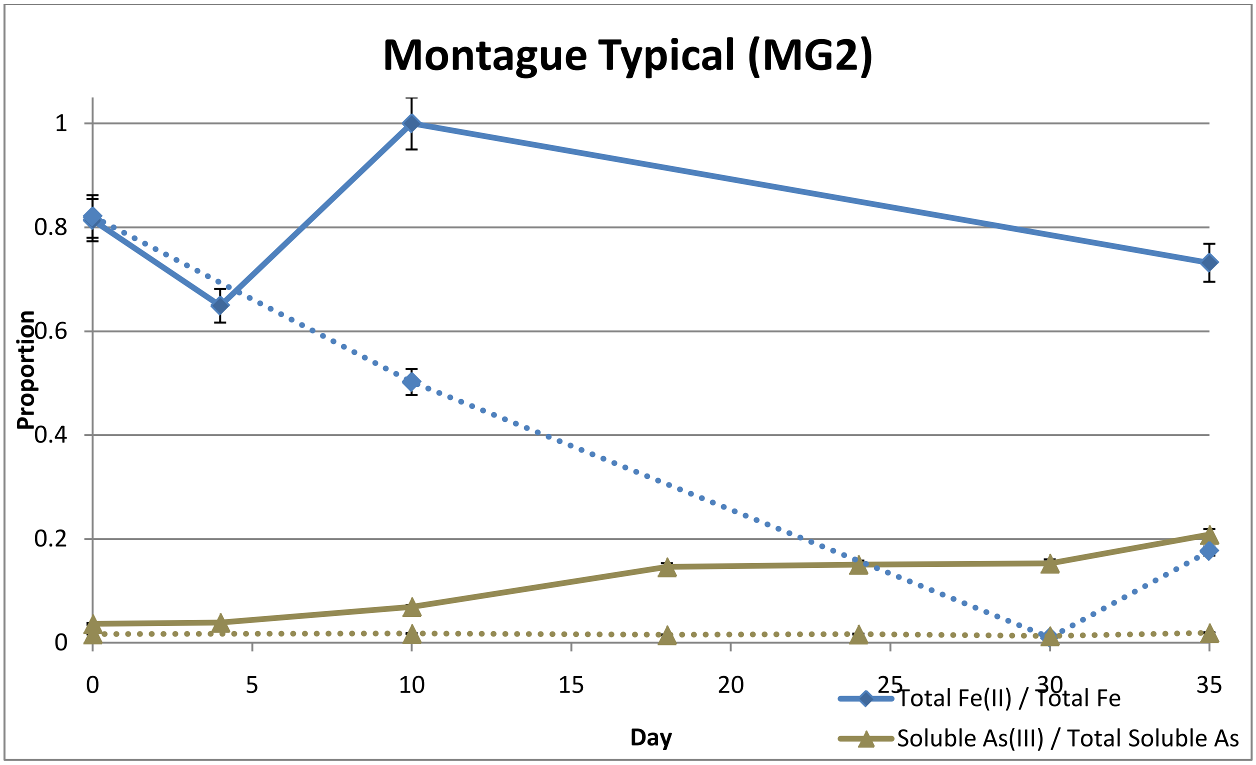

Figure 19: Changes in iron and arsenic speciation during the microcosm experiment for the "Typical" end-member, Goldenville (MG2). Changes in the control experiment are shown by the dotted line.

Table 12: Relationship of various geochemical changes during the microcosm experiment with the "Typical" end-member, Montague (MG2).

MG2	Soluble Fe(II)/ Total Soluble Fe	Total Fe(II)/ Total Fe	Soluble As(III)/ Total Soluble As	Soluble As/ Total As	Soluble Fe/ Total Fe	Soluble S/ Total S	Soluble Ca/ Total Ca	Soluble Si/ Total Si	Soluble Al/ Total Al	Soluble K/ Total K	Soluble Mg/ Total Mg	Soluble Mn/ Total Mn	Soluble Na/ Total Na
Soluble Fe(II)/ Total Soluble Fe	1.00												
Total Fe(II)/Total Fe	0.32	1.00											
Soluble As(III)/ Total Soluble As	0.33	0.03	1.00										
Soluble As/ Total As	0.21	0.00	0.10	1.00									
Soluble Fe/ Total Fe	**0.80**	0.10	0.15	0.02	1.00								
Soluble S/ Total S	0.12	0.04	0.16	**0.83**	0.02	1.00							
Soluble Ca/ Total Ca	0.14	0.02	0.02	**0.84**	0.00	**0.81**	1.00						
Soluble Si/ Total Si	0.11	0.02	0.02	0.64	0.01	0.58	0.60	1.00					
Soluble Al/ Total Al	0.00	0.24	0.18	0.14	0.05	0.10	0.12	0.42	1.00				
Soluble K/ Total K	0.08	0.10	0.01	0.63	0.00	0.61	**0.87**	0.41	0.07	1.00			
Soluble Mg/ Total Mg	0.34	0.04	0.26	**0.84**	0.04	0.65	0.74	0.27	0.01	0.65	1.00		
Soluble Mn/ Total Mn	0.23	0.03	0.08	**0.96**	0.02	**0.89**	**0.87**	0.73	0.20	0.62	0.73	1.00	
Soluble Na/ Total Na	0.00	0.02	0.19	0.00	0.00	0.04	0.05	0.14	0.14	0.09	0.02	0.01	1.00

Geochemical Modelling:

The chemical conditions of the aqueous phase from each sampling date and for each microcosm were used to determine selected mineral saturation indices using PHREEQC. All of the microcosms were found to be under saturated with respect to several arsenic-rich minerals, but some systems were saturated with respect to scorodite ($FeAsO_4.2H_2O$) and $Mn_3(AsO_4)_2.8H_2O$ (Tables 12 - 17). Iron bearing minerals goethite and hematite were also predicted to form in the various microcosms (Tables 12 - 17), but pyrite was not.

Table 13: Trends in saturation indices of important minerals during the microcosm experiment for the Hardpan end-member, Goldenville (GD1).

Hardpan	GD1	Day						
Mineral	Formula	0	4	10	18	24	30	35
Native As	As	-11.97	-11.48	-11.1	-8.54	-11.92	-11.09	-11.57
	As_2O_5	-16.18	-14.97	-14.83	-13.18	-14.99	-14.56	-14.69
	As_2S_3	-52.01	-47.96	-51.42				
Orpiment	As_2O_3	-50.61	-46.56	-50.02				
Arsenolite	As_2O_3	-5.92	-5.64	-3.76	-2.73	-2.55	-2.49	-2.58
Claudetite	A_s2O_5	-5.96	-5.68	-3.8	-2.77	-2.59	-2.53	-2.62
Realgar	AsS	-20.52	-19.01	-20.04				
Scorodite	$FeAsO_4.2H_2O$	-1.67	-1.36	-2.98	-2.7	-1.33	-1.51	0.71
	$Mn_3(AsO_4)_2.8H_2O$	-14.42	-13.89	-12.64	-14.55	-9.4	-10.59	-9.68
	$Ca_3(AsO_4)_2.4H_2O$	-21.26	-21.08	-20.05	-22.03	-17.05	-18.3	-17.51
Mansfieldite	$AlAsO_4.2H_2O$	-5.23	-4.95	-4.4	-5.06	-2.57	-5.56	-5.07
Pyrite	FeS_2	-25.46	-21.53	-23.92				
Hematite	Fe_2O_3	10.34	9.74	5.78	4.25	9.16	8.23	12.16
Goethite	α–FeOOH	4.16	3.87	1.88	1.11	3.57	3.1	5.07

Table 14: Trends in saturation indices of important minerals during the microcosm experiment for the "Typical" end-member, Goldenville (GD2).

Typical	GD2	Day							
Mineral	Formula	0	5	9	15	19	22	27	35
Native As	As	-27.1	-26.91	-25.01	-24.68	-25.77	-30.61	-27.92	-28.05
	As_2O_5	-26.06	-25.8	-24.31	-22.88	-24.67	-30.58	-27.01	-27.37
	As_2S_3	-198.09	-194.98	-180.87	-172.86	-185.05	-231.95	-204.96	-207.62
Orpiment	As_2O_3	-196.69	-193.58	-179.47	-171.46	-183.64	-230.55	-203.56	-206.22
Arsenolite	As_2O_3	-7.69	-8.03	-6.81	-8.01	-7.67	-8.05	-8.06	-7.73
Claudetite	A_s2O_5	-7.73	-8.07	-6.85	-8.05	-7.71	-8.09	-8.1	-7.77
Realgar	AsS	-74.27	-73.17	-67.83	-65.05	-69.47	-86.72	-76.83	-77.76
Scorodite	$FeAsO_4.2H_2O$	0.48	-1.59	-0.53	*1.93*	-0.19		-2.17	
	$Mn_3(AsO_4)_2.8H_2O$	*1.6*	*1.24*	*0.37*	*1.48*	*1.65*	*5.02*	*3.24*	*3.61*
	$Ca_3(AsO_4)2.4H_2O$	-1.49	-1.55	-2.65	-3.01	-2.26	1.16	-0.66	-0.34
Mansfieldite	$AlAsO_4.2H_2O$	-2.65	-3.77	-2.61	0.21	-2.6	-7.21	-4.41	-4.81
Pyrite	FeS_2	-102.37					-120.47		-107.08
Hematite	Fe_2O_3	24.53	20.12	20.76	24.26	21.79		20.18	
Goethite	α–FeOOH	11.26	9.05	9.38	11.12	9.89		9.08	

Table 15: Trends in saturation indices of important minerals during the microcosm experiment for the High Ca:As end-member, Goldenville (GD3).

High Ca:As	GD3	Day							
Mineral	Formula	0	5	9	15	19	22	27	35
Native As	As	-25.31	-25.79	-26.15	-25.28	-25.11	-24.9	-25.09	-25.06
	As_2O_5	-26.53	-28.34	-27.27	-27.68	-27.07	-27.06	-27.11	-27.13
	As_2S_3	-177.14	-189.72	-194.34	-187.37	-184.68	-184.68	-188.11	-189.47
Orpiment	As_2O_3	-175.74	-188.32	-192.94	-185.97	-183.28	-183.27	-186.71	-188.07
Arsenolite	As_2O_3	-8	-6.11	-5.87	-5.45	-5.71	-5.24	-4.89	-4.57
Claudetite	A_s2O_5	-8.04	-6.15	-5.91	-5.49	-5.75	-5.28	-4.93	-4.61
Realgar	AsS	-66.68	-71.03	-72.7	-70.08	-69.13	-69.06	-70.27	-70.71
Scorodite	$FeAsO_4.2H_2O$		-3.47	-2.22	-2.12	-1.84		-1.83	-1.67
	$Mn_3(AsO_4)_2.8H_2O$	-6.42		*4.35*	*2.76*	*2.95*	*3.06*	*3.76*	*3.85*
	$Ca_3(AsO_4)_2.4H_2O$	-5.11	-3.85	-1.79	-3.5	-3.41	-3.19	-2.58	-2.36
Mansfieldite	$AlAsO_4.2H_2O$	-3.95		-2.75	-4.32	-3.92	-4.53	-4.67	-4.72
Pyrite	FeS_2	-91.34					-69.47		
Hematite	Fe_2O_3		18.89	20.34	20.95	20.9		20.96	21.31
Goethite	α–FeOOH			9.17	9.47	9.45		9.48	9.65

Table 16: Trends in saturation indices of important minerals during the microcosm experiment for the Saturated end-member, Goldenville (GD4).

Saturated	GD4	Day						
Mineral	Formula	0	4	10	18	24	30	35
Native As	As	-20.99	-19.26		-20.18	-20.72	-20.6	-22.37
	As_2O_5	-22.86	-20.88		-21.89	-21.84	-22.24	-23.16
	As_2S_3	-155.6	-141.71		-153.92	-158.78	-157.83	-165.95
Orpiment	As_2O_3	-154.2	-140.31		-153.89	-157.38	-153.43	
Arsenolite	As_2O_3	-3.11	-2.32		-1.89	-2	-1.95	-1.86
Claudetite	A_s2O_5	-3.15	-2.36		-1.93	-2.04	-1.99	-1.9
Realgar	AsS	-58.06	-52.86		-57.67	-59.03	-58.68	-61.64
Scorodite	$FeAsO_4.2H_2O$	0.66	1.41		1.16	1.22	1.64	1.76
	$Mn_3(AsO_4)_2.8H_2O$	-0.56	-0.8		0.48	1.23	0.36	1.16
	$Ca_3(AsO_4)_2.4H_2O$	-5.8	-6.12		-4.68	-3.75	-4.46	-3.76
Mansfieldite	$AlAsO_4.2H_2O$	-0.97	-0.38		-0.89	-0.9	-0.91	-1.53
Pyrite	FeS_2	-81.56	-74.19	-74.73	-80.92	-83.82	-83.13	-87.53
Hematite	Fe_2O_3	21.68	21.2	19.75	21.82	21.78	23.1	24.34
Goethite	α–FeOOH	9.84	9.6	8.87	9.9	9.89	10.55	11.17

Table 17: Trends in saturation indices of important minerals during the microcosm experiment for the "Typical" end-member, Montague (MG2).

Typical	MG2	Day						
Mineral	Formula	0	4	10	18	24	30	35
Native As	As	-25.04	-25.98	-25.57	-25.32	-25.24	-26.03	-25.78
	As_2O_5	-24.69	-25.67	-25.51	-25.63	-25.51	-26.46	-26.38
	As_2S_3	-178.57	-189.36	-187.44	-187.51	-187.26	-195.35	-194.16
Orpiment	As_2O_3	-177.17	-187.96	-186.04	-186.11	-186.85	-193.95	-192.76
Arsenolite	As_2O_3	-7.41	-7.07	-6.54	-5.86	-5.76	-5.72	-5.41
Claudetite	A_s2O_5	-7.45	-7.11	-6.58	-5.9	-5.8	-5.76	-5.45
Realgar	AsS	-67.07	-70.98	-70.2	-70.14	-70.03	-72.99	-72.52
Scorodite	$FeAsO_4.2H_2O$	-0.8				-1.79	1.97	-2.48
	$Mn_3(AsO_4)_2.8H_2O$		-0.31	0.62	-0.37	1.37	1.99	1.94
	$Ca_3(AsO_4)_2.4H_2O$	-4.22	-2.9	-2.9	-3.01	-2.84	-2.19	-2.27
Mansfieldite	$AlAsO_4.2H_2O$	-2.72	-3.86	-3.74	-3.52	-3.75	-4.49	-4.43
Pyrite	FeS_2	-90.98	-97.98	-96.92	-97.24	-96.2	-101.99	-100.06
Hematite	Fe_2O_3	20.6	28.12	27.61	28.01	19.44	28.13	28.66
Goethite	α–FeOOH	9.3	13.05	12.8	13	8.72	13.06	8.46

4

Discussion

Microcosms:

The effect of sterilization on the mineralogy of the tailings

Sterile controls for the microcosm experiments were created by autoclaving the tailings samples. Autoclaving is a standard method for sterilizing environmental sediment and water samples; however, the process involves heating the samples to ~120^{o}C, which can affect the mineralogy of poorly ordered minerals (such as ferrihydrite and other amorphous iron oxides) in arsenic rich tailings (Radloff et al. 2007). As a result, X-Ray Diffraction (XRD) was used to check for mineralogical changes after autoclave sterilisation. Figure 13 shows the XRD patterns of the tailings samples before (blue) and after (red) autoclaving. In all samples, no significant change was seen in the mineralogy of the tailings due to autoclaving, which indicates that heat sterilisation was an efficient and effective method for our samples. However, it should be noted that many secondary iron and arsenic minerals (including many amorphous compounds) are not well resolved by bulk XRD analysis (Drahota and Filippi, 2009). These minerals are best resolved using more complex techniques, such as synchrotron-based technique and micro-XRD (Drahota and Filippi, 2009). These more complex techniques were not practically possible in this study. The similarity of the XRD patterns and iron and arsenic speciation measured at day 0 (Figs. 13, 14 - 19, Appendix) indicates that the sterilisation of the tailings samples by autoclaving appeared to have little affect on the mineralogy of the samples and that it is an effective sterilisation technique for the microcosm experiments used in this study.

Initial carbon concentrations in the microcosm experiments

Both organic and inorganic carbon concentrations in the Goldenville surface waters from the Gegogan Brook used in the microcosm construction were within the normal range of concentrations measured in wetlands and headwater streams (Table 7, Wetzel, 2002). Both wetlands and headwater streams are known to support diverse microbial communities (Wetzel, 2002). The carbon concentrations measured in this study imply that the tailings sites (and associated microcosms) are also capable of supporting diverse microbial communities, including metal reducing bacteria. The high carbon concentration allowed microcosms to proceed without carbon amendment.

Insufficient surface water was obtained from the Montague site to determine any carbon concentrations, all water collected was needed in microcosm construction. These concentrations are likely close to that of the Goldenville site as the two sites are very similar, and the streams are of the same approximate size and morphology. Additionally, the Montague stream (Mitchell Brook) enters the tailings site from a heavy forested area (Fig. 6) indicating that carbon concentrations may be higher at this site than at Goldenville.

Release and Reduction of Arsenic and Iron:

Hardpan (GD1):

Microbial reduction of both iron and arsenic was observed in the Hardpan end-member biotic microcosm (Fig. 15). Both soluble Fe(II) and As(III) concentrations increased over 35 days, i.e., from 0.38 ppm to 362 ppm and 0.22 ppm to 8.67 ppm, respectively. The reduction was essentially driven by bacterial activity because the autoclaved sterilized control microcosms for this sample did not show the same level of Fe(II) and As(III) increase overtime (Fe(II) rose from 3.06 to 30.4 ppm, while As(III) rose from 0.72 to 3.37 ppm) (Appendix). The increased released of iron and arsenic is therefore a clear evidence of metal reducing bacterial activity in the gold mine tailings. The main Fe-As mineral in the hardpan end-member is scorodite ($FeAsO_4 \cdot 2H_2O$) (Walker et al., 2009). In the aqueous phase of the microcosm, the saturation index of scorodite varied from -1.67 to -0.71 over the 35 day experiment (Table 13, Appendix), which indicates that scorodite was unstable and dissolving under the microcosm conditions over the course of the experiments. Such findings likely explain why some Fe and As were released into solution in the abiotic (i.e., sterilised) systems.

Results also show that the release of soluble arsenic (measured by the ratio of soluble As to total As) in the system is related to the reduction of iron (R^2=0.62), but not as strongly related to the reduction of arsenic (R^2=0.32, Table 8). This may mean that some of the arsenic being released into solution is related to the activity of iron reducing bacteria, not arsenic reducing bacteria. The reduction of Fe(III) to Fe(II) in many Fe-As minerals can release arsenic into solution (Oremland et al., 2005). Arsenic can also be released in this way through the microbial reduction of iron oxides to which As(V) has sorbed (Islam et al., 2005b). Both the reductive dissolution of Fe-As minerals, and the release of sorbed As(V) have been observed in arsenic contaminated sediments in the West Bengal region of Bangledesh (Islam et al., 2005b, Héry et al., 2008).

Results do indicate that arsenic reduction might be driven by the activity of arsenic reducing bacteria, since gene specific PCR showed the presence of both arsenic resistant microorganisms (ARMs) and Dissimilatory Arsenic Reducing Bacteria (DARPs) in this end-member (Figs. 8 and 9). The activity of these specific bacteria in the microcosm experiment is not known, but their presence indicate that they possibly contributed to the reduction of arsenate to arsenite in the system. The reduction and release of arsenic in the system is most strongly related to the reduction and release of iron (Table 8), which suggests that the arsenic reducing bacteria are likely reducing arsenic from scorodite, which is the main Fe-As mineral in the system (Walker et al., 2009).

Iron reducing bacteria have been known to access Fe(III) in scorodite, reducing it to Fe(II) (Cummings et al., 1999). The process is also known to release the arsenate from the mineral (Cummings et al., 1999). Once released, the As(V) can be easily accessed and reduced by arsenic reducing microorganisms (Cummings et al., 1999). This is likely another mechanism for arsenic reduction and release of As(III) from the hardpan in the microcosm experiment.

There is also evidence of sulphur release in the biotic and abiotic systems. The soluble sulphur concentration in the experimental microcosm rose from 28.6 to 100 ppm from day 0 to day 10 (when the data became unreliable), while the control abiotic system showed less sulphur release (36.7-70.5 ppm) over the same period of time (Appendix). This sulphur release is correlated to the release of iron (R^2=0.89), but not arsenic (R^2=0.09, Appendix). Given the fact that the speciation of sulphur was not performed (i.e., sulphate and sulphide concentrations were not determined), it remains difficult to discriminate between abiotic and biotic reactions responsible for the release of sulphur during the microcosm experiment. However, saturation indices for sulphide minerals in this sample (pyrite, realgar, etc) were negative throughout the experiment (Table 13), indicating that these minerals are likely unstable under the microcosm conditions. This might explain why sulphur release is seen in both the experimental and control systems.

“Typical” tailings (GD2 and MG2):

Two samples of the “Typical” tailings end-member were used in the microcosm experiments to create a direct comparison between the behaviour of the Goldenville tailings and the Montague tailings. The response of these samples over the 35 day experiments was often similar, as expected from their similar geochemistry and mineralogy (DeSisto, 2008; Walker et al., 2009; Kavalench, 2010).

There was, however, one important difference in the behaviour of the “Typical” end-members in the microcosm experiments: the Montague (MG2) sample showed evidence of microbial arsenic reduction (Fig. 19), while the Goldenville sample (GD2) did not (Fig. 18). Both samples; however, showed evidence of microbial iron reduction (Figs. 18 and 19), which followed a similar pattern: after a delay of several days (5-10), a period of rapid iron reduction occurred (between days 5-15), followed by a slow decrease in reduced iron proportion up to day 35 (Figs. 18 and 19).

The reduction of arsenic in the Montague sample was slow, and steady (Fig. 19). The soluble As(III) concentration rose from 0.04 to 0.41 ppm from day 0 to day 35 while the control concentration remained at ~0 ppm. There was no evidence of microbial arsenic reduction in both the biotic and abiotic Goldenville "Typical" sample (GD2) microcosms (Fig. 18). The release of arsenic in the Montague "Typical" sample was related to the release of sulphur, calcium, and silicon (R^2=0.83, 0.84, 0.64, respectively), and the release of sulphur was related to the release of calcium (R^2=0.81, Table 12, Appendix). This implies that the release of arsenic may be related to the dissolution of arsenic bearing sulphide, silicate, or calcium mineral. PHREEQC modelling shows that the saturation indices for such minerals were negative (implying dissolution), except in the case of Ca-As minerals (Table 17). Therefore, the release of arsenic (likely in the more mobile arsenite form) is probably related to the dissolution of complex arsenic secondary minerals, such as arsenosiderite ($Ca_2Fe_3O_2(AsO_4)_3 \cdot 3H_2O$) and pharmacosiderite ($K[Fe_4(OH)_4(AsO_4)_3] \cdot 6.5H_2O$) in the Montague Typical end-member.

The release and reduction or iron in the Montague Typical sample is not related to the release and reduction of arsenic, as it was the case in the Hardpan sample (Tables 8 and 12, Appendix). This means that bacteria-mediated dissolution of scorodite in the Hardpan end-member is not occurring in the "Typical" end-member. This could be simply due to the difference in the abundance of scorodite and the arsenic concentration between the two samples. The arsenic concentration in the Hardpan end-member can be as much as 300 000 ppm, while the Typical end-member typically has concentrations in the 10 000 – 45 000 ppm range (Table 2, DeSisto, 2008; Walker et al., 2009; Kavalench, 2010).

On the other hand, the Goldenville end-member did not show any evidence of microbial arsenic release. The concentration of As(III) did not rise in either the experimental or control microcosms (Fig. 18). However, there is a relationship between iron release and arsenic release in this sample (R^2=0.76), suggesting there might be scorodite dissolution in this sample (Table 11). As with the Hardpan end-member, this dissolution of scorodite is likely driven by the activity of iron reducing bacteria (as mentioned earlier). However, in the Goldenville Typical end-member there is no related microbial arsenic reduction: As(V) to As(III).

There are also strong relationships between the release of arsenic and many other elements (S, Si, Ca, Al, K, Mg, Mn; Table 11, Appendix) in the Goldenville Typical end member. This means that there may be dissolution of complex arsenic bearing minerals, such as arsenosiderite ($Ca_2Fe_3O_2(AsO_4)_3 \cdot 3H_2O$) and pharmacosiderite ($K[Fe_4(OH)_4(AsO_4)_3] \cdot 6.5H_2O$) (Drahota and Filippi, 2009). Modelling shows that the saturation indices for similar complex minerals (mansfieldfite, $Ca_3(AsO_4)_2 \cdot 4H_2O$) remain negative over the course of the experiment (Table 11). However, as it was seen in both the experimental and control microcosms, this dissolution is not driven by the activity of metal reducing bacteria.

Gene specific PCR did show the presence of DARPs in the Goldenville Typical end-member (Fig. 8). The lack of microbial arsenic reduction in the microcosm experiment with this sample could be due to (1) lack of activity on the part of these bacteria, (2) lack of these bacteria in the sample used for the microcosm due to high heterogeneity in the composition of the tailings. Although the microcosms were conducted under anaerobic conditions, and amended with high carbon site water, it may be that the DARPs in this sample were not active despite these seemingly ideal conditions. DARPs are known to be active even in non-ideal conditions (including in the presence of oxygen), but must always have access to arsenate (Meunier et al., 2010). The dissolution of iron from scorodite would seem to create accessible arsenate, but it is possible that this arsenate quickly sorbed onto other mineral particles (including goethite, which has the potential to form in the microcosm (Table 17)), making them inaccessible to the DARPs (Meunier et al., 2010). The accessibility of arsenic (or other metals) to the DARPs (or other metal reducing bacteria), is an important factor in determining the activity of these microbes, and the amount of metal reduced and/or released in the system (Meunier et al., 2010).

High Ca:As (GD3):

Studies on the human bio-accessibility of the tailings have shown the High Ca:As end-member to have the most bio-accessible arsenic (Meunier et al., 2010). The study assessed the accessibility of toxins to human intestinal flora through sequential digestions that replicate conditions found in the human digestion track (Meunier et al., 2010). Because these bioaccessibility studies focussed on accessibility by intestinal bacteria, there is a possible link between these studies and the accessibility of metals by environmental bacteria.

Therefore, it is not a surprise that the High Ca:As end-member showed the highest amount of microbial arsenic reduction in the microcosm experiments (Fig. 17). As(III) concentrations rose from 0.02 ppm to 1.06 ppm from Day 0 to 35, while the control remained ~0.02 ppm (Fig. 18, Appendix).

The important characteristic of the High Ca:As end-member is the concentration of calcium in the tailings. As the host rocks of the original gold-bearing quartz veins are meta-turbidites, there are often high calcium zones within these greenschist facies rocks (Bierlein et al., 2004). Since different parts of these host rocks were exploited at different times in the mining history of the region, this has created high calcium zones within the resulting tailings. The high calcium concentration has dramatic effects on the resulting arsenic secondary mineralogy: while in other end-member, scorodite is usually the dominant arsenic phase, in the High Ca:As end-member, Ca-As minerals are likely to form, including yukonite ($Ca_7Fe_{12}(AsO4)_2(OH)_{20}{\cdot}15H_20$) and arsenosiderite ($Ca_2Fe_3O_2(AsO_4)_3{\cdot}3H_2O$) (Jamieson et al., 2006, Meunier et al., 2010).

The higher bio-accessibility of the High Ca:As end-member has been linked to this different secondary mineralogy in previous studies (Meunier et al., 2010). Most important was the mineral yukonite ($Ca_7Fe_{12}(AsO4)_2(OH)_{20}{\cdot}15H_20$), which is particularly bio-accessible (Meunier et al., 2010). This mineral is found in abundance in the High Ca:As tailings end-member at both the Goldenville and Montague sites (Walker et al., 2009). This end-member is also high in the potassium arsenic mineral pharmacosiderite ($K[Fe_4(OH)_4(AsO_4)_3]{\cdot}6.5H_2O$) (Walker et al., 2009).

The high rate of microbial arsenic reduction in the High Ca:As end-member is likely linked to the presence of these highly bio-accessibility minerals (especially yukonite). The release of arsenic into solution in this microcosm is related to the release of calcium (R^2=0.46), implying the dissolution of a As-Ca mineral, such as yukonite (($Ca_7Fe_{12}(AsO4)_2(OH)_{20}{\cdot}15H_20$), Table 10, Appendix). The release of arsenic is also related to the release of aluminum (R^2=0.67), potassium (R^2=0.52), magnesium (R^2=0.60, and manganese (R^2=0.67, Table 10, Appendix). This is evidence of the microbial reduction of other secondary minerals found in this end-member, such as pharmacosiderite, which would explain the relationship between potassium and arsenic release.

The PHREEQC database does not include these uncommon and poorly studied/characterised, secondary minerals. However, there are similar minerals/compounds such as mansfieldite ($AlAsO_4{\cdot}2H_2O$) and $Ca_3(AsO_4)_2{\cdot}4H_2O$, which are included in the geochemical modelling of this microcosm. The saturation index of $Ca_3(AsO_4)_2{\cdot}4H_2O$ was negative over the course of the microcosm experiment, meaning this mineral is likely dissolving if present (Table 15). These saturation indices and the relationship between the release of arsenic and the release of both manganese and calcium are strong evidence of the microbial mediated reductive dissolution of Ca-As and Mn-As secondary minerals in this end-member.

PHREEQC also shows the saturation index for scorodite was negative throughout the course of the experiment (Table 15), which indicates that As release is not solely due to microbial reduction, but also mineral dissolution. However, this mineral is not a major arsenic bearing mineral in the High Ca:As end-member and its dissolution likely contributed to a very small release of As overtime. The small amount of this mineral in the Ca-As end-member partly explains the lack of relationship between the reduction of arsenic and iron in the microcosm experiment. Gene specific PCR showed the presence of DARPs in the High Ca:As end-member (Fig. 8, sample GD7 and GD8) and it is likely that the release of arsenic was the result of activity of dissimilatory metal reducers.

Saturated (GD4):

The only tailings end-member which showed no evidence of either iron or arsenic reduction was the saturated end-member (Fig. 16). This is not surprising, as this end-member is defined as being permanently under reducing, anoxic conditions (Kavalench, 2010). Therefore, all iron and arsenic bearing minerals were already in their reduced forms at the beginning of the experiment, leaving no metal to reduce (either by bacteria, or not). This is shown by the ratio of reduced metal to oxidised remaining at near 1 throughout the course of the experiment (Fig. 16).

Molecular microbiology and its challenges

Microbiology techniques exist to confirm the existence and activity of metal cycling bacteria. 16S rDNA techniques (like those discussed below) can identify and confirm the existence of specific bacteria, while RNA techniques can confirm the activity of bacteria. The advantage of RNA techniques is that they rely on the detection of unstable RNA molecules (rather than the more stable, double-helix structured DNA (Lovely, 2003). Since RNA degrades quickly when the bacteria are not active, RNA techniques provide a measure of microbial activity, while DNA techniques simply provide a confirmation of their existence.

DNA amplification of 16S, *arrA*, and *arsC* genes were attempted for microcosm samples in an attempt to track changes in the microbial ecology of the samples over the course of the microcosms. However, these approaches failed at the DNA amplification (PCR) step, likely due to the inhibitory effect of arsenic and other heavy metals, discussed below.

Sequencing:

Metal reducing bacteria:

Eight (8) colonies from the Goldenville High Ca:As end member sample yielded sequences belonging to the *Geobacter* family (Fig. 12). This taxon was the first group of bacteria confirmed to have reducing capabilities (Mahadeva et al., 2011). *Geobacter* species are known to live in a wide variety of environments, including arsenic contaminated sediments (Héry et al., 2008; Mahadeva et al., 2011). The silt- to sand-sized tailings in this study are similar to arsenic contaminated sediments, so the presence of *Geobacter* in these samples is not surprising. There is evidence of at least three different members of the *Geobacter* family. Colonies G7-1,4,5,7,9, and 11 are all shown to be roughly the same in phylogeny , while samples G72 and G712 are dissimilar both from the above group and one another (Fig. 12).

Geobacter are known to have both iron and arsenic reducing capacities (Héry et al., 2008). *G. uraniireducens* is known to have arsenic reducing abilities, while many other members of the *Geobacter* family contain the genes for this process (Héry et al., 2008). Both *G. bemidjiensis* and *G. metallireducens* are thought to be involved in the reduction of arsenic in contaminated systems (Héry et al., 2008). The presence of these bacteria in the environmental samples supports the microcosm findings of arsenic reduction related to microbial action.

DARPs belonging to the *Geobacter* family are present in at least one end-member and are likely involved in the microbial reduction of arsenic seen in this end-member's microcosm (Fig. 12). This High Ca:As end-member showed more microbial reduction of arsenic than any other sample (Figs. 15 - 19), so it is no surprise that the most metal reducing – and likely arsenic reducing – bacteria were identified in this sample.

Geobacter are also known to survive in many environments (including high heat and in the presence of oxygen), so it is likely that these bacteria are present, or could be present, in other samples from these sites. Inefficiencies in both PCR and sequencing methods discussed below may be responsible for the non-identification of this taxon in other samples; however, its presence in the High Ca:As end-member may support a confirmation the DARPs identified in samples by *arrA* PCR (below) as *Geobacter* members.

Methylobacterium:

Members of the *Methylobacterium* family of anaerobic bacteria were found in the Hardpan end-member (Fig. 12). These bacteria are often found in soils and sediments (Lidstrom and Chistoserdova, 2002). Methylobacterium are important in carbon cycling in the environment as they are known to use carbon compounds such as methanol, methylamine, C2, C_3, and C_4 to grow (Lidstrom and Chistoserdova, 2002). Some members of the Methylobacerium family have been shown to be arsenic resistant (Huang et al., 2010). In addition, Battaglia-Brunet et al. (2006) identified a member of the *Methylobacterium* family in a bioreactor amended with mine waters which makes its detection in the hardpan sample not surprising.

Bacteriodetes incertae sedis:

Members of the *Bacterioidetes incertae sedis* phylum were found in the High Ca:As end-member (Fig. 12). This phylum includes the facultative anaerobes *Cytophaga* and *Prolixibacter* (Fig. 12, Holmes et al., 2007). *Cytophaga*are are known cellulose degrading bacteria, while *Prolixibacter* is known to ferment sugars in marine sediments (Holmes et al., 2007). *Prolixibacter* may be important in the reduction and cycling of metals, as the fermentation of sugars can provide new carbon sources (such as acetate) for dissimilatory metal reducers (Holmes et al., 2007). The presence of these anaerobes in the tailings supports the hypothesis that metal reducing bacteria would be present. Presence of other anaerobic bacteria in the samples shows that the environment is suitable for growth and development of anaerobic bacteria, including metal reducers.

Proteobacteria:

Many samples were found to contain *Proteobacteria*, a group of common soil bacteria (Fig. 12). One colony (M5-5) was also found to be similar to the known arsenic and iron reducer *Schewanella putrefaciens* (Fig. 12). Given the high levels of arsenic and iron in the samples, it is very possible that *Schewanella* is present and an active metal reducer in the tailings. In a previous experiment using As-rich Bacteriogenic Iron Oxides (BIOS) retrieved from the Goldenville site, it was shown that *Schewanella putrefaciens* CN-32 was capable of reducing both Fe(III) and As(V) in the oxides (Marshall, 2009). This strengthens the possibility that active *Schewanella* populations are present in the tailings (Marshall, 2009).

There were also iron oxidizing bacteria from the *actinobacter* group (Fig. 12). The bacteria *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* are known iron oxidizing bacteria which have previously been isolated from mine tailings environments (Johnson et al., 2009). These are acid tolerant, anaerobic bacteria which are also known to catalyze the dissimilatory iron reduction, by bacteria such as *Geobacter* (Johnson et al., 2009). Other P*roteobacteria* found in the samples included acid tolerant *Acidisphaera* species (Hiraishi et al., 2000), and ethanol converting A*cetobacter* species (Fig. 12).

Unknown:

No close matches for colonies M5-4 and M5-7 were found using the ribosomal database project or NCBI Blast system. These bacteria are simply listed as "unknown" and included in the phylogenetic tree so future analysis may identify these sequences (Fig. 12).

Microbial Ecology:

While sequencing provides important data by identifying bacteria present in samples to the species level, this technique is both time consuming and expensive; the cost makes sequencing prohibitive in large scale projects. Instead diversity and richness of microbial communities can be estimated using T-RFLP. Additionally, the presence of metal reducing bacteria in a sample can be determined using gene specific PCR, and restriction analysis of these amplified genes can provide diversity data for specific functional groups of bacteria (Singh et al., 2006).

Digested 16S rDNA fragments were analysed to determine the relative abundance of all present fragment lengths, which represent unique operational taxanomic units (OTUs). The lower cost of this technique allowed for far more samples to be analysed than with cloning and sequencing.

Overall, the communities in each sample were seen to vary (Figs. 10 and 11). This shows that there are different bacteria thriving in different areas of the tailings. The trends in the richness (#OTUs) and Diversity (Shannon-Weaver) provide interesting clues about the makeup of these communities.

The richness and diversity showed a positive trend with respect to As(V) concentration and overall arsenic concentrations (Table 4). This would usually be unexpected as As(V) is known to impede oxidative phosphorylation (Oremland and Stolz, 2003). However, the presence of arsenic reducers in these samples would change this usual result. Arsenic reducing bacteria require arsenate to be present in order to thrive, so a relationship between As(V) concentration and bacterial diversity may be an indicator of arsenic reducing bacteria being present in the high arsenic areas within the tailings. Sequencing shows that these arsenic reducers likely belong to the *Geobacter* or *Schewanella* families (Fig. 12).

Additionally there was a relationship between soluble iron and richness (R^2=0.14, F=1.44, p=0.03, Table 4). This may be due to the presence of iron reducing bacteria within the samples which would thrive in high iron concentrations. These iron reducers are likely from the *Geobacter* family, as shown by sequencing (Fig. 12).

Permutational multivariate analysis of variance was used to determine which geochemical variables are most related to the similarities between the microbial communities. Like diversity and richness, similarity was related to soluble arsenic concentration (R^2=0.1253, p=0.0597, Table 5). This further strengthens the conclusion that these bacterial communities include arsenic reducing bacteria.

The T-RFLP analysis was performed for multiple samples across all end-members from both sites. The trends in the data indicate that there could be arsenic and iron reducing bacteria present in any, and/or all of the end-members. While sequencing was only successful in identifying metal reducers at a few sites, this technique was able to show evidence of this microbial activity at all sites.

Methodological Issues:

To further resolve the diversity and richness of the metal reducing bacteria, the same restriction analysis was to be performed on amplified *arrA* and *arsC* genes, specific to DARPs and ARMs respectively. However, amplification of these genes proved difficult, and only two trials of the PCR were completed. No digestion analysis was possible from these results as the trials were not performed with labelled primers.

The intent was to use amplified group-specific genes for many of the important functional groups being considered in this study: iron reducers and oxidizers, arsenic reducers. This would have been a fast and easy method to identify where these bacteria were present, and where the most diverse communities of these functional groups were found. Instead, the

T-RFLP methodology only provided results for overall bacterial diversity through amplification of the 16S gene.

There were also several issues faced with the amplification of 16S genes from the tailings samples. As a result, no single DNA extraction and PCR recipe were found to work for all samples, and optimization was required for every sample type. This was both resource and time consuming and thus limited the amount of data available from these techniques.

The problems with gene amplification are likely due to the high arsenic concentration of the tailings. Arsenic is a known PCR inhibitor and other studies on similar samples have also run into problems with amplification (J. Drysdale and Dr. C. Saltikov, pers. communication). While no discussion of the mechanism of PCR inhibition by arsenic exists in literature, speculation here is that the mechanism may be related to the similarities between phosphate and arsenate. In theory, arsenate may be able to substitute for phosphate in the replication of DNA (Wolfe-Simon, 2009), which could easily disrupt the sensitive PCR system. This inhibition can be avoided by washing the samples prior to DNA amplification; this washing step should remove some of the arsenic and possibly make PCR more efficient. Several washing protocols were trialed in this study, ultimately the repetition of steps 15 and 16 in the UltraCLean Soil DNA Extraction Kit (MoBio #12800) proved most useful. This is an alcohol based rinse step meant to remove many contaminants from the samples. Other methods used involved extensive rinses with ammonium oxalate and ddH_2O (Boothman, 2007). These methods did not yield significantly more DNA than the repetition of steps 15 and 16 to justify the considerable resources and time commitment associated with extensive washing. Also, extensive alcohol washing may actually reduce efficiency of PCR, as alcohol is known to degrade DNA (Brooks, 2006).

Future Approaches:

Despite the difficulty of extraction and amplification of DNA from arsenic rich samples, the use of molecular microbiology to analyse for the presence and diversity of arsenic reducers in mine wastes systems should still be attempted in future studies of these sites and others. The potential role that bacteria can play in the reduction of arsenic and release of toxic arsenite is important in assessing the bioaccessibility of arsenic and can inform remediation efforts (Meunier et al., 2010). Molecular microbiology provides a method by which arsenic reducers can be identified and quantified in mine waste systems.

Future studies at the Montague and Goldenville sites should focus on the use of RNA rather than DNA, both T-RFLP and cloning and sequencing. RNA is less stable than DNA, thus the presence of RNA from arsenic reducers shows that these bacteria are alive and active, while presence of DNA may occur even if these bacteria are long dead or not active (Lovely, 2003). RNA extraction, amplification, and analysis provide a more accurate prediction of microbial activity (Lovely, 2003). This would be especially effective in conjunction with microcosm experiments where the activity of various bacteria could be tracked through an experiment using RNA techniques. Due to the issues faced with DNA amplification, it was not possible to attempt RNA extraction and amplification in this study.

Implications for Remediation Efforts:

The impact of microbial release of As(III) into the environment surrounding either tailings site could be devastating, and it is therefore an important consideration in developing effective remediation strategies. Understanding which parts of the tailings are more likely to reduce and/or release arsenic into the environment can help inform remediation decisions. The microcosm experiments demonstrated that not all the tailings end-members are created equal with respect to the reduction and release of arsenic.

Any remediation effort that would see the Hardpan end-member placed totally, or partially, under reducing, anoxic conditions would likely result in the massive release of As(III) into the surrounding environments. The use of a Geosynthetic Clay Liner (GCL) has been proposed for these sites. The use of such a layer would raise the anoxic boundary placing the otherwise oxidised hardpan under reducing conditions. This would promote the microbial reduction of arsenate to the more mobile and toxic arsenite form. If a GCL is used, it would be important to ensure that flow of groundwater under the liner is reduced to prevent this toxic arsenic from migrating into the wetlands, embayments, and human settlement areas near both sites.

The increased bio-accessibility of the arsenic bearing minerals in the High Ca:As end-member makes it a particular concern for remediation. Since large amounts of toxic, mobile arsenite could be released from this end-member, it is important to avoid placing the tailings under reducing, anoxic conditions where the metal reducing bacteria can thrive. Monitoring of this end-member is also important, as these bacteria are known to be active in non-ideal conditions, including oxic environments (Macur et al., 2001).

However, the High Ca:As end-member represents a minority of the bulk of the tailings; the Typical end-member represents the vast majority of tailings at both Goldenville and Montague. The reduction and release of arsenic seen in the Montague Typical microcosms, although much less than that seen in either the hardpan or High Ca:As end-members, is nonetheless important. Given that there is many times more Typical tailings at the site than either the hardpan or the High Ca:As, this moderate reduction and release of arsenic may actually represent more total release since there is so much more of these tailings.

Although both the Hardpan and High Ca:As end-members require special attention due to the amount of arsenic reduction and bioaccessibility of the arsenic, this attention should not preclude attention given to the Typical tailings. The Typical tailings, if covered by a GCL may indeed release more arsenic than the Hardpan would under these conditions.

Additionally, the pH of the microcosm experiments, as well as measurements taken in the field, demonstrates that acidity is not a major issue at these tailings except in and around the Hardpan end-member. The site surface water and groundwater are usually sub-neutral (pH=6-7). However, evidence of reduction and release of toxic arsenic from the higher pH systems (Typical, High Ca:As) shows that liming (or other methods of pH increase) would not address the issue of arsenic release from the Hardpan end-member. Since the arsenic concentrations – not the pH – is the major health and environmental issue at these sites, remediation efforts which do not directly address this issue would be unwise. This is in keeping with previous studies which showed that increasing the pH of acidic tailings by up to 4 units did nothing to abate the activity of metal reducing bacteria (Macur et al., 2001). In fact, increasing the pH can result in increased microbial arsenic release (Macur et al., 2001).

In modern mining operations, tailings similar to these would be immediately placed under water in tailings ponds to immobilize metals and prevent acidic drainage. As this was not done with the tailings (and would be impractical to do now), remediation strategies may differ greatly from the expected in order to properly address some of the issues linked to improper initial management..

5

Conclusions

Given the increased toxicity and mobility of arsenite over arsenate (Henke, 2009b), determining the oxidation state of arsenic in these contaminated mine tailings sites is an important step in the creation of effective remediation strategies. Additionally, understanding arsenic cycling and triggers of arsenic reduction and release reveal the dynamics of arsenic in these historic tailings. The capacity of many bacteria to mediate and trigger the release of arsenite from contaminated sites means that study of bacterial communities in contaminated Nova Scotian tailings is of great importance to both the human and ecosystem health of regions surrounding historic tailings sites.

Both arsenic reducers and iron reducers have been shown to be both present and active in these tailings. The activity and diversity of these bacteria varies throughout these compositionally diverse sites, which means that remediation strategies cannot treat the tailings as homogenous. Instead remediation strategies must take into account the areas of high microbial arsenic release and those areas with low arsenic release.

Overall, the High Ca:As end-member shows the most microbial reduction and release of arsenic of all the end-members (Figs.15 – 19). However, as this end-member does not represent a large proportion of the tailings (Figs. 6 ad 7), it may be that there is more arsenic released in total from the "Typical" tailings, which represent the bulk of the tailings at both sites. This would be especially true at the Montague site which showed evidence for microbial arsenic reduction (Fig. 19), while the Goldenville "Typical" end-member did not (Fig. 18).

The bacteria responsible for this metal reduction are from the *Geobacter* and *Schewanella* families (Fig. 12). *Geobacter* have previously been shown to reduce both arsenic and mediate the release of arsenic through the reduction of iron in Fe-As minerals in similarly contaminated sites (Islam et al., 2004; Héry et al., 2008). A similar process is likely occurring here as many of the Fe-As minerals existing at these sites are under saturated in the site water (Tables 13 – 17). Therefore, the microbial release of arsenic from these tailings is not wholly dependent on the activity of arsenic reducing bacteria, but also on the activity of iron reducing bacteria.

The detection of these bacteria and their activity at both the Montague and Goldenville sites creates a new concern for remediation. However, many methodological issues were faced in this study, so there is still much to be learned about the microbial ecology of metal reducing bacteria in mine tailings from these sites. Continued study of the sites, and monitoring during remediation trials would be a logical extension of the work conducted here.

The end members defined for the Montague and Goldenville sites are not restricted to just these sites; they have also been identified at many other historic Nova Scotian gold mine tailings. The findings of this study can easily be extended to inform remediation strategies at other sites within the province, and other historic tailings sites across Canada. The important role that bacteria can play in altering the toxicity and bioaccessibility of arsenic in tailings systems makes microbiological investigations of tailings an important step in geoenvironmental modelling of mine wastes and ore deposits.

References:

Anderson, C.R. and G.M. Cook (2004) "Isolation and Characterization of Arsenate-Reducing Bacteria from Arsenic-Contaminated Sites in New Zealand," Current Microbiology, **48**(5): 341-347.

Battaglia-Brunet, F., Y. Itard, F. Garrido, F. Delorme, C. Crouzet, C. Greffié, and C. Joulian (2006) "A Simple Biogeochemical Process Removing Arsenic from a Mine Drainage Water," Geomicrobiology Journal, **23**: 201-211.

Bierlein, FP and AB Christie, PK Smith (2004) "A comparison of orogenic gold mineralization in central Victoria (AUS), western South Island (NZ) and Nova Scotia (CAN): implications for variations in the endowment of Palaezoic metamorphic terrains," Ore Geology Reviews, **25**: 125.

Brooks, P. (2006) "DNA Damage, DNA Repair, and Alcohol Toxicity – A Review," Alcoholism: Clinical and Experimental Research, **6**: 1073-1082.

Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. S. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity and J.M. Tiedje (2008), "The Ribosomal Database Project: improved alignments and new tools for rRNA analysis," Nucleic Acids Research, **37**(supplement 1): D141-D145.

Cummings, S. N., R. F. Bopp, H. J. Simpson, D. A. Chaky, D. C. Walsh, C. Chin Choy, L. Tolley, and A. Yarme (1999) "Twentieth Century Atmosphere Metal Fluxes into Central Park Lake, New York City," Environmental Science & Technology, **33**(5): 657-662.

Dale, J.M. and B. Freedman (1982) "Arsenic Pollution Associated with Tailings at an Abandoned Gold Mine in Halifax County, Nova Scotia," Proc. N.S. Instit. Sci., **32**: 337-349.

DeSisto, S. L. (2008) "Dynamic Arsenic Cycling in Scorodite-Bearing Hardpan Cements, Montague Gold Mines, Nova Scotia," M.Sc. Thesis, Queen's University, Kingston, Sup: H. Jamieson.

Drahota, P., and M. Filippi (2009) "Secondary Arsenic Minerals in the Environment: A Review," Environmental International, **35:** 1243-1255.

Drysdale, J., (2009-2011) Personal Correspondence and Conversation.

Goodwin, T.A., M.B. Parsons and R.B. Taylor (2008) **Environmental Geology of Halifax and Surrounds,** EdGEO Field Excusion: Guidebook, Nova Scotia Department of Natural Resources.

Haffert, L., and D. Craw (2008) "Mineralogical Controls on Environmental Mobility of Arsenic from historic Mine Processing Residues, New Zealand," Applied Geochemistry, **23:** 1467-1483.

Henke, K.R. (2009a) "Introduction" in **Arsenic:** 1-10, Ed. Henke, K.R.: John Wiley & Sons: West Sussex, UK.

Henke, K.R. (2009b) "Arsenic in Natural Environments" in **Arsenic:** 69-236, Ed. Henke, K.R.: John Wiley & Sons: West Sussex, UK.

Héry, M, A.G. Gault, H.A.L. Rowland, G. Lear, D.A. Polya and J.R. Lloyd (2008) "Molecular and Cultivation-Dependent Analysis of Metal-Reducing Bacteria Implicated in Arsenic Mobilisation in South-East Asian Aquifers," Applied Geochemistry, **23**: 3215-3223.

Hiraishi, A., Y. Matsuzawa, T. Kanbe, and N. Wakao, (2000) "*Acidisphaera rubifaciens* gen. nov., sp. nov., and aerobic bacteriochlorophyll-containing bacterium isolated from acidic environments," International Journal of Systematic and Evolutionary Microbiology, **50**: 1539-1546.

Huang, A., M. Teplitski, B. Rathinasabapathi, and L. Ma (2010) "Characterization of Arsenic-Resistant Bacteria from the Rhizosphere of Arsenic Hyperaccumulator *Pteris vittata*," Canadian Journal of Microbiology, **56**: 236-246.

Islam, F.S., A.G. Gault, C. Boothman, D.A. Polya, J.M. Charmock, D. Chatterjee and J.R. Lloyd (2004) "Role of Metal-Reducing Bacteria in Arsenic Release from Bengal Delta Sediments," Nature, **430:** 68-71.

Islam, F. S., C. Boothman, A. G. Gault, D. A. Polya, and J. R. Lloyd (2005) "Potential role of the Fe(III)-reducing Bacteria *Geobacter* and *Geothrix* in Controlling Arsenic Solubility in Bengal Delta Sediments," Mineralogical Magazine, **69**(5): 865-875.

Jamieson, H.E., M.C. Corriveau, M.B. Parsons, I. Koch and K.J. Reimer (2006) "Mineralogy and Bioaccessibility or Arsenic-Bearing Secondary Phases in Gold Mine Tailings," Abstract from Goldschmidt Conference Abstracts 2006: A289.

Johnson, D. B., P. Bacelar-Nicolau, N. Okibe, A. Thomas, and K. B. Hallberg (2009), "*Ferrimicrobium acidiphilum* gen. nov., sp. nov. And *Ferrithrix thermotolerans* gen. nov., sp. nov.: Hetertrophic, Iron-Reducing, Extremely Acidophilic Actinobacteria," International Journal of Systematic and Evolutionary Microbiology, **59**: 1082-1089.

Kavalench, J. L. (2010) "Effect of Tailings Mineralogy and Infiltration Water Chemistry on Arsenic Release from Historic Gold Mine Tailings," M.Sc. Thesis, Queen's University, Kingston, Sup: H. Jamieson.

Kontak, DJ and PK Smith, R Kerrich, PF Williams (1990) "Integrated model for Meguma Group lode gold deposits, Nova Scotia, Canada," Geology, **18**: 238.

Lee, J., S. Lee, H. Chon, K. Kim and J. Lee (2009) "Enhancement of Arsenic Mobility by Indigenous Bacteria from Mine Tailings as Response to Organic Supply," Environment International, **35:** 496-501.

Lidstrom, M.E. and L. Chistoserdova (2002) "Plants in the Pink: Cytokin Production by *Methylobacterium*," Journal of Bacteriology, **184**(7): 1818.

Lovely, D. R. (2003) "Cleaning Up with Genomics: Applying Molecular Biology to Bioremediation," Nature Reviews Microbiology, **1**: 35-44.

Macur, R.E., J.T. Wheeler, T.R. McDermott and W.P. Inskeep (2001) "Microbial Populations Associated with the Reduction and Enhanced Mobilization of Arsenic in Mine Tailings," Environ. Sci. Technol., **35:** 3676-3682.

Macy, J.M., K. Nunan, K.D. Hagen, D.R. Dixon, P.J. Horbour, M. Cahill and L.I. Sly (1996) "*Chrysiogene arsenates* gen. nov., sp. Nov., a New Arsenate-Respiring Bacterium Isolated from Gold Mine Wastewater," International Journal of Systematic Bacteriology, **46**(4): 1153-1157.

Malasarn, D, C.W. Saltikov, K.M. Campbell, J.M. Santini, J.G. Hering, D.K. Newman (2004) "*arrA* Is a Reliable Marker for As(V) Respiration," Science, **306**: 455.

Marshall, S. (2009) "The Release of As from Bacteriogenic Iron Oxides (BIOS) under Reducing Conditions" B.Sc. Thesis, University of Ottawa, Ottawa, Sup. D. Fortin.

Meunier, L, S.R. Walker, J. Wragg, M.B. Parsons, I. Koch, H.E. Jamieson and K.J. Reimer (2010) "Effects of Soil Composition and Mineralogy on the Bioaccessibility of Arsenic from Tailings and Soil in Gold Mine Districts of Nova Scotia," Environ. Sci. Technol., **44**: 2667-2674

Mills, R.F. (1997) "Preliminary Investigation of Abandoned Mill Tailings at the Montague Gold District, Nova Scotia: Implications for Development Potential," Nova Scotia Dept. of Natural Resources: Report of Activities: 109-113.

Mullis, K (1994) Preface to **The Polymerase Chain Reaction,** Ed. Mullis, K, F. Ferré, R.A. Gibbs: Brikhäuser: Boston.

Opel, K.L., D. Chung, and B.R. McCord (2010) "A Study of PCR Inhibition Mechanisms Using Real Time PCR," J. Forensic. Sci., **55**(1): 25-33.

Oremland, R.S. and J.F. Stolz (2003) "The Ecology of Arsenic," Science, **300**: 939-944.

Oremland, R.S., J.F. Stolz and J.T. Hollibaugh (2005) "The Microbial Arsenic Cycle in Mono Lake, California," FEMS Microbiology Ecology, **48**: 15-27.

Promega Corporation (2010), **Technical Manual: pGEM®-T and pGEM®-T East Vector Systems**, Promega Coporation, Madison, WI: available online: http://www.promega.com/tbs/tm042/tm042.pdf.

Radloff, K. A., Z. Cheng, M. W. Rahman, K. M. Ahmed, B. J. Mailloux, A. R. Juhl, P. Schlosser, and A. Van Geen, (2007) "Mobilization of Arsenic During One-Year Incubations of Grey Aquifer Sands from Araihazar, Bangladesh," Environmental Science and Technology, **41**: 3639-3645.

Röling W.F.M and I.M. Head (2005) "Prokaryotic Systematics: PCR and Sequence Analysis of Amplified 16S rRNA Genes," in **Molecular Microbial Ecology:** 25-57, Ed. Osborn, A.M. and C.J. Smith: Taylor & Francis Group: New York.

Saltikov, C. (2010) Personal Correspondence.

Silver, S. and L.T. Phung (2005) "Genes and Enzymes Involved in Bacterial Oxidaion and Reduction of Inorganic Arsenic," Applied and Environmental Microbiology, **71**(2): 599-608.

Singh, B. K., L. Nazaries, S. Munro, I. C. Anderson, and C. D. Campbell (2006) "Use of Multiplex Terminal Restriction Fragment Length Polymorphism for Rapid and Simultaneous Analysis of

Different Components of the Soil Microbial Community," Applied and Environmental Microbiology, **72**(11): 7278-7285.

Smith, P.K., M.B. Parsons, and T.A. Goodwin (2005) "Geology and Environmental Geochemistry of Lode Gold Deposits in Nova Scotia," GAC/MAC/CSPG/CSSS Joint Annual Meeting: Pre- and post- conference field trips: Field Trip FT-B5.

St.Jean, G. (2003) "Automated Quantitative and Isotopic (13C) Analysis of Dissolved Inorganic and Dissolved Organic Carbon in Continuous-flow Using a Total Organic Carbon Analyser," Rapic Communications in Mass Spectrometry, **17**: 419-428.

Sun, Y., E.A. Polishchuk, U. Radoja and W.R. Cullen (2004) "Identification and Quantification of *arsC* Genes in Environmental Samples by using Real-Time PCR," Journal of Microbiological Methods, **58**: 335-349.

United States Geologic Survey (USGS) (2010), "PHREEQC" web resource: http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/index.html, modified Sept. 27, 2010.

Utting, D.J., T.A. Goodwin, and D. Whalen (2009) "Potential Identification of Mine Openings Using Remote Sensing Topographic LiDAR, Montague Gold Distrcit (NTS 11D/12), Halifax Regional Municipality," Nova Scotia Dept. of Natural Resources: Report of Activities: 125-132.

Walker, S.R., M.B. Parsons, H.E. Jamieson, and A. Lanzirotti (2009) "Arsenic Mineralogy of Near-Surface Tailings and Soils: Influence on Arsenic Mobility and Bioaccessibility in the Nova Scotia Gold Mining Districts," The Canadian Mineralogist, **47:** 533-556.

Wetzel, Robert G. 2001. *Limnology: Lake and River Ecosystems*. Toronto: Academic Press.

Wolfe-Simon, F., P.C.W. Davies, and A.D. Anbar (2009) "Did Nature also Choose Arsenic?" International Journal of Astrobiology, **8**(2): 69-74.

Wolfe-Simon, F., J.S. Blum, T.R. Kulp, G.W. Gordon, S.E. Hoeft, J. Pett-Ridge, J.F. Stolz, S.M. Webb, P.K. Weber, P.C.W. Davies, A.D. Anbat and R.S. Oremland (2010) "A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus," Sciencexpress: Dec. 2, 2010: 1-9.

Wong, H.K.T.A. Gauthier and J.O. Nriagu (1999) "Dispersion and Toxicity of Metals from Abandoned Gold Mine Tailings at Goldenville, Nova Scotia, Canada," The Sciences of the Total Environment, **228**: 35-47.

Appendix:

Microcosm Metal Concentrations

T-RFLP Data

DNA Extraction and Amplification Trials

Location	Sample	Date	Day	pH	Sol Fe(II) ppm	Sol T Fe ppm	Sol Fe(II)/T Fe	Tot Fe(II) ppm	Tot T Fe ppm	Tot Fe(II)/ T Fe	Sol Fe/ T Fe	Sol As(III) ppm	Sol T As ppm
GD1	A	16/11/2010	0	3	0.377006	0.725939	0.519335	82.75583	1219.972	0.0678342	0.00119	0.363818	0.92716
GD1	B	16/11/2010	0	2.9	0.377006	0.695005	0.542451	54.54662	1316.114	0.0414452	0.001056	0.08094	0.906937
GD1	C1	16/11/2010	0	2.81	3.291957	3.9947	0.824081	148.5773	1682.908	0.088286	0.004747	0.648986	1.74699
GD1	C2	16/11/2010	0	2.79	2.821803	3.45206	0.817426	92.15889	1103.996	0.0834776	0.006254	0.799772	1.92731
GD1	A	20/11/2010	4	2.88	36.49423	36.9712	0.987099	1099.205	1699.818	0.6466607	0.0435	0.314775	2.61299
GD1	B	20/11/2010	4	2.79	36.87517	37.3948	0.986104	489.7004	1630.408	0.3003545	0.045872	0.283917	2.63291
GD1	C1	20/11/2010	4	2.61	9.256968	9.7254	0.951834	175.4243	2024.16	0.0866652	0.009609	1.50804	3.36964
GD1	C2	20/11/2010	4	2.56	7.923676	8.46172	0.936414	146.8537	1704.622	0.0861503	0.009928	1.94835	3.95146
GD1	A	26/11/2010	10	3.02	112.8132	106.76	1.056699	1622.439	1691.08	0.9594098	0.126263	2.52134	6.55174
GD1	C1	26/11/2010	10	2.61	39.10639	15.6955	2.491567		1651.85		0.019004	2.18246	4.65814
GD1	A	04/12/2010	18	2.49	219.1391	173.264	1.26477	2150.73	2243.68	0.9585724	0.154446	10.4828	19.1302
GD1	B	04/12/2010	18	2.19	184.7703	173.264	1.066409	1744.113	2876.52	0.6063274	0.120468	5.28093	12.6441
GD1	C1	04/12/2010	18	2.69	27.44838	21.7096	1.264343	195.0967	4044.82	0.0482337	0.010735	2.7849	5.38851
GD1	C2	04/12/2010	18	2.65	18.25109	17.9332	1.017727	156.3713	1556.24	0.1004802	0.023047	2.93355	5.68791
GD1	A	12/10/2010	24	3.74	304.6903	304.6903	1	3291.247	2142.26	1	0.284457	10.3613	20.3494
GD1	B	12/10/2010	24	3.25	252.0545	252.0545	1	2741.473	1443.48	1	0.349232	8.31391	16.424
GD1	C1	12/10/2010	24	2.68	21.3532	24.7034	0.864383					2.95669	5.72678
GD1	C2	12/10/2010	24	2.62	21.86225	20.4821	1.067383	379.4822	2145.94	0.1768373	0.019089	3.47393	6.05984
GD1	A	16/12/2010	30	3.23	325.0213	325.0213	1	4829.455	2290.28	1	0.283827	8.52678	16.6301
GD1	B	16/12/2010	30	3.23	304.6187	304.6187	1	2887.282	1796.308	1	0.339161	11.1585	19.7246
GD1	C1	16/12/2010	30	2.71	28.59459	29.8298	0.958592	395.8063	2140.96	0.1848733	0.027866	3.29283	7.38056
GD1	C2	16/12/2010	30	2.67	36.0494	23.6736	1.522768	395.8063	1936.026	0.2044426	0.024456	3.34987	6.3088
GD1	A	21/12/2010	35	3.45	381.5654	381.5654	1	4465.571	3154.88	1	0.241889	7.31311	17.4043
GD1	B	21/12/2010	35	3.3	343.335	343.335	1	4043.024	3294.36	1	0.208438	10.0362	19.2733
GD1	C1	21/12/2010	35	2.72	33.26657	31.6497	1.051086	481.5629	2568.48	0.1874895	0.024645	3.2488	6.07413
GD1	C2	21/12/2010	35	2.67	27.63262	26.3001	1.050666	280.3504	2872.76	0.0975892	0.01831	3.49366	6.42553
GD4	A	16/11/2010	0	6.52	0.094914	1.04908	0.090473	186.1896	101.8622	1	0.020598	0.272504	1.26459
GD4	B	16/11/2010	0	6.33	4.232264	4.99692	0.846974	289.6233			0.142975	10.9814	12.0963
GD4	C1	16/11/2010	0	6.5	0.282975	0.954111	0.296585	252.011	144.115	1	0.013241	6.45682	7.48761
GD4	C2	16/11/2010	0		0.188945	1.0632	0.177713	223.8018	210.782	1.0617692	0.010088	5.32195	6.69748
GD4	A	20/11/2010	4	6.04	5.066621	5.00808	1.011689	423.0357	218.856	1	0.045766	7.35473	9.21929
GD4	B	20/11/2010	4	5.92	7.161795	9.65174	0.742021	499.2239			0.133076	20.2533	24.8347
GD4	C1	20/11/2010	4	6.53	1.257214	1.48841	0.844669	261.1359	157.418	1	0.01891	9.98532	11.7723

Location	Sample	Date	Day	Sol As(III)/ T As	T As ppm	Sol As/ T As	Sol Mn ppm	T Mn ppm	Sol/T Mn ppm	Sol Al ppm	T Al ppm	Sol/T Al ppm
GD1	A	16/11/2010	0	0.39240045	1178.94	0.000786	1.15844	3.12978	0.370135	0.735747	66.1684	0.011119
GD1	B	16/11/2010	0	0.08924545	1291.246	0.000702	0.997563	3.0104	0.331372	0.698232	73.1704	0.009543
GD1	C1	16/11/2010	0	0.3714881	1682.424	0.001038	1.388	3.6114	0.384338	0.609542	82.7136	0.007369
GD1	C2	16/11/2010	0	0.41496801	1100.46	0.001751	1.02156	2.55244	0.400229	0.51366	56.0162	0.00917
GD1	A	20/11/2010	4	0.12046544	1646.86	0.001587	1.43478	3.86698	0.371034	1.09963	92.7548	0.011855
GD1	B	20/11/2010	4	0.10783392	1572.118	0.001675	1.26691	3.58986	0.352913	1.05618	92.0028	0.01148
GD1	C1	20/11/2010	4	0.44753742	2003.7	0.001682	1.54331	4.21058	0.366531	1.61595	105.6806	0.015291
GD1	C2	20/11/2010	4	0.49307092	1697.294	0.002328	1.1253	3.3105	0.339918	1.50413	89.4218	0.016821
GD1	A	26/11/2010	10	0.38483517	1652.162	0.003966	1.56191	3.96462	0.393962	1.18948	95.783	0.012418
GD1	C1	26/11/2010	10	0.46852606	1598.284	0.002914	1.59153	4.00178	0.397706	2.45706	89.5182	0.027448
GD1	A	04/12/2010	18	0.54797127	2124.18	0.009006	1.58031	4.74008	0.333393	0.639069	125.045	0.005111
GD1	B	04/12/2010	18	0.41765962	2712.82	0.004661	1.39809	5.41144	0.258358	0.927031	163.1228	0.005683
GD1	C1	04/12/2010	18	0.22025292	3970.32	0.001357	1.62562	6.92318	0.234808	3.23026	205.94	0.015685
GD1	C2	04/12/2010	18	0.54440838	1520.808	0.00374	1.19845	3.50946	0.341491	3.16039	86.627	0.036483
GD1	A	12/10/2010	24	1.82163572	2014.4	0.010102	1.52905	4.56778	0.334747	0.457085	127.555	0.003583
GD1	B	12/10/2010	24	0.50620494	1302.358	0.012611	1.40362	3.4408	0.407934	0.789532	84.3184	0.009364
GD1	C1	12/10/2010	24	0.51629188			1.62343	8.43152	0.192543	3.67903	276.486	0.013306
GD1	C2	12/10/2010	24	0.57327091	2075.18	0.00292	1.18356	4.11758	0.287441	3.54792	116.1548	0.030545
GD1	A	16/12/2010	30	0.51273173	2005.5	0.008292	1.3921	4.83956	0.28765	0.362649	126.5012	0.002867
GD1	B	16/12/2010	30	0.56571489	1535.438	0.012846	1.38386	3.6686	0.377217	0.633295	100.2468	0.006317
GD1	C1	16/12/2010	30	0.44614907	2026.66	0.003642	1.61323	4.7375	0.340523	4.07592	120.9444	0.033701
GD1	C2	16/12/2010	30	0.53098371	1882.502	0.003351	1.20443	3.8616	0.311899	4.04149	108.4786	0.037256
GD1	A	21/12/2010	35	0.42018984	2922.08	0.005956	1.58166	5.97372	0.26477	0.416112	183.773	0.002264
GD1	B	21/12/2010	35	0.52073075	3129.14	0.006159	1.42681	5.88028	0.242643	0.602834	191.2184	0.003153
GD1	C1	21/12/2010	35	0.53485849	2450.86	0.002478	1.65871	5.11882	0.324041	4.44657	143.4222	0.031003
GD1	C2	21/12/2010	35	0.54371546	2821.5	0.002277	1.24013	4.77762	0.259571	4.47956	149.6374	0.029936
GD4	A	16/11/2010	0	0.21548802	33.045	0.038269	0.338685	1.448588	0.233804	0.049612	17.94362	0.002765
GD4	B	16/11/2010	0	0.90783132	21.352	0.566518	0.782172	1.108252	0.705771	0.05391	14.95838	0.003604
GD4	C1	16/11/2010	0	0.86233391	41.9914	0.178313	0.451543	2.86556	0.157576	0.05769	30.5482	0.001888
GD4	C2	16/11/2010	0	0.79461977	45.1964	0.148186	0.413443	3.59328	0.11506	0.069045	54.857	0.001259
GD4	A	20/11/2010	4	0.79775449	68.5226	0.134544	0.761205	2.71408	0.280465	0.025722	38.848	0.000662
GD4	B	20/11/2010	4	0.81552425	50.0774	0.495926	0.936625	2.52426	0.371049	0.042234	32.434	0.001302
GD4	C1	20/11/2010	4	0.84820468	45.5296	0.258564	0.461522	2.70708	0.170487	0.055112	34.3284	0.001605

Location	Sample	Date	Day	Sol S ppm	T S ppm	Sol/T S ppm	Sol Si ppm	T Si ppm	Sol/T Si ppm	Sol Ca ppm	T Ca ppm	Sol/T Ca ppm	Sol Na ppm
GD1	A	16/11/2010	0	27.9765	81.976	0.341277	3.93102	92.9612	0.042287	10.839	12.66718	0.855676	10.8425
GD1	B	16/11/2010	0	29.1469	84.3766	0.345438	3.93435	101.5438	0.038745	10.7059	12.80476	0.836088	10.921
GD1	C1	16/11/2010	0	37.4701	92.2128	0.406344	3.5798	113.1264	0.031644	11.1343	12.5938	0.88411	11.0172
GD1	C2	16/11/2010	0	35.8733	78.364	0.457778	3.53599	81.9236	0.043162	10.5353	11.58262	0.909578	10.8571
GD1	A	20/11/2010	4	56.1734	113.8504	0.493397	5.6568	128.2558	0.044106	10.3106	14.18902	0.72666	10.2652
GD1	B	20/11/2010	4	56.5568	113.1162	0.499989	5.70591	122.6912	0.046506	10.1959	13.41176	0.760221	10.3114
GD1	C1	20/11/2010	4	57.6154	121.5886	0.473855	5.60822	134.7212	0.041628	11.1438	13.29334	0.838299	10.6309
GD1	C2	20/11/2010	4	59.1464	111.8876	0.528623	5.63688	117.4048	0.048012	10.5473	12.00218	0.878782	10.4852
GD1	A	26/11/2010	10	99.9699	140.1508	0.713302	7.65284	128.3212	0.059638	10.1727	13.55452	0.750502	10.2665
GD1	C1	26/11/2010	10	70.5219	127.7072	0.552216	7.71037	121.296	0.063567	11.1674	13.41232	0.832623	10.6413
GD1	A	04/12/2010	18		195.1886		10.0357	153.9614	0.065183	9.57943	16.64314	0.575578	9.84355
GD1	B	04/12/2010	18		196.9838		9.46493	178.4608	0.053036	9.41982	17.77364	0.529988	9.71938
GD1	C1	04/12/2010	18	77.4786	231.804	0.334242	10.0752	197.1268	0.05111	11.1715	17.61806	0.634094	10.6221
GD1	C2	04/12/2010	18	76.8191	147.6002	0.520454	10.2275	120.3972	0.084948	10.5864	13.04454	0.811558	10.494
GD1	A	12/10/2010	24		203.426		11.2406	148.7378	0.075573	8.95847	13.03006	0.687523	9.68622
GD1	B	12/10/2010	24		180.229		11.0608	118.9176	0.093012	9.10676	12.37832	0.735702	9.78713
GD1	C1	12/10/2010	24	76.7884	265.382	0.28935	11.6121	209.39	0.055457	10.9891	19.22546	0.571591	10.7816
GD1	C2	12/10/2010	24	76.3005	174.036	0.438418	11.4095	149.008	0.07657	10.2622	14.3341	0.715929	10.5589
GD1	A	16/12/2010	30		254.782		11.9034	162.8262	0.073105	8.04998	12.03586	0.668833	8.94264
GD1	B	16/12/2010	30		219.002		12.3139	130.79	0.09415	8.84545	9.55268	0.925965	9.55291
GD1	C1	16/12/2010	30	78.1742	155.916	0.501387	13.2435	151.58	0.08737	10.8717	12.84862	0.846138	10.6102
GD1	C2	16/12/2010	30	78.2087	148.2532	0.527535	13.1295	142.3704	0.092221	10.3666	11.9914	0.864503	10.6667
GD1	A	21/12/2010	35		262.95		14.5444	190.4354	0.076374	8.86242	15.28056	0.57998	9.70218
GD1	B	21/12/2010	35		269.28		13.6976	193.663	0.070729	8.98833	13.6972	0.656217	9.64804
GD1	C1	21/12/2010	35	80.0997	162.818	0.491959	14.4553	165.9436	0.08711	11.1214	12.69024	0.876374	10.755
GD1	C2	21/12/2010	35	80.6109	175.7168	0.458755	14.4425	173.864	0.083068	10.5558	12.95094	0.815061	11.1463
GD4	A	16/11/2010	0	6.8276	16.33904	0.41787	2.96541	31.5048	0.094126	17.2566	29.6418	0.582171	10.2061
GD4	B	16/11/2010	0	6.18075	15.85562	0.389814	3.06737	27.8652	0.110079	19.1149	27.2026	0.702687	10.1829
GD4	C1	16/11/2010	0	7.00968	19.769	0.354579	3.18339	45.7562	0.069573	18.135	36.3588	0.498779	10.4668
GD4	C2	16/11/2010	0	6.94221	22.0166	0.315317	3.15771	74.7782	0.042228	17.537	39.1972	0.447404	10.4291
GD4	A	20/11/2010	4	7.11864	23.9842	0.296805	3.00175	56.2128	0.0534	27.7538	48.7848	0.568903	9.93181
GD4	B	20/11/2010	4	6.26013	20.8292	0.300546	3.15611	49.2126	0.064132	23.4755	39.5782	0.593142	9.87883
GD4	C1	20/11/2010	4	7.14671	19.34154	0.369501	3.11466	50.849	0.061253	22.7492	38.8198	0.586021	10.0728

Location	Sample	Date	Day	T Na ppm	Sol/T Na ppm	Sol K ppm	T K ppm	Sol/T K ppm	Sol Mg ppm	T Mg ppm	Sol/T Mg ppm
GD1	A	16/11/2010	0	25.3068	0.428442	1.64118	16.59988	0.098867	1.95485	42.5138	0.045982
GD1	B	16/11/2010	0	25.1688	0.43391	1.57537	17.82052	0.088402	1.92887	46.784	0.041229
GD1	C1	16/11/2010	0	25.3624	0.434391	1.68014	21.095	0.079646	2.34774	52.9968	0.0443
GD1	C2	16/11/2010	0	25.214	0.430598	1.5775	14.46746	0.109038	2.13277	36.0076	0.059231
GD1	A	20/11/2010	4	26.124	0.392941	1.78157	24.4448	0.072881	2.17038	59.4522	0.036506
GD1	B	20/11/2010	4	25.1574	0.409875	2.37249	22.1178	0.107266	2.15414	58.7644	0.036657
GD1	C1	20/11/2010	4	25.2788	0.420546	2.07571	24.9736	0.083116	2.79369	68.169	0.040982
GD1	C2	20/11/2010	4	24.8232	0.422395	1.96477	21.7644	0.090274	2.58037	57.5546	0.044833
GD1	A	26/11/2010	10	24.982	0.410956	1.87264	22.934	0.081653	2.44	62.0076	0.03935
GD1	C1	26/11/2010	10	26.1232	0.407351	2.48822	21.2022	0.117357	3.06416	57.0914	0.053671
GD1	A	04/12/2010	18	24.5376	0.401162	2.00683	29.39	0.068283	2.70727	79.9116	0.033878
GD1	B	04/12/2010	18	26.01	0.373679	1.53706	37.0952	0.041436	2.6023	102.0684	0.025496
GD1	C1	04/12/2010	18	27.0304	0.392969	2.69785	46.5876	0.057909	3.37374	128.937	0.026166
GD1	C2	04/12/2010	18	27.1174	0.386984	2.61023	21.0256	0.124145	3.26025	54.3716	0.059962
GD1	A	12/10/2010	24	24.6464	0.393007	2.15724	28.126	0.076699	2.79162	82.0036	0.034043
GD1	B	12/10/2010	24	24.387	0.401326	1.52324	19.75014	0.077126	2.75642	53.994	0.05105
GD1	C1	12/10/2010	24	25.833	0.417358	2.76242	60.9106	0.045352	3.55076	175.862	0.020191
GD1	C2	12/10/2010	24	25.0094	0.422197	2.60397	26.9912	0.096475	3.293	73.2748	0.04494
GD1	A	16/12/2010	30	24.6926	0.362159	2.26825	28.5976	0.079316	2.77589	81.6974	0.033978
GD1	B	16/12/2010	30	21.6686	0.440864	1.58446	22.4532	0.070567	2.94878	65.0628	0.045322
GD1	C1	16/12/2010	30	26.022	0.40774	2.89462	25.927	0.111645	3.81173	76.1796	0.050036
GD1	C2	16/12/2010	30	26.4274	0.403623	2.74846	24.0098	0.114472	3.57941	67.867	0.052742
GD1	A	21/12/2010	35	25.4498	0.381228	2.72622	40.0242	0.068114	3.28467	117.2392	0.028017
GD1	B	21/12/2010	35	24.7198	0.390296	1.73357	40.804	0.042485	3.17333	121.2488	0.026172
GD1	C1	21/12/2010	35	23.9072	0.449864	2.99076	30.5728	0.097824	4.03482	89.4762	0.045094
GD1	C2	21/12/2010	35	24.1782	0.461006	2.83178	33.763	0.083872	3.82517	93.8388	0.040763
GD4	A	16/11/2010	0	24.51	0.416406	2.20621	5.5929	0.394466	1.06218	11.74382	0.090446
GD4	B	16/11/2010	0	24.4722	0.416101	1.33056	4.71452	0.282226	1.50342	10.19094	0.147525
GD4	C1	16/11/2010	0	24.63	0.424961	1.53245	7.65006	0.200319	1.25858	19.11112	0.065856
GD4	C2	16/11/2010	0	22.948	0.454467	1.42944	9.2637	0.154306	1.21584	34.664	0.035075
GD4	A	20/11/2010	4	24.4924	0.405506	2.95051	9.77872	0.301728	1.37234	24.7014	0.055557
GD4	B	20/11/2010	4	25.159	0.392656	1.58144	7.23774	0.218499	1.59108	20.743	0.076704
GD4	C1	20/11/2010	4	25.2148	0.39948	1.69753	7.24662	0.234251	1.2938	21.2788	0.060802

Location	Sample	Date	Day	pH	Sol Fe(II) ppm	Sol T Fe ppm	Sol Fe(II)/T Fe	Tot Fe(II) ppm	Tot T Fe ppm	Tot Fe(II)/ T Fe	Sol Fe/ T Fe	Sol As(III) ppm	Sol T As ppm
GD4	C2	20/11/2010	4	6.54	1.352449	1.18309	1.14315	289.7065	334.066	0.8672133	0.007083	8.4599	10.0559
GD4	A	26/11/2010	10	6.04	7.004403	5.61698	1.247005		210.45		0.053381		12.716
GD4	C1	26/11/2010	10	6.37	1.015227	1.37441	0.738664	472.5167	172.8184	1	0.015906		14.1953
GD4	A	04/12/2010	18	6.29	8.76337	5.2114	1.681577	311.2729	358.324	0.8686912	0.029088	15.3658	17.5023
GD4	B	04/12/2010	18	6.42	9.344251	7.01431	1.33217	379.0424	128.2064	1	0.109422	30.2322	33.7606
GD4	C1	04/12/2010	18	6.77	1.308729	1.14479	1.143205	146.6899	316.962		0.007224	13.8752	15.6952
GD4	C2	04/12/2010	18	6.88	1.88961	1.04166	1.814037	166.0526	431.436		0.004829	12.0222	13.2868
GD4	A	12/10/2010	24	6.47	1.194826	2.14323	0.557489		257.214		0.016665	8.64526	14.0545
GD4	B	12/10/2010	24	6.56	6.183514	6.89507	0.896802		235.736		0.058498	31.665	35.6673
GD4	C1	12/10/2010	24	6.78	0	0.817071	0		295.686		0.005527	14.1118	15.9496
GD4	C2	12/10/2010	24	7.04	0	0.770806	0		320.23		0.004814	12.442	13.758
GD4	A	16/12/2010	30	6.27		1.06582		964.7258	380.188	1	0.005607	9.121	12.8181
GD4	B	16/12/2010	30	6.71	5.739721	5.23884	1.095609	817.5915			0.042252	33.2811	34.9733
GD4	C1	16/12/2010	30	7.12	2.502765	0.251486	9.951907	572.3675	289.336	1	0.001738	13.0783	14.6786
GD4	C2	16/12/2010	30	7.2	0.442884	0.5723	0.773867		433.01		0.002643	12.7024	13.6246
GD4	A	21/12/2010	35	6.82		1.54096		642.5329	356.956	1	0.008634	13.1049	15.1663
GD4	B	21/12/2010	35	6.7	5.700455	6.70426	0.850274		211.162		0.063499	34.1546	36.4275
GD4	C1	21/12/2010	35	6.93	0.167111	0.455632	0.366768	209.9261	299.758	0.7003184	0.00304	13.5244	15.3109
GD4	C2	21/12/2010	35	7.1	0.167111	0.504303	0.331371	240.1079	250.06	0.9602013	0.004033	11.5903	13.7691
MG2	A	16/11/2010	0		0.000883	0.166165	0.005315	693.9552	737.556	0.9408848	0.000451	0.039063	1.1463
MG2	B	16/11/2010	0	7.06	0	0.028746	0	402.4601	585.84	0.6869796	9.81E-05	0.039278	1.01242
MG2	C1	16/11/2010	0	7.42	0.188945	0.006382	29.60585	458.8785	623.29	0.7362199	2.05E-05	0.019236	1.71201
MG2	C2	16/11/2010	0	7.56	0.000883	0.034612	0.025517	618.7307	683.17	0.905676	0.000101	0.036435	1.59573
MG2	A	20/11/2010	4	7.35	0.685803	0.010265	66.80982	832.547	650.626	1.2796092	3.16E-05	0.076919	1.45983
MG2	B	20/11/2010	4	7.51	0.590568	0.014885	39.67536	175.4243	588.626	0.2980233	5.06E-05	0.039197	1.57169
MG2	C1	20/11/2010	4	7.64	0.685803	0.007285	94.13903	299.23	704.562	0.4247036	2.07E-05		
MG2	C2	20/11/2010	4	7.6	0.685803	0.004003	171.3222	4.000973	580.318	0.0068944	1.38E-05		
MG2	A	26/11/2010	10	7.38	1.414505	0.018318	77.2194	448.56	410.066	1.0938727	8.93E-05	0.108955	1.57135
MG2	C1	26/11/2010	10	7.49	0.136814	0.022028	6.210909	256.9064	511.572	0.50219	8.61E-05	0.059863	3.36338
MG2	A	04/12/2010	18	7.34	1.59917	0.001119	1429.106	166.0526	415.106	0.4000247	5.39E-06	0.307068	1.54686
MG2	B	04/12/2010	18	7.48	1.570126	0.001266	1240.226	146.6899	747.334	0.1962843	3.39E-06	0.165159	1.76435
MG2	C1	04/12/2010	18	7.7	0.340594	0.037667	9.042236	1.469669	458.914	0.0032025	0.000164	0.054717	3.57756
MG2	C2	04/12/2010	18	7.48	0.146967	0.001978	74.30075	553.3067	390.79	1.4158671	1.01E-05	0.053113	3.44423

Location	Sample	Date	Day	Sol As(III)/ T As	T As ppm	Sol As/ T As	Sol Mn ppm	T Mn ppm	Sol/T Mn ppm	Sol Al ppm	T Al ppm	Sol/T Al ppm
GD4	C2	20/11/2010	4	0.8412872	65.4384	0.15367	0.420761	5.21398	0.080699	0.060644	89.3404	0.000679
GD4	A	26/11/2010	10		66.948	0.189938	0.828687	3.13114	0.26466	0.082461	38.1832	0.00216
GD4	C1	26/11/2010	10		53.6052	0.264812	0.416625	2.9414	0.141642	0.074223	37.1178	0.002
GD4	A	04/12/2010	18	0.87793033	98.433	0.177809	0.849812	5.28486	0.160801	0.025108	70.4352	0.000356
GD4	B	04/12/2010	18	0.89548764	46.1798	0.731069	0.852591	2.50098	0.340903	0.009151	29.6722	0.000308
GD4	C1	04/12/2010	18	0.88404098	74.6326	0.2103	0.406102	4.84658	0.083791	0.094657	75.5386	0.001253
GD4	C2	04/12/2010	18	0.90482283	82.1774	0.161684	0.385276	6.42632	0.059953	0.048308	118.1066	0.000409
GD4	A	12/10/2010	24	0.61512398	81.728	0.171967	0.505288	3.62944	0.139219	0.022085	46.3578	0.000476
GD4	B	12/10/2010	24	0.88778797	68.0132	0.524417	0.821086	3.72482	0.220436	0.015293	55.9432	0.000273
GD4	C1	12/10/2010	24	0.88477454	72.1432	0.221083	0.377153	4.41338	0.085457	0.031981	68.3718	0.000468
GD4	C2	12/10/2010	24	0.90434656	64.7172	0.212586	0.337882	4.85502	0.069594	0.034257	89.0482	0.000385
GD4	A	16/12/2010	30	0.71157192	109.968	0.116562	0.312787	5.22458	0.059868	0.028187	74.14	0.00038
GD4	B	16/12/2010	30	0.95161452	77.61	0.450629	0.720021	3.85546	0.186754	0.022547	55.9562	0.000403
GD4	C1	16/12/2010	30	0.89097734	78.0754	0.188005	0.236996	4.46888	0.053033	0.037144	61.186	0.000607
GD4	C2	16/12/2010	30	0.93231361	90.8736	0.149929	0.289235	6.3853	0.045297	0.110183	113.4982	0.000971
GD4	A	21/12/2010	35	0.86408023	98.812	0.153486	0.361424	4.96336	0.072818	0.035887	71.5598	0.000501
GD4	B	21/12/2010	35	0.93760483	58.5976	0.621655	0.752669	3.59242	0.209516	0.016747	55.2558	0.000303
GD4	C1	21/12/2010	35	0.88331842	79.7516	0.191982	0.282763	4.57836	0.061761	0.032087	66.4198	0.000483
GD4	C2	21/12/2010	35	0.84176163	64.9612	0.211959	0.286023	4.01338	0.071267	0.039412	63.4152	0.000621
MG2	A	16/11/2010	0	0.03407747	214.104	0.005354	0.003465	16.1313	0.000215	0.018407	176.9186	0.000104
MG2	B	16/11/2010	0	0.03879615	196.1956	0.00516	0.002419	12.8344	0.000188	0.013672	123.8606	0.00011
MG2	C1	16/11/2010	0	0.01123592	182.5216	0.00938	0.008128	13.47338	0.000603	0.012833	132.5024	9.69E-05
MG2	C2	16/11/2010	0	0.02283281	187.9402	0.008491	0.009181	14.19664	0.000647	0.026228	161.3016	0.000163
MG2	A	20/11/2010	4	0.05269038	190.4326	0.007666	0.110152	13.69514	0.008043	0.008879	154.858	5.73E-05
MG2	B	20/11/2010	4	0.0249394	201.964	0.007782	0.08549	12.9449	0.006604	0.01592	124.9256	0.000127
MG2	C1	20/11/2010	4				0.009922	15.347	0.000647	0.015726	152.5124	0.000103
MG2	C2	20/11/2010	4				0.009843	12.51914	0.000786	0.011845	138.2936	8.57E-05
MG2	A	26/11/2010	10	0.06933847	125.5704	0.012514	0.226055	10.3049	0.021937	0.012593	92.4416	0.000136
MG2	C1	26/11/2010	10	0.01779846	153.2638	0.021945	0.241772	12.39356	0.019508	0.008305	108.4806	7.66E-05
MG2	A	04/12/2010	18	0.19851053	138.0664	0.011204	0.19573	13.19786	0.01483	0.009431	86.9474	0.000108
MG2	B	04/12/2010	18	0.09360898	248.954	0.007087	0.010178	17.58334	0.000579	0.022361	143.8358	0.000155
MG2	C1	04/12/2010	18	0.0152945	144.8526	0.024698	0.011246	11.9158	0.000944	0.025739	91.3492	0.000282
MG2	C2	04/12/2010	18	0.01542086	122.9792	0.028007	0.011926	9.88812	0.001206	0.010824	90.891	0.000119

Location	Sample	Date	Day	Sol S ppm	T S ppm	Sol/T S ppm	Sol Si ppm	T Si ppm	Sol/T Si ppm	Sol Ca ppm	T Ca ppm	Sol/T Ca ppm	Sol Na ppm
GD4	C2	20/11/2010	4	7.05019	34.1546	0.20642	3.12728	115.4108	0.027097	22.0784	56.5576	0.39037	10.097
GD4	A	26/11/2010	10	6.59733	23.069	0.285982	3.45194	55.5554	0.062135	30.8188	51.2532	0.601305	9.65991
GD4	C1	26/11/2010	10	7.31955	20.4876	0.357267	3.17161	54.3412	0.058365	25.9104	46.9226	0.552194	10.1947
GD4	A	04/12/2010	18	4.57137	35.786	0.127742	3.25439	93.57	0.03478	32.9708	70.275	0.469168	9.82711
GD4	B	04/12/2010	18	1.19251	16.91508	0.0705	3.11933	43.4446	0.0718	24.3316	32.915	0.739225	9.72851
GD4	C1	04/12/2010	18	7.31015	34.2218	0.213611	3.11703	98.7786	0.031556	27.827	66.0288	0.421437	10.1432
GD4	C2	04/12/2010	18	7.2017	39.5644	0.182025	3.08677	140.3084	0.022	26.6185	74.6318	0.356664	10.0636
GD4	A	12/10/2010	24	4.65556	24.3198	0.191431	3.21844	65.6452	0.049028	27.7863	54.8508	0.50658	9.81693
GD4	B	12/10/2010	24	0.635767	27.6234	0.023016	3.08674	77.7982	0.039676	23.3766	48.8166	0.478866	9.70432
GD4	C1	12/10/2010	24	6.97207	33.2556	0.209651	3.04672	92.4464	0.032957	27.7437	59.345	0.467499	10.058
GD4	C2	12/10/2010	24	6.79744	30.9062	0.219938	3.04917	114.4108	0.026651	26.8152	59.7738	0.448611	10.0549
GD4	A	16/12/2010	30	4.68315	33.9658	0.137878	3.07576	99.2388	0.030994	23.0915	67.5464	0.341861	9.51935
GD4	B	16/12/2010	30	0.556132	26.3072	0.02114	3.06379	77.706	0.039428	22.6871	43.598	0.52037	9.6838
GD4	C1	16/12/2010	30	6.89619	27.4436	0.251286	2.93913	82.5758	0.035593	26.175	58.2268	0.449535	9.89675
GD4	C2	16/12/2010	30	7.07807	38.3576	0.184528	3.11625	142.2864	0.021901	27.2999	73.616	0.370842	10.1374
GD4	A	21/12/2010	35	4.21898	33.221	0.126997	2.97314	95.5542	0.031115	23.1127	59.7028	0.387129	9.54693
GD4	B	21/12/2010	35	0.572864	26.3072	0.021776	2.99953	78.1758	0.038369	22.606	46.2592	0.488681	9.5753
GD4	C1	21/12/2010	35	6.75192	30.079	0.224473	2.9506	89.9574	0.0328	27.0783	62.6498	0.432217	9.99406
GD4	C2	21/12/2010	35	6.68855	23.2406	0.287796	3.01435	86.2366	0.034954	27.6651	61.2912	0.451371	9.97556
MG2	A	16/11/2010	0	8.69267	69.8598	0.12443	2.1944	192.9852	0.011371	14.7638	131.0126	0.11269	31.3688
MG2	B	16/11/2010	0	8.56173	67.9734	0.125957	2.09605	155.0054	0.013522	13.9306	107.7842	0.129245	31.4722
MG2	C1	16/11/2010	0	10.0323	56.7886	0.17666	2.00562	149.3944	0.013425	13.6503	102.4804	0.133199	31.7995
MG2	C2	16/11/2010	0	9.92123	53.792	0.184437	2.02553	172.0874	0.01177	13.7823	112.4606	0.122552	31.8908
MG2	A	20/11/2010	4	8.98857	56.569	0.158896	2.15888	161.6806	0.013353	16.0601	112.7104	0.14249	30.564
MG2	B	20/11/2010	4	8.8081	65.2352	0.135021	2.03824	156.473	0.013026	15.0467	107.0914	0.140503	30.9021
MG2	C1	20/11/2010	4	10.496	58.8724	0.178284	2.06236	160.6002	0.012842	14.5638	115.0654	0.12657	32.1064
MG2	C2	20/11/2010	4	10.2201	43.1686	0.236748	2.01545	161.9052	0.012448	14.3159	96.8926	0.14775	31.5334
MG2	A	26/11/2010	10	8.8135	32.4072	0.271961	1.98412	126.4414	0.015692	17.5374	80.1826	0.218718	30.0058
MG2	C1	26/11/2010	10	8.72297	36.294	0.240342	1.97399	136.2876	0.014484	17.6356	89.7408	0.196517	31.3057
MG2	A	04/12/2010	18	8.41823	25.5242	0.329814	1.91081	135.3282	0.01412	15.68	89.7086	0.174788	31.384
MG2	B	04/12/2010	18	10.233	54.1352	0.189027	2.02726	136.003	0.014906	14.8468	131.9996	0.112476	31.2511
MG2	C1	04/12/2010	18	9.90824	27.889	0.355274	2.03218	131.842	0.015414	15.1671	84.7804	0.178899	32.0117
MG2	C2	04/12/2010	18	10.1921	22.8136	0.446755	2.08952	130.9612	0.015955	15.8007	74.8736	0.211032	33.4002

Location	Sample	Date	Day	T Na ppm	Sol/T Na ppm	Sol K ppm	T K ppm	Sol/T K ppm	Sol Mg ppm	T Mg ppm	Sol/T Mg ppm
GD4	C2	20/11/2010	4	26.5896	0.379735	1.57939	16.7564	0.094256	1.26057	56.4524	0.02233
GD4	A	26/11/2010	10	24.3492	0.396724	3.07462	9.6825	0.317544	1.46828	24.0612	0.061023
GD4	C1	26/11/2010	10	24.741	0.412057	2.36917	8.39358	0.28226	1.36386	22.8278	0.059746
GD4	A	04/12/2010	18	25.996	0.378024	3.09342	15.15004	0.204186	1.51158	44.6398	0.033862
GD4	B	04/12/2010	18	20.6486	0.471146	1.67642	6.1662	0.271872	1.55575	18.55868	0.083829
GD4	C1	04/12/2010	18	24.282	0.417725	1.79099	14.34184	0.124879	1.3789	47.9258	0.028772
GD4	C2	04/12/2010	18	24.846	0.405039	1.68512	20.818	0.080945	1.33448	74.9472	0.017806
GD4	A	12/10/2010	24	23.7506	0.413334	2.90807	10.30398	0.282228	1.31718	29.3112	0.044938
GD4	B	12/10/2010	24	24.6874	0.393088	1.63214	11.2155	0.145525	1.50038	35.4454	0.042329
GD4	C1	12/10/2010	24	25.5652	0.393425	1.77412	13.04432	0.136007	1.34714	43.2664	0.031136
GD4	C2	12/10/2010	24	24.496	0.410471	1.55961	15.26554	0.102165	1.30382	56.4632	0.023092
GD4	A	16/12/2010	30	23.6534	0.402452	2.74661	14.60826	0.188018	1.12566	46.7866	0.024059
GD4	B	16/12/2010	30	23.662	0.409255	1.59871	10.4882	0.152429	1.4734	35.213	0.041843
GD4	C1	16/12/2010	30	25.2198	0.39242	1.72646	11.46472	0.150589	1.24343	37.777	0.032915
GD4	C2	16/12/2010	30	25.5886	0.396169	2.02388	19.13764	0.105754	1.37268	71.436	0.019216
GD4	A	21/12/2010	35	24.55	0.388877	2.72739	13.98604	0.195008	1.13498	45.043	0.025198
GD4	B	21/12/2010	35	25.6836	0.372818	1.59925	11.56882	0.138238	1.46847	34.737	0.042274
GD4	C1	21/12/2010	35	25.097	0.398217	1.69768	12.58324	0.134916	1.28458	41.3604	0.031058
GD4	C2	21/12/2010	35	27.1486	0.367443	1.58322	11.81652	0.133984	1.30144	39.0984	0.033286
MG2	A	16/11/2010	0	52.3942	0.598707	4.03773	68.0572	0.059328	5.31301	140.4324	0.037833
MG2	B	16/11/2010	0	50.8532	0.618883	2.66911	46.8454	0.056977	5.00748	102.0316	0.049078
MG2	C1	16/11/2010	0	48.798	0.651656	2.65469	48.8584	0.054334	5.40365	105.5534	0.051194
MG2	C2	16/11/2010	0	52.953	0.602247	2.80198	59.4528	0.047129	5.43767	123.3226	0.044093
MG2	A	20/11/2010	4	48.0614	0.635937	4.06777	58.7552	0.069233	6.09035	122.3612	0.049774
MG2	B	20/11/2010	4	50.8998	0.607116	2.89729	46.7778	0.061937	5.7156	102.0228	0.056023
MG2	C1	20/11/2010	4	51.0562	0.628844	2.84412	55.0034	0.051708	6.01189	120.5648	0.049864
MG2	C2	20/11/2010	4	50.7918	0.620836	2.81243	50.7738	0.055391	5.8807	105.324	0.055834
MG2	A	26/11/2010	10	46.8148	0.640947	6.39096	39.078	0.163544	6.53874	75.2644	0.086877
MG2	C1	26/11/2010	10	51.242	0.610938	4.98586	40.3374	0.123604	6.74052	85.7802	0.078579
MG2	A	04/12/2010	18	52.6032	0.596618	3.01242	36.328	0.082923	6.06731	71.198	0.085217
MG2	B	04/12/2010	18	46.6926	0.669294	2.89717	52.947	0.054718	6.17963	113.5362	0.054429
MG2	C1	04/12/2010	18	51.4756	0.621881	3.019	34.8678	0.086584	6.33997	73.2928	0.086502
MG2	C2	04/12/2010	18	52.164	0.640292	2.99171	35.04	0.08538	6.56068	68.3346	0.096008

Location	Sample	Date	Day	pH	Sol Fe(II) ppm	Sol T Fe ppm	Sol Fe(II)/T Fe	Tot Fe(II) ppm	Tot T Fe ppm	Tot Fe(II)/ T Fe	Sol Fe/ T Fe	Sol As(III) ppm	Sol T As ppm
MG2	A	12/10/2010	24	7.33	0	0.018083	0		257.214		0.000141	0.338573	1.73424
MG2	B	12/10/2010	24	7.47	0	0.025142	0		235.736		0.000213	0.204161	1.92045
MG2	C1	12/10/2010	24	7.61	0	0.021342	0		295.686		0.000144	0.058803	3.76634
MG2	C2	12/10/2010	24	7.64	0	0.004867	0		320.23		3.04E-05	0.061957	3.46029
MG2	A	16/12/2010	30	7.67	0.540974	0.011592	46.66784		555.186		4.18E-05	0.326526	1.81984
MG2	B	16/12/2010	30	7.67	0.540974	0.009058	59.72329		298.992		6.06E-05	0.254419	2.00923
MG2	C1	16/12/2010	30	7.84	0.344794	0.019654	17.54322	768.5467	410.652	1	9.57E-05	0.059701	3.75805
MG2	C2	16/12/2010	30	7.86	0.344794	0.010468	32.93794	3.447944	337.304	0.0102221	6.21E-05	0.037864	3.49907
MG2	A	21/12/2010	35	7.62	0	0.009546	0	340.7142	640.18	0.5322162	2.98E-05	0.378325	1.83087
MG2	B	21/12/2010	35	7.66	0	0.009763	0	894.0486	960.026	0.9312754	2.03E-05	0.439864	2.08977
MG2	C1	21/12/2010	35	7.76	0	0.049867	0	199.8654	1131.468	0.1766426	8.81E-05	0.073865	3.92561
MG2	C2	21/12/2010	35	7.75	0	0.006831	0					0.071017	3.55617
GD2	A	27/09/2010	0	7.4	0.521854	6.68974	0.078008	106.8571	688.928	0.1551064	0.019421	0.031217	3.43062
GD2	B	27/09/2010	0	7.99	0.521854	0.241065	2.164787	192.3475	823.346	0.2336169	0.000586	0.035486	2.12077
GD2	C1	27/09/2010	0	7.98	0.236886	0.214249	1.10566	97.3582	933.906	0.1042484	0.000459	0.041277	3.481
GD2	C2	27/09/2010	0	7.52	0.236886	0.275223	0.860707	201.8465	1012.078	0.1994377	0.000544	0.043741	3.44118
GD2	A	02/10/2010	5	7.48	0	0.044832	0	421.1706	1077.81	0.3907652	8.32E-05	0.019038	2.35598
GD2	B	02/10/2010	5	7.67	0	0.034984	0	388.2627	1143.526	0.3395311	6.12E-05	0.015262	2.30248
GD2	C1	02/10/2010	5	7.57	0	0.041673	0	267.6003	2079.38	0.1286924	4.01E-05	0.005449	4.01516
GD2	C2	02/10/2010	5	7.85	0	0.042005	0	190.8152	1902.598	0.1002919	4.42E-05	0.016522	4.02246
GD2	A	05/10/2010	9	6.78	0	0.154752	0	849.3762	2038.68	0.4166305	0.000152	0.050485	2.36231
GD2	B	05/10/2010	9	7.51	0	0.065815	0	745.1123	775.112	0.9612963	0.00017	0.11213	2.29298
GD2	C1	05/10/2010	9	7.85	0	0.074699	0	692.9804	1170.272	0.5921532	0.000128	0.044002	4.06463
GD2	A	12/10/2010	15	6.24	0	0.068349	0	842.0795	604.59	1	0.000226	0.030133	2.26374
GD2	B	12/10/2010	15	7.44	0	17.4463	0	486.7712	613.646	0.7932444	0.056861	0.019407	5.64144
GD2	C1	12/10/2010	15	7.65	0	0.055853	0	377.0254	945.088	0.3989315	0.000118	0.006774	4.01613
GD2	A	16/10/2010	19		0.003084	0.63988	0.00482	559.0513	1783.05	0.3135365	0.000718	0.041997	2.32029
GD2	B	16/10/2010	19	7.26	0	0.025739	0	518.9062	1568.428	0.3308448	3.28E-05	0.01892	2.36945
GD2	C1	16/10/2010	19	7.42	0.003084	0.279999	0.011014	167.6366	1575.466	0.1064045	0.000355	0.007725	4.07691
GD2	C2	16/10/2010	19	7.58	0	0.112781	0	137.5278	1153.46	0.1192307	0.000196		4.03862
GD2	A	22/10/2010	22	9.37	0.278359	0.013859	20.08509	1842.979	719.352	1	3.85E-05	0.019045	2.38208
GD2	B	22/10/2010	22	8.25	0.470146	0.006166	76.24817	1881.337	489.9	1	2.52E-05	0.0332	2.40482
GD2	C1	22/10/2010	22	8.01	0.56604	0.239748	2.360978	136.0755	2026.3	0.0671547	0.000237	-0.01042	4.1361

Location	Sample	Date	Day	Sol As(III)/ T As	T As ppm	Sol As/ T As	Sol Mn ppm	T Mn ppm	Sol/T Mn ppm	Sol Al ppm	T Al ppm	Sol/T Al ppm
MG2	A	12/10/2010	24	0.19522846	142.556	0.012165	0.249946	12.87604	0.019412	0.00671	91.969	7.3E-05
MG2	B	12/10/2010	24	0.10630894	146.5788	0.013102	0.206927	10.29958	0.020091	0.016077	79.6566	0.000202
MG2	C1	12/10/2010	24	0.01561277	121.6544	0.030959	0.011034	9.99088	0.001104	0.016481	77.2604	0.000213
MG2	C2	12/10/2010	24	0.01790515	148.8998	0.023239	0.01179	12.1456	0.000971	0.009041	118.5524	7.63E-05
MG2	A	16/12/2010	30	0.17942566	174.169	0.010449	0.248415	13.663	0.018182	0.008172	123.1422	6.64E-05
MG2	B	16/12/2010	30	0.12662513	110.443	0.018192	0.201546	7.81784	0.02578	0.008363	57.6654	0.000145
MG2	C1	16/12/2010	30	0.01588616	134.006	0.028044	0.011326	11.13902	0.001017	0.014968	85.7656	0.000175
MG2	C2	16/12/2010	30	0.01082116	110.1148	0.031777	0.011282	8.80444	0.001281	0.010586	81.053	0.000131
MG2	A	21/12/2010	35	0.20663674	197.3138	0.009279	0.253937	15.37494	0.016516	0.00827	142.7416	5.79E-05
MG2	B	21/12/2010	35	0.21048441	315.1	0.006632	0.206353	21.0324	0.009811	0.008939	196.2766	4.55E-05
MG2	C1	21/12/2010	35	0.01881618	323.914	0.012119	0.011253	24.4542	0.00046	0.029179	247.026	0.000118
MG2	C2	21/12/2010	35	0.01997008								
GD2	A	27/09/2010	0	0.00909952	111.4984	0.030768	0.189342	15.386	0.012306	0.891405	157.1348	0.005673
GD2	B	27/09/2010	0	0.0167326	130.963	0.016194	0.01108	17.7165	0.000625	0.033753	186.8622	0.000181
GD2	C1	27/09/2010	0	0.0118578	151.1072	0.023037	0.011114	20.004	0.000556	0.035047	214.122	0.000164
GD2	C2	27/09/2010	0	0.01271105	175.2086	0.01964	0.017989	21.5956	0.000833	0.039904	211.202	0.000189
GD2	A	02/10/2010	5	0.00808071	175.5248	0.013422	0.107094	23.7936	0.004501	0.022394	268.764	8.33E-05
GD2	B	02/10/2010	5	0.0066285	183.9826	0.012515	0.10648	26.2122	0.004062	0.020016	280.992	7.12E-05
GD2	C1	02/10/2010	5	0.00135711	341.686	0.011751	0.004228	45.5888	9.27E-05	0.014974	471.3	3.18E-05
GD2	C2	02/10/2010	5	0.00410744	336.08	0.011969	0.006257	42.1994	0.000148	0.01313	386.328	3.4E-05
GD2	A	05/10/2010	9	0.02137103	337.276	0.007004	0.134487	46.5244	0.002891	0.017092	454.798	3.76E-05
GD2	B	05/10/2010	9	0.04890143	124.4138	0.01843	0.123886	17.8791	0.006929	0.018199	171.2372	0.000106
GD2	C1	05/10/2010	9	0.01082559	201.02	0.02022	0.004178	26.3276	0.000159	0.013961	246.176	5.67E-05
GD2	A	12/10/2010	15	0.01331116	97.6906	0.023173	0.226183	14.69948	0.015387	0.032154	138.519	0.000232
GD2	B	12/10/2010	15	0.00344008	100.478	0.056146	0.65205	14.1185	0.046184	2.69919	131.9096	0.020462
GD2	C1	12/10/2010	15	0.0016867	163.9408	0.024497	0.005149	22.2728	0.000231	0.020846	199.236	0.000105
GD2	A	16/10/2010	19	0.01809989	298.334	0.007777	0.293816	41.8436	0.007022	0.050016	403.016	0.000124
GD2	B	16/10/2010	19	0.00798498	258.52	0.009165	0.257019	38.2256	0.006724	0.031895	369.428	8.63E-05
GD2	C1	16/10/2010	19	0.00189482	270.584	0.015067	0.006749	37.894	0.000178	0.145832	324.982	0.000449
GD2	C2	16/10/2010	19		193.007	0.020925	0.014361	27.8078	0.000516	0.039564	272.59	0.000145
GD2	A	22/10/2010	22	0.00799511	102.6546	0.023205	0.277572	36.527	0.007599	0.027126	145.1164	0.000187
GD2	B	22/10/2010	22	0.01380561	83.619	0.028759	0.273935	17.61856	0.015548	0.02347	96.6268	0.000243
GD2	C1	22/10/2010	22	-0.00251928	239.73	0.017253	0.015572	94.1364	0.000165	0.012913	426.324	3.03E-05

Location	Sample	Date	Day	Sol S ppm	T S ppm	Sol/T S ppm	Sol Si ppm	T Si ppm	Sol/T Si ppm	Sol Ca ppm	T Ca ppm	Sol/T Ca ppm	Sol Na ppm
MG2	A	12/10/2010	24	8.48149	25.5886	0.331456	1.94462	130.1194	0.014945	17.3541	85.1836	0.203726	30.3967
MG2	B	12/10/2010	24	8.38483	31.5472	0.265787	1.91431	121.0428	0.015815	15.5169	83.1998	0.186502	30.538
MG2	C1	12/10/2010	24	9.68139	21.5814	0.448599	2.00301	117.0182	0.017117	15.1842	70.2096	0.21627	31.1038
MG2	C2	12/10/2010	24	9.68667	25.3868	0.381563	1.98682	156.4062	0.012703	15.3337	88.9768	0.172334	31.5633
MG2	A	16/12/2010	30	8.26215	35.4982	0.232748	1.92854	160.1562	0.012042	16.8945	103.0248	0.163985	29.7795
MG2	B	16/12/2010	30	8.10006	21.2118	0.381866	1.88295	93.02	0.020242	15.219	65.582	0.232061	29.7648
MG2	C1	16/12/2010	30	9.66386	21.9776	0.439714	1.98242	129.5082	0.015307	15.3323	75.9368	0.201909	30.8269
MG2	C2	16/12/2010	30	9.54836	18.42594	0.518202	1.9474	122.9832	0.015835	15.3139	66.1832	0.231387	31.0481
MG2	A	21/12/2010	35	8.21709	40.602	0.202381	1.96566	175.1832	0.011221	17.251	117.1914	0.147204	30.174
MG2	B	21/12/2010	35	8.05202	86.8546	0.092707	1.93036	187.786	0.01028	15.6001	167.2962	0.093248	30.2985
MG2	C1	21/12/2010	35	9.61125	93.4378	0.102863	2.00038	190.6762	0.010491	15.7345	178.8498	0.087976	31.1447
MG2	C2	21/12/2010	35										
GD2	A	27/09/2010	0	7.76553	16.8088	0.461992	4.40018	138.2728	0.031822	17.0355	88.5658	0.192349	11.1359
GD2	B	27/09/2010	0	7.99589	19.88578	0.402091	3.14498	134.968	0.023302	16.8416	110.0554	0.153028	11.0795
GD2	C1	27/09/2010	0	9.43061	21.535	0.43792	3.02495	144.5392	0.020928	17.5424	119.7054	0.146546	11.449
GD2	C2	27/09/2010	0	9.60439	23.3616	0.411119	3.00584	147.6182	0.020362	17.7203	128.5472	0.137851	11.4875
GD2	A	02/10/2010	5	7.89952	24.2762	0.325402	2.2699	150.7072	0.015062	23.287	140.9378	0.165229	10.9998
GD2	B	02/10/2010	5	8.22392	28.046	0.29323	2.27116	173.7716	0.01307	23.6474	169.1506	0.139801	11.1128
GD2	C1	02/10/2010	5	9.74577	18.49862	0.526838	2.11576	137.5996	0.015376	22.9663	263.804	0.087058	11.3838
GD2	C2	02/10/2010	5	10.2059	16.35274	0.624109	2.04508	147.6852	0.013848	23.6667	250.784	0.094371	11.4978
GD2	A	05/10/2010	9	7.8314	15.26016	0.513193	1.98763	165.2692	0.012027	22.9437	248.64	0.092277	10.913
GD2	B	05/10/2010	9	8.05603	18.88852	0.426504	1.99226	144.8148	0.013757	23.1815	102.3002	0.226603	10.9277
GD2	C1	05/10/2010	9	9.69815	27.1296	0.357475	1.94922	189.446	0.010289	23.3617	159.409	0.146552	11.079
GD2	A	12/10/2010	15	7.79109	14.5881	0.534072	1.74083	133.293	0.01306	23.8719	87.8094	0.27186	10.6016
GD2	B	12/10/2010	15	8.05913	15.50818	0.51967	5.81883	132.9012	0.043783	26.5169	86.157	0.307774	10.6509
GD2	C1	12/10/2010	15	9.57543	21.2256	0.451126	1.87318	158.2304	0.011838	23.7726	129.9944	0.182874	11.0351
GD2	A	16/10/2010	19	8.0277	31.9944	0.25091	1.73248	139.2968	0.012437	25.1168	215.178	0.116726	10.8618
GD2	B	16/10/2010	19	8.24266	30.4104	0.271047	1.65299	101.267	0.016323	25.2147	204.946	0.123031	10.9227
GD2	C1	16/10/2010	19	9.69673	28.9272	0.335211	2.06975	126.0564	0.016419	24.3826	204.496	0.119233	11.1652
GD2	C2	16/10/2010	19	10.2113	25.8568	0.394917	1.81658	131.9532	0.013767	24.392	158.346	0.154042	11.36
GD2	A	22/10/2010	22	8.15749	28.8522	0.282734	1.6783	134.6608	0.012463	25.919	84.2078	0.307798	11.1117
GD2	B	22/10/2010	22	8.35079	12.5659	0.66456	1.6342	107.3236	0.015227	25.9224	78.0502	0.332125	11.1217
GD2	C1	22/10/2010	22	9.86844	36.7896	0.26824	1.87211	116.2336	0.016106	25.0495	196.2582	0.127635	11.375

Location	Sample	Date	Day	T Na ppm	Sol/T Na ppm	Sol K ppm	T K ppm	Sol/T K ppm	Sol Mg ppm	T Mg ppm	Sol/T Mg ppm
MG2	A	12/10/2010	24	47.3724	0.641654	4.7686	37.178	0.128264	6.77572	72.3704	0.093626
MG2	B	12/10/2010	24	50.07	0.609906	3.00702	31.0344	0.096893	6.1653	64.5696	0.095483
MG2	C1	12/10/2010	24	50.1654	0.620025	2.90053	30.8918	0.093893	6.47028	59.2638	0.109178
MG2	C2	12/10/2010	24	52.429	0.60202	2.88595	44.6282	0.064667	6.48005	86.5682	0.074855
MG2	A	16/12/2010	30	50.1062	0.594328	4.30073	47.5772	0.090395	6.67837	94.9252	0.070354
MG2	B	16/12/2010	30	50.4784	0.589654	2.99422	23.91	0.125229	6.0736	46.526	0.130542
MG2	C1	16/12/2010	30	51.1824	0.602295	2.92143	32.842	0.088954	6.50358	65.2212	0.099716
MG2	C2	16/12/2010	30	52.0058	0.597012	2.85533	32.3612	0.088233	6.46128	59.5906	0.108428
MG2	A	21/12/2010	35	50.983	0.591844	4.15433	54.0094	0.076919	6.91339	110.1488	0.062764
MG2	B	21/12/2010	35	56.7676	0.533729	3.03987	70.701	0.042996	6.30289	156.0428	0.040392
MG2	C1	21/12/2010	35	50.388	0.618098	2.92214	86.5668	0.033756	6.73782	193.5814	0.034806
MG2	C2	21/12/2010	35								
GD2	A	27/09/2010	0	21.6534	0.51428	2.23189	28.0562	0.079551	1.77799	97.7502	0.018189
GD2	B	27/09/2010	0	19.46476	0.569208	1.60884	33.9728	0.047357	1.31204	117.4786	0.011168
GD2	C1	27/09/2010	0	22.314	0.513086	1.70865	38.453	0.044435	1.03769	133.9206	0.007749
GD2	C2	27/09/2010	0	23.4538	0.489793	1.75833	38.215	0.046012	1.03032	132.5196	0.007775
GD2	A	02/10/2010	5	21.7808	0.505023	1.83511	48.3418	0.037961	1.65483	170.2942	0.009717
GD2	B	02/10/2010	5	21.1584	0.525219	1.83862	56.9694	0.032274	1.65568	179.769	0.00921
GD2	C1	02/10/2010	5	22.1308	0.514387	1.89666	80.3518	0.023604	1.18101	300.686	0.003928
GD2	C2	02/10/2010	5	21.763	0.528319	1.86454	68.4458	0.027241	1.18711	247.26	0.004801
GD2	A	05/10/2010	9	23.0464	0.473523	2.06625	73.3006	0.028189	1.65393	288.114	0.005741
GD2	B	05/10/2010	9	19.67836	0.555316	2.07208	30.1672	0.068687	1.65082	106.6096	0.015485
GD2	C1	05/10/2010	9	22.9262	0.483246	2.13083	45.6188	0.046709	1.19389	155.5248	0.007677
GD2	A	12/10/2010	15	20.053	0.528679	2.25951	24.5646	0.091982	1.75198	85.3522	0.020526
GD2	B	12/10/2010	15	20.987	0.5075	3.16821	23.535	0.134617	3.38148	81.6514	0.041414
GD2	C1	12/10/2010	15	22.724	0.485614	1.90546	35.196	0.054139	1.2094	124.0892	0.009746
GD2	A	16/10/2010	19	21.4726	0.505845	2.33772	64.2086	0.036408	1.84839	253.676	0.007286
GD2	B	16/10/2010	19	20.939	0.521644	2.34772	64.619	0.036332	1.82151	234.512	0.007767
GD2	C1	16/10/2010	19	21.4012	0.521709	2.1527	54.3246	0.039627	1.33258	204.828	0.006506
GD2	C2	16/10/2010	19	19.76308	0.574809	2.07128	52.0346	0.039806	1.24391	173.5492	0.007167
GD2	A	22/10/2010	22	20.367	0.545574	2.70071	28.717	0.094046	1.88188	92.395	0.020368
GD2	B	22/10/2010	22	20.799	0.534723	2.43116	17.04072	0.142668	1.84794	60.114	0.030741
GD2	C1	22/10/2010	22	21.811	0.521526	2.05871	76.776	0.026814	1.27919	273.608	0.004675

Location	Sample	Date	Day	pH	Sol Fe(II) ppm	Sol T Fe ppm	Sol Fe(II)/T Fe	Tot Fe(II) ppm	Tot T Fe ppm	Tot Fe(II)/ T Fe	Sol Fe/ T Fe	Sol As(III) ppm	Sol T As ppm
GD2	C2	22/10/2010	22	7.46	0.56604	0.021539	26.27976	97.71813	1472.356	0.0663685	2.93E-05	0.001285	4.10677
GD2	A	24/10/2010	27	7.87	0	0.023634	0	1306.001	1452.21	0.8993195	3.25E-05	0.025498	2.42514
GD2	B	24/10/2010	27	7.94	0	0.066362	0	1082.364	850.542	1	0.000156	0.01877	2.4966
GD2	C1	24/10/2010	27	7.93	0	0.033632	0	81.32561	1284.266	0.0633246	5.24E-05	0.004179	4.11305
GD2	C2	24/10/2010	27	7.88	0	0.026438	0	60.02691	1015.208	0.0591277	5.21E-05	0.005302	4.05622
GD2	A	01/11/2010	35	7.97	0.923796	0.012835	71.97474	231.5853	1027.864	0.2253074	2.5E-05	0.040699	2.59173
GD2	B	01/11/2010	35	8.04	1.79765	0.022774	78.93431	852.9928	964.27	0.8845995	4.72E-05	0.016936	2.66449
GD2	C1	01/11/2010	35	8.02	0.826701	0.026644	31.02766	66.52396	822.536	0.0808767	6.48E-05	-0.00618	4.35637
GD2	C2	01/11/2010	35	7.93	1.894745	0.032927	57.54381	134.4904	715.25	0.1880327	9.21E-05	-0.00363	4.36527
GD3	A	27/09/2010	0	7.15	0.236886	0.06872	3.447126	334.8315	1105.334	0.3029234	0.000124	0.025664	0.215956
GD3	B	27/09/2010	0	6.95	0.236886	0.054123	4.376817	315.8337	2432	0.1298658	4.45E-05	0.022511	0.118659
GD3	C1	27/09/2010	0	6.97	0.331876	0.056201	5.905159	135.3539			0.002082	0.032778	0.315133
GD3	C2	27/09/2010	0	6.6	0.236886	0.055243	4.288081	287.3369	986.95	0.2911362	0.000112	0.05604	0.245762
GD3	A	02/10/2010	5	7.41	0	0.01186	0	607.6488	1410.812	0.4307086	1.68E-05	0.135698	0.285724
GD3	B	02/10/2010	5	7.64	0	0.007563	0		2123.42	0	7.12E-06	0.225102	0.318787
GD3	C1	02/10/2010	5	7.63	0	0.02288	0	300.5082	2234.18	0.1345049	2.05E-05	0.014059	0.239511
GD3	C2	02/10/2010	5	5.54	0	0.031027	0	278.5696	1473.39	0.1890671	4.21E-05	0.001264	0.241948
GD3	A	05/10/2010	9	7.67	0	0.048592	0	471.4195	724.968	0.6502625	0.000134	0.181822	0.336001
GD3	B	05/10/2010	9	7.7	0	12.5378	0		580.538		0.043194	0.299259	1.7935
GD3	C1	05/10/2010	9	7.72	0	0.054254	0	536.5845	1286.648	0.4170406	8.43E-05	0.018091	0.261723
GD3	A	12/10/2010	15	7.41	0	0.145818	0	248.9885	1124.356	0.2214499	0.000259	0.1171	0.339313
GD3	B	12/10/2010	15	7.52	0	0.082009	0		914.064		0.000179	0.648965	0.832069
GD3	C1	12/10/2010	15	7.64	0	0.022768	0	230.6975	1147.374	0.2010657	3.97E-05	0.014651	0.275147
GD3	A	16/10/2010	19	7.37	0.003084	0.148215	0.020807	518.9062	1839.166	0.2821421	0.000161	0.147007	0.418748
GD3	B	16/10/2010	19	7.36	0.003084	0.073659	0.041868		2584.96	0	5.7E-05	0.405239	0.672466
GD3	C1	16/10/2010	19	7.49	0	0.293797	0	137.5278	1823.802	0.0754072	0.000322	0.012402	0.288542
GD3	C2	16/10/2010	19	7.53	0	0.027045	0	147.5641	1930.912	0.076422	2.8E-05	0.026369	0.28613
GD3	A	22/10/2010	22	7.37	0.661933	0.011531	57.40466		0			0.409778	0.658448
GD3	B	22/10/2010	22	7.38	0.56604	0.089021	6.358496		1072.454		0.000166	0.553816	0.881404
GD3	C1	22/10/2010	22	7.5	0.56604	0.010878	52.03527	136.0755	1202.324	0.1131771	1.81E-05	0.023569	0.279289
GD3	C2	22/10/2010	22	7.77	0.182466	0.079777	2.287198	222.3796	2428.82	0.0915587	6.57E-05	0.00026	0.300039
GD3	A	24/10/2010	27	7.53	0	0.071611	0		1183.3		0.000121	0.696775	1.0887
GD3	B	24/10/2010	27	7.46	0	0.138471	0		1452.258		0.000191	0.762773	1.24106

Location	Sample	Date	Day	Sol As(III)/ T As	T As ppm	Sol As/ T As	Sol Mn ppm	T Mn ppm	Sol/T Mn ppm	Sol Al ppm	T Al ppm	Sol/T Al ppm
GD2	C2	22/10/2010	22	0.0003129	268.832	0.015276	0.009632	32.6142	0.000295	0.012204	294.772	4.14E-05
GD2	A	24/10/2010	27	0.01051403	243.962	0.009941	0.298195	37.8818	0.007872	0.026643	307.082	8.68E-05
GD2	B	24/10/2010	27	0.00751822	140.3094	0.017794	0.289851	21.8796	0.013248	0.048085	157.2228	0.000306
GD2	C1	24/10/2010	27	0.00101603	212.888	0.01932	0.006647	30.7682	0.000216	0.316076	246.87	0.00128
GD2	C2	24/10/2010	27	0.00130713	181.7786	0.022314	0.013118	24.613	0.000533	0.013458	175.2882	7.68E-05
GD2	A	01/11/2010	35	0.01570341	170.336	0.015215	0.337515	26.586	0.012695	0.024623	201.9	0.000122
GD2	B	01/11/2010	35	0.00635619	160.7244	0.016578	0.325434	25.6072	0.012709	0.028644	195.1438	0.000147
GD2	C1	01/11/2010	35	-0.00141746	142.7168	0.030525	0.006896	21.8466	0.000316	0.018579	134.0204	0.000139
GD2	C2	01/11/2010	35	-0.00083042	127.208	0.034316	0.01047	18.36616	0.00057	0.016462	109.6778	0.00015
GD3	A	27/09/2010	0	0.11883902	142.7778	0.001513	0.006885	56.584	0.000122	0.01079	245.592	4.39E-05
GD3	B	27/09/2010	0	0.18971169	267.722	0.000443	0.00432	103.618	4.17E-05	0.003447	567.658	6.07E-06
GD3	C1	27/09/2010	0	0.10401323	7.2684	0.043357	0.04876	2.38244	0.020466	0.00438	12.56324	0.000349
GD3	C2	27/09/2010	0	0.22802549	115.5594	0.002127	0.12422	52.4792	0.002367	1.05527	220.448	0.004787
GD3	A	02/10/2010	5	0.47492685	185.8184	0.001538	2.01136	77.0694	0.026098	0.003172	299.55	1.06E-05
GD3	B	02/10/2010	5	0.70612039	224.092	0.001423	3.52047	80.4398	0.043765	0.005741	537.76	1.07E-05
GD3	C1	02/10/2010	5	0.05869877	252.254	0.000949	0.042675	99.805	0.000428	0.021394	506.366	4.23E-05
GD3	C2	02/10/2010	5	0.00522426	184.051	0.001315	0.154454	81.904	0.001886	0.001079	295.606	3.65E-06
GD3	A	05/10/2010	9	0.54113529	101.027	0.003326	2.39663	43.3502	0.055285	0.001792	143.4968	1.25E-05
GD3	B	05/10/2010	9	0.16685754	70.9372	0.025283	4.69151	29.2496	0.160396	3.05515	126.079	0.024232
GD3	C1	05/10/2010	9	0.0691227	157.3596	0.001663	0.154976	65.2606	0.002375	0.004632	257.148	1.8E-05
GD3	A	12/10/2010	15	0.34510909	149.2382	0.002274	2.77513	60.1292	0.046153	0.067069	233.402	0.000287
GD3	B	12/10/2010	15	0.77994133	109.0448	0.007631	4.94363	39.1326	0.12633	0.00545	220.386	2.47E-05
GD3	C1	12/10/2010	15	0.0532479	141.2734	0.001948	1.47833	47.5654	0.03108	0.00993	236.716	4.19E-05
GD3	A	16/10/2010	19	0.35106317	243.018	0.001723	3.45283	95.8924	0.036007	0.060016	384.742	0.000156
GD3	B	16/10/2010	19	0.60261634	292.16	0.002302	5.40329	106.7718	0.050606	0.024517	610.946	4.01E-05
GD3	C1	16/10/2010	19	0.04298161	223.756	0.00129	1.91092	91.5	0.020884	0.147014	364.484	0.000403
GD3	C2	16/10/2010	19	0.09215741	243.966	0.001173	0.210323	106.7098	0.001971	0.00848	406.564	2.09E-05
GD3	A	22/10/2010	22	0.6223392			3.85796			0.006027		
GD3	B	22/10/2010	22	0.62833389	136.0378	0.006479	5.13181	48.4566	0.105905	0.01362	239.986	5.68E-05
GD3	C1	22/10/2010	22	0.08438929	201.718	0.001385	2.28251	28.7854	0.079294	0.007587	226.604	3.35E-05
GD3	C2	22/10/2010	22	0.00086655	279.974	0.001072	0.306913	128.3078	0.002392	0.008432	491.598	1.72E-05
GD3	A	24/10/2010	27	0.64000643	160.7358	0.006773	3.95242	66.5044	0.059431	0.007549	218.31	3.46E-05
GD3	B	24/10/2010	27	0.61461412	163.2228	0.007603	4.84705	67.6388	0.071661	0.007033	285.68	2.46E-05

Location	Sample	Date	Day	Sol S ppm	T S ppm	Sol/T S ppm	Sol Si ppm	T Si ppm	Sol/T Si ppm	Sol Ca ppm	T Ca ppm	Sol/T Ca ppm	Sol Na ppm
GD2	C2	22/10/2010	22	10.3312	31.7612	0.325277	1.80394	162.7852	0.011082	25.1336	206.69	0.1216	11.5516
GD2	A	24/10/2010	27	8.21215	31.189	0.263303	1.65321	183.4702	0.009011	26.2942	188.8906	0.139203	11.0768
GD2	B	24/10/2010	27	8.35732	19.4552	0.429567	1.605	142.8462	0.011236	26.3156	103.2876	0.25478	11.1509
GD2	C1	24/10/2010	27	9.84553	27.4898	0.358152	1.86699	166.7508	0.011196	25.2461	164.6748	0.153309	11.3574
GD2	C2	24/10/2010	27	10.2013	22.0458	0.462732	1.76455	151.039	0.011683	24.9536	127.0466	0.196413	11.3525
GD2	A	01/11/2010	35	8.79556	21.1596	0.415677	1.73374	157.8966	0.01098	28.9633	119.4412	0.24249	12.2041
GD2	B	01/11/2010	35	8.87416	22.5732	0.393128	1.65761	142.3306	0.011646	28.314	120.294	0.235373	11.9788
GD2	C1	01/11/2010	35	10.4786	18.17386	0.576575	1.95434	139.6688	0.013993	27.6315	101.6194	0.271912	12.0175
GD2	C2	01/11/2010	35	11.0121	16.16298	0.681316	1.87913	118.6698	0.015835	27.6163	87.4606	0.315757	12.1807
GD3	A	27/09/2010	0	49.3406	35.0606	1	4.50068	158.8958	0.028325	55.7272	128.3512	0.434177	15.2405
GD3	B	27/09/2010	0	40.8417	50.7268	0.805131	3.21764	127.6494	0.025207	45.9062	236.13	0.194411	11.0586
GD3	C1	27/09/2010	0	41.7676	27.0164	1	3.82873	23.6004	0.162232	45.1894	38.8192	1	11.5784
GD3	C2	27/09/2010	0	42.5499	28.8832	1	3.92806	135.0674	0.029082	45.7453	101.8102	0.449319	11.6352
GD3	A	02/10/2010	5	38.9854	40.2542	0.96848	2.54092	166.5766	0.015254	54.0076	163.53	0.330261	11.1605
GD3	B	02/10/2010	5	45.9076	55.1736	0.832057	2.31818	87.2242	0.026577	61.2779	217.144	0.282199	11.0383
GD3	C1	02/10/2010	5	46.6501	44.2296	1.054726	3.54161	136.731	0.025902	58.0365	232.316	0.249817	11.1131
GD3	C2	02/10/2010	5	44.8478	34.6412	1	3.59599	179.5054	0.020033	53.3699	150.042	0.3557	11.4344
GD3	A	05/10/2010	9	38.2488	40.5964	0.942172	2.51261	145.4868	0.01727	53.9741	106.8928	0.504937	11.0078
GD3	B	05/10/2010	9	45.1558	47.352	0.95362	6.62052	141.454	0.046803	63.5248	96.5602	0.657878	10.9398
GD3	C1	05/10/2010	9	45.7216	38.5238	1	3.28055	144.2922	0.022735	56.759	141.0138	0.402507	10.7681
GD3	A	12/10/2010	15	36.5516	32.9968	1	2.64073	174.9524	0.015094	52.5081	131.3232	0.399839	10.4229
GD3	B	12/10/2010	15	43.5371	40.979	1	2.65446	151.6434	0.017505	64.0266	110.6562	0.578608	10.702
GD3	C1	12/10/2010	15	49.1157	48.4972	1	2.62057	180.8164	0.014493	62.1144	139.5982	0.444951	11.454
GD3	A	16/10/2010	19	37.315	32.5552	1	2.74842	105.6418	0.026016	55.7049	174.9988	0.318316	10.8806
GD3	B	16/10/2010	19	43.2732	42.504	1	3.0679	73.1818	0.041922	68.048	224.202	0.303512	11.3714
GD3	C1	16/10/2010	19	49.927	33.9802	1	2.60597	111.9626	0.023275	63.314	178.5732	0.354555	11.6963
GD3	C2	16/10/2010	19	45.982	32.103	1	3.607	92.0238	0.039196	56.6714	174.381	0.324986	11.571
GD3	A	22/10/2010	22	36.5265			2.68184						
GD3	B	22/10/2010	22	39.8134	38.7898	1	3.18494	141.4582	0.022515	68.0185	113.9554	0.596887	11.4089
GD3	C1	22/10/2010	22	51.9023	25.7754	1	2.35605	172.2244	0.01368	67.0611	157.9012	0.424703	12.2063
GD3	C2	22/10/2010	22	46.466	32.5082	1	3.61179	87.9792	0.041053	57.7278	195.6472	0.295061	12.0106
GD3	A	24/10/2010	27	30.7199	36.3778	0.844468	2.64107	164.0718	0.016097	56.8089	121.6184	0.467108	10.9241
GD3	B	24/10/2010	27	32.7168	40.5824	0.806182	3.16128	128.6442	0.024574	66.5588	129.271	0.514878	11.4855

Location	Sample	Date	Day	T Na ppm	Sol/T Na ppm	Sol K ppm	T K ppm	Sol/T K ppm	Sol Mg ppm	T Mg ppm	Sol/T Mg ppm
GD2	C2	22/10/2010	22	20.4314	0.565385	2.02491	56.7872	0.035658	1.25401	188.8678	0.00664
GD2	A	24/10/2010	27	21.0064	0.527306	2.77737	61.374	0.045253	1.89879	195.0216	0.009736
GD2	B	24/10/2010	27	18.41162	0.605645	2.66299	27.7932	0.095814	1.86991	98.1258	0.019056
GD2	C1	24/10/2010	27	20.9554	0.54198	2.43593	47.3996	0.051391	1.30503	157.046	0.00831
GD2	C2	24/10/2010	27	19.66324	0.577346	2.03395	31.5032	0.064563	1.2569	110.3586	0.011389
GD2	A	01/11/2010	35	19.56864	0.623656	3.4289	33.712	0.101712	2.07144	126.1328	0.016423
GD2	B	01/11/2010	35	18.842	0.63575	2.97856	35.9736	0.082798	1.99496	123.1968	0.016193
GD2	C1	01/11/2010	35	19.62638	0.612314	2.42298	22.908	0.10577	1.4066	82.6958	0.017009
GD2	C2	01/11/2010	35	19.18498	0.634908	2.24901	18.38662	0.122318	1.3781	68.8504	0.020016
GD3	A	27/09/2010	0	20.9388	0.727859	6.83463	49.3786	0.138413	5.68462	158.13	0.035949
GD3	B	27/09/2010	0	23.241	0.475823	5.34408	110.3008	0.04845	4.5182	369.1	0.012241
GD3	C1	27/09/2010	0	21.1902	0.546404	4.05104	5.87476	0.689567	4.09483	9.57334	0.427733
GD3	C2	27/09/2010	0	21.1856	0.549203	4.31756	40.7918	0.105844	4.31875	140.8264	0.030667
GD3	A	02/10/2010	5	21.9126	0.509319	7.00496	63.608	0.110127	5.24094	192.9402	0.027164
GD3	B	02/10/2010	5	20.3318	0.542908	7.84178	105.8686	0.074071	6.03384	343.688	0.017556
GD3	C1	02/10/2010	5	22.8976	0.485339	5.17698	107.1392	0.04832	4.8209	330.584	0.014583
GD3	C2	02/10/2010	5	21.684	0.52732	5.20307	57.4078	0.090634	4.87418	189.3148	0.025746
GD3	A	05/10/2010	9	20.3416	0.541147	7.2281	30.615	0.236097	5.27076	91.8742	0.057369
GD3	B	05/10/2010	9	22.41	0.488166	8.98822	29.3542	0.306199	8.04749	81.5226	0.098715
GD3	C1	05/10/2010	9	20.8544	0.516347	5.25029	49.1102	0.106908	4.8795	165.4402	0.029494
GD3	A	12/10/2010	15	20.9662	0.497129	7.82208	48.4832	0.161336	5.17842	149.7556	0.034579
GD3	B	12/10/2010	15	20.6226	0.518945	8.36549	42.997	0.19456	6.23113	141.2938	0.044101
GD3	C1	12/10/2010	15	22.2812	0.514066	7.72382	51.4224	0.150203	5.68864	153.028	0.037174
GD3	A	16/10/2010	19	22.173	0.490714	8.06658	64.8068	0.124471	5.49004	243.906	0.022509
GD3	B	16/10/2010	19	21.7752	0.522218	8.85772	97.0754	0.091246	6.62281	391.286	0.016926
GD3	C1	16/10/2010	19	20.9326	0.55876	8.34413	62.497	0.133512	6.0584	233.296	0.025969
GD3	C2	16/10/2010	19	22.973	0.503678	5.93183	66.5272	0.089164	5.43165	258.32	0.021027
GD3	A	22/10/2010	22								
GD3	B	22/10/2010	22	20.5608	0.554886	8.93338	42.7998	0.208725	6.59607	152.485	0.043257
GD3	C1	22/10/2010	22	20.9764	0.581906	8.93976	44.8452	0.199347	6.35847	142.7148	0.044554
GD3	C2	22/10/2010	22	19.80872	0.606329	6.04215	90.5628	0.066718	5.55039	314.272	0.017661
GD3	A	24/10/2010	27	19.48384	0.560675	8.24508	43.3978	0.189988	5.56822	139.1884	0.040005
GD3	B	24/10/2010	27	17.98696	0.638546	8.80858	51.8038	0.170037	6.5042	183.561	0.035433

Location	Sample	Date	Day	pH	Sol Fe(II) ppm	Sol T Fe ppm	Sol Fe(II)/T Fe	Tot Fe(II) ppm	Tot T Fe ppm	Tot Fe(II)/ T Fe	Sol Fe/ T Fe	Sol As(III) ppm	Sol T As ppm
GD3	C1	24/10/2010	27	7.59	0	0.0112	0	81.32561	2355.18	0.0345305	9.51E-06		0.274572
GD3	C2	24/10/2010	27	7.59	0	0.040205	0	70.67626	1116.63	0.0632943	7.2E-05	0.017903	0.307844
GD3	A	01/11/2010	35	7.54	0	0.076131	0		888.17		0.000171	0.474517	1.01674
GD3	B	01/11/2010	35	7.54	0	0.246602	0		1326.966		0.000372	1.64986	2.12468
GD3	C1	01/11/2010	35	7.66	0	0.013103	0	76.23345	1949.576	0.0391026	1.34E-05		0.285489
GD3	C2	01/11/2010	35	7.42	0	0.013966	0	105.3619	1556.088	0.0677095	1.8E-05	0.014872	0.323063

Location	Sample	Date	Day	Sol As(III)/ T As	T As ppm	Sol As/ T As	Sol Mn ppm	T Mn ppm	Sol/T Mn ppm	Sol Al ppm	T Al ppm	Sol/T Al ppm
GD3	C1	24/10/2010	27		254.134	0.00108	2.6619	116.3962	0.022869	0.008944	490.996	1.82E-05
GD3	C2	24/10/2010	27	0.05815608	137.3082	0.002242	0.299572	60.2636	0.004971	0.011134	214.674	5.19E-05
GD3	A	01/11/2010	35	0.46670437	116.732	0.00871	3.95031	48.9224	0.080746	0.013752	160.7644	8.55E-05
GD3	B	01/11/2010	35	0.77652164	152.5268	0.01393	3.34448	67.1404	0.049813	0.013407	267.628	5.01E-05
GD3	C1	01/11/2010	35		221.274	0.00129	3.12594	106.286	0.029411	0.00875	359.806	2.43E-05
GD3	C2	01/11/2010	35	0.04603436	188.7228	0.001712	0.364132	87.6694	0.004153	0.003678	280.596	1.31E-05

Location	Sample	Date	Day	Sol S	T S	Sol/T S	Sol Si	T Si	Sol/T Si	Sol Ca	T Ca	Sol/T Ca	Sol Na
				ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
GD3	C1	24/10/2010	27	48.5905	34.359	1	2.02507	92.6056	0.021868	62.2672	212.308	0.293287	11.3952
GD3	C2	24/10/2010	27	48.1206	37.6942	1	3.7185	171.5256	0.021679	60.8201	119.8344	0.507535	12.0414
GD3	A	01/11/2010	35	28.4899	27.553	1	2.86091	135.6226	0.021095	59.8501	87.6094	0.683147	11.9846
GD3	B	01/11/2010	35	19.7729	37.2668	0.530577	2.9342	151.6408	0.01935	52.6559	117.9566	0.446401	11.5017
GD3	C1	01/11/2010	35	50.3682	32.0784	1	1.94712	115.9548	0.016792	66.5506	168.4684	0.395033	12.0091
GD3	C2	01/11/2010	35	48.4563	32.4514	1	3.70506	119.3114	0.031054	61.6455	139.738	0.441151	12.0568

Location	Sample	Date	Day	T Na ppm	Sol/T Na ppm	Sol K ppm	T K ppm	Sol/T K ppm	Sol Mg ppm	T Mg ppm	Sol/T Mg ppm
GD3	C1	24/10/2010	27	19.45936	0.58559	8.28554	94.6146	0.087571	6.15687	316.674	0.019442
GD3	C2	24/10/2010	27	20.1562	0.597404	6.44875	44.085	0.14628	5.89732	138.5302	0.042571
GD3	A	01/11/2010	35	18.61844	0.643695	9.24255	30.0942	0.307121	5.77547	102.1294	0.056551
GD3	B	01/11/2010	35	20.2064	0.569211	8.27894	46.141	0.179427	5.75386	170.8184	0.033684
GD3	C1	01/11/2010	35	19.47716	0.616573	8.54583	69.7922	0.122447	6.53347	231.706	0.028197
GD3	C2	01/11/2010	35	19.20072	0.627935	6.71625	50.8894	0.131977	6.094	180.937	0.03368

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Fragment Size Data for 50 Samples

Date: 2010.07.28
Submission No: 10-060123

Client Name: Paul Beddoes

Sample File Name	Size	Height	Area	Data Point
J35_C05.fsa	67.27	608	7149	4733
	85.24	186	4319	4990
P1_A06.fsa	66.34	473	4603	4705
	67.27	729	8956	4718
	85.34	586	14764	4978
	126.28	600	5209	5597
	128.42	268	2257	5630
	140.2	132	1055	5811
	144.91	224	2343	5883
	260.63	183	2365	7695
P2_B06.fsa	66.33	489	4764	4734
	67.34	1071	11398	4748
	84.92	506	12293	5001
	144.06	151	1281	5901
	212.84	490	4580	6970
	215.58	403	3676	7012
	216.84	117	1105	7031
P4_C06.fsa	66.34	390	3917	4699
	67.29	422	4701	4712
	84.93	133	3263	4962
P5_D06.fsa	67.27	1090	16387	4715
	85.31	356	8992	4972
	279.37	107	1136	7966
P6_E06.fsa	67.31	1873	27393	4710
	85.05	478	11847	4963
P10_F06.fsa	66.42	471	4707	4716
	67.29	835	9987	4728
	74.25	138	1290	4825
	85.05	451	11165	4980
	87.6	180	1337	5017
	131.74	117	940	5679
	167.8	363	3364	6226
	261.02	164	1530	7675
	262.86	379	3490	7704
	444.22	152	1798	10626
	446.33	146	1813	10660
	447.69	123	1518	10682
	483.68	243	3196	11262
	485.67	377	5554	11294
P13_G06.fsa	67.34	1068	20056	4724
	85.26	709	17333	4979

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Fragment Size Data for 50 Samples

Date: 2010.07.28
Submission No: 10-060123

Client Name: Paul Beddoes

Sample File Name	Size	Height	Area	Data Point
	89.03	107	1007	5034
	91.28	267	2419	5067
	117.01	110	1453	5451
	145.04	138	1190	5878
	276.66	167	1709	7922
	291.23	173	2048	8156
	438.79	621	7788	10544
	440.93	742	9426	10579
	485.98	130	1180	11306
	488.04	190	2567	11339
P26_H06.fsa	66.46	567	5527	4746
	67.32	835	10359	4758
	85.24	551	13352	5017
P28_A07.fsa	67.16	976	12818	4775
	85.26	263	6146	5036
	87.44	776	5934	5068
	89.53	497	3830	5099
	483.49	189	2771	11427
	485.58	286	4094	11461
P29_B07.fsa	53.57	120	1404	4560
	67.27	1211	18055	4743
	71.32	139	1135	4800
	74.2	101	867	4841
	85.12	415	10119	5000
	152.84	242	2193	6033
	167.61	121	969	6261
	525.95	126	1453	12058
	528.01	182	2252	12091
P30_C07.fsa	66.4	678	6552	4768
	67.26	1150	14656	4780
	85.14	675	16737	5037
	127.06	503	4075	5672
	129.19	250	2043	5705
	135.79	153	1338	5807
	152.88	211	1865	6069
	527.88	136	1523	12113
P33_D07.fsa	66.29	588	5917	4726
	67.23	941	11777	4739
	85.23	539	13872	4997
	212.52	426	3656	6953
	215.33	407	3373	6996

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Fragment Size Data for 50 Samples

Date: 2010.07.28
Submission No: 10-060123

Client Name: Paul Beddoes

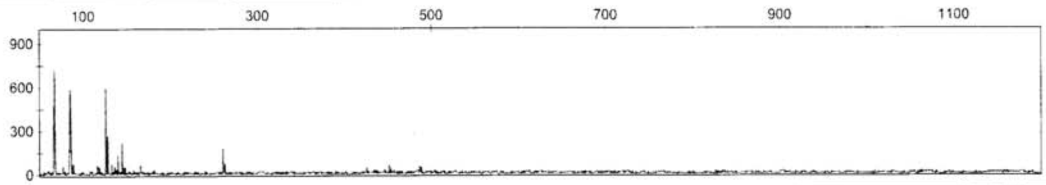
Sample File Name	Size	Height	Area	Data Point
	216.53	103	820	7014
	281.67	178	1660	8052
P34_E07.fsa	66.27	181	1855	4766
	67.21	286	3236	4779
	84.54	148	3727	5027
P35_F07.fsa	53.87	149	1610	4601
	67.25	552	7106	4782
	84.94	335	8265	5036
	373.35	109	263	9585
P38_G07.fsa	67.28	1036	15994	4819
	85.61	775	19476	5087
	132.22	145	1483	5806
	135.79	212	2682	5862
	451.93	107	1424	10986
	453.88	153	2795	11018

The fragment sizes are 50 - 1200 bp; the (height) signal cut-off is 50.

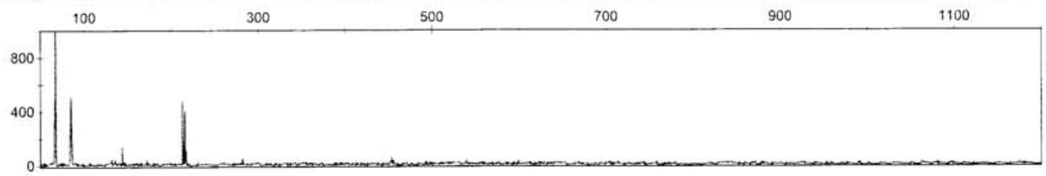
Data reviewed by: Jiping Li

Agriculture and Food laboratory - Guelph, ON N1H 8J7 -www.labservices.uoguelph.ca

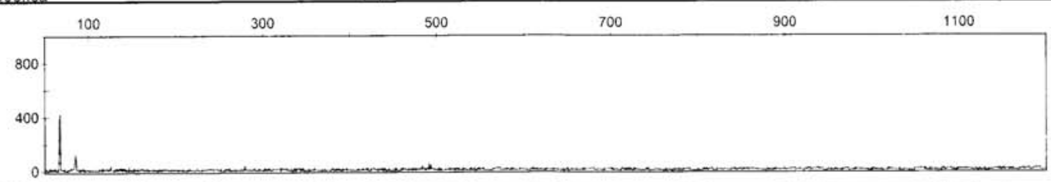
Sample File	Sample Name	SQ
P1_A06.fsa	P1	



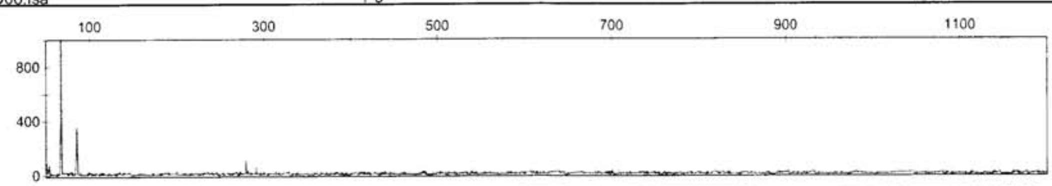
Sample File	Sample Name	SQ
P2_B06.fsa	P2	



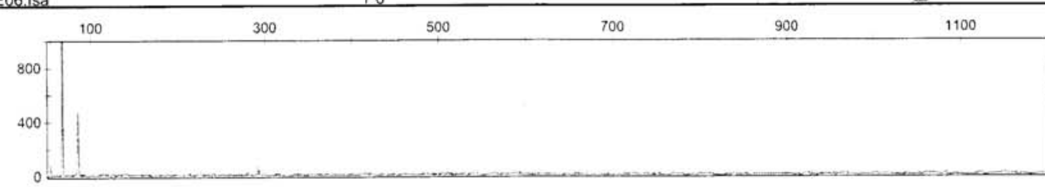
Sample File	Sample Name	SQ
P4_C06.fsa	P4	



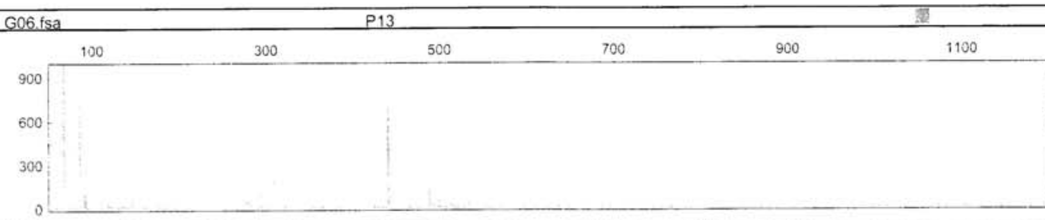
Sample File	Sample Name	SQ
P5_D06.fsa	P5	



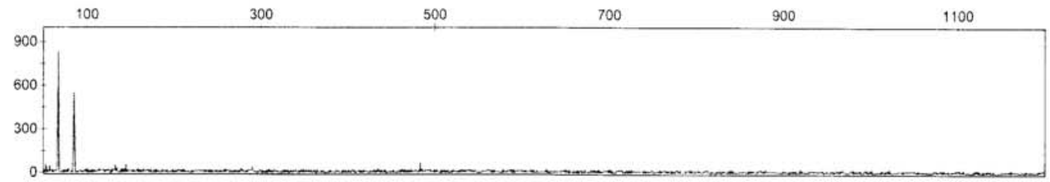
Sample File	Sample Name	SQ
P6_E06.fsa	P6	



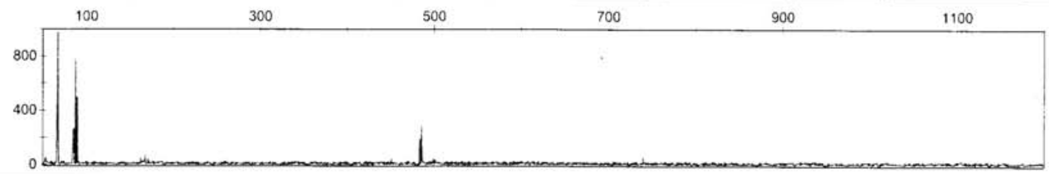
Sample File	Sample Name	SQ
P13_G06.fsa	P13	



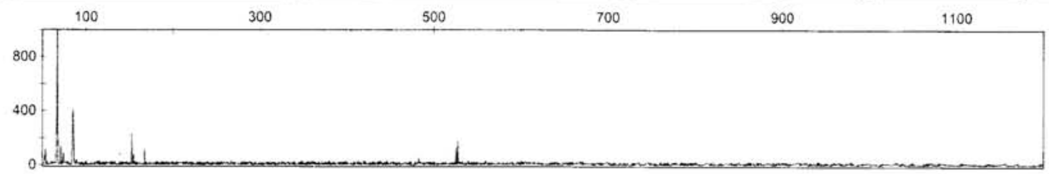
Sample File	Sample Name	SQ
P26_H06.fsa	P26	



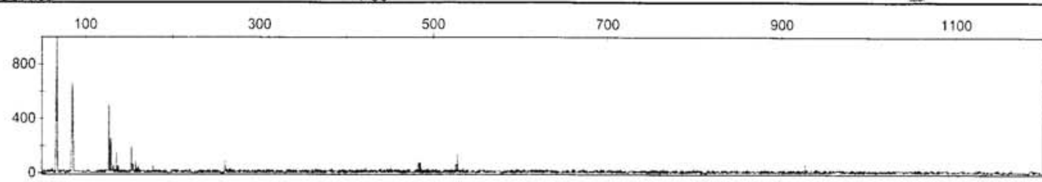
Sample File	Sample Name	SQ
P28_A07.fsa	P28	



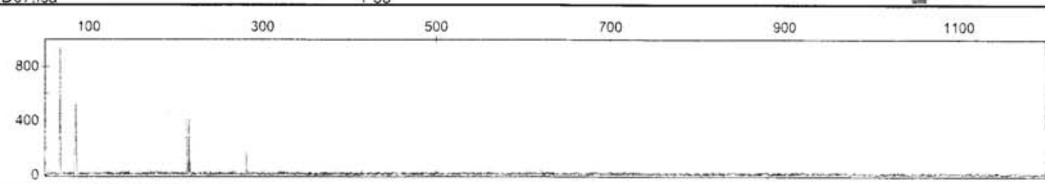
Sample File	Sample Name	SQ
P29_B07.fsa	P29	



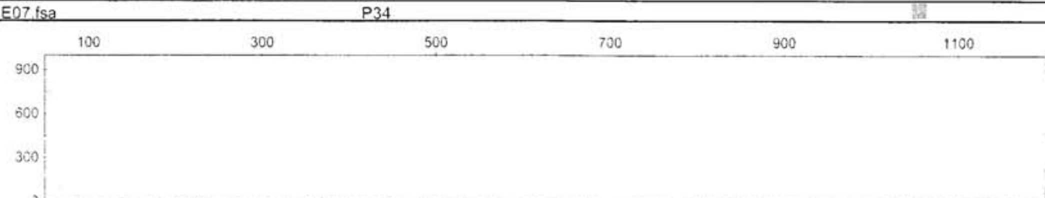
Sample File	Sample Name	SQ
P30_C07.fsa	P30	



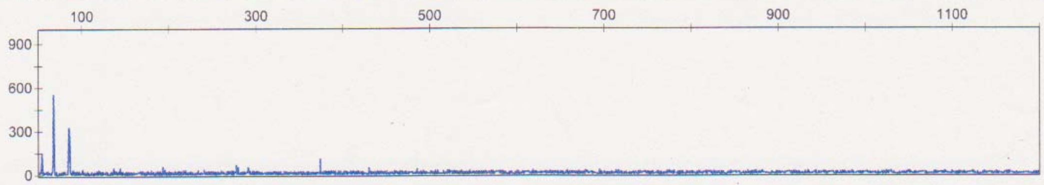
Sample File	Sample Name	SQ
P33_D07.fsa	P33	



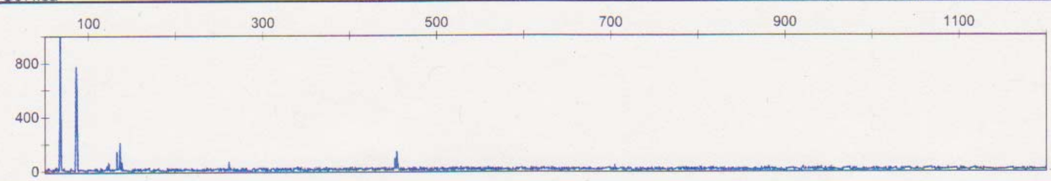
Sample File	Sample Name	SQ
P34_E07.fsa	P34	



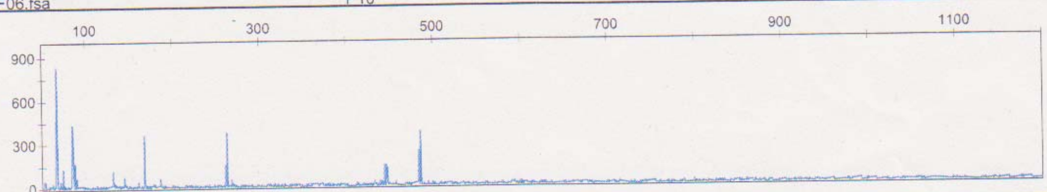
Sample File	Sample Name	SQ
P35_F07.fsa	P35	



P38_G07.fsa	P38	



P10_F06.fsa	P10	



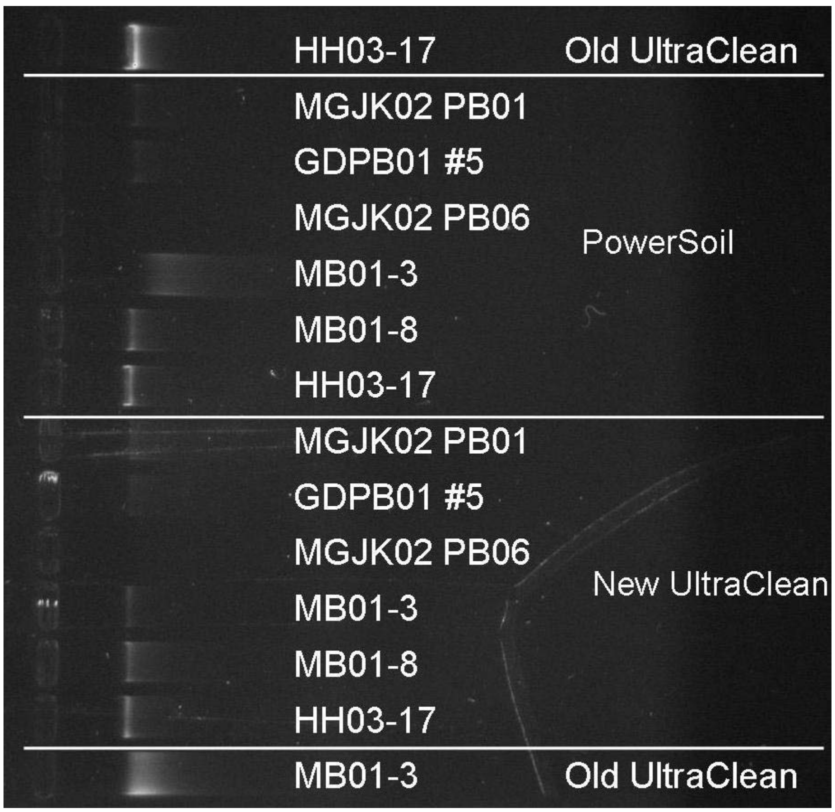
DNA Extraction and Amplification Trials:

As many different DNA extraction and amplification (PCR) methods were tried in this study, a summary of the most commonly used and successful methods follows:

DNA Extraction:

MoBio Kits:

Both the UltraClean Soil DNA Isolation Kit and the PowerSoil DNA Isolation Kit were trialed with tailings samples. The below genomic gel shows that there was little difference between the genomic DNA recovered using these kits:



As previous studies involving arsenic contaminated samples have used the UltraClean kit, this method was chosen.

As the tailings samples were rich in arsenic, a known inhibitor of PCR (Opel et al., 2010), several different washing and rinsing methods were trialed in an attempt to remove the arsenic in the extraction step, so that it would not interfere with PCR.

Ammonium Oxalate Method (Boothman, 2009, Personal Correspondence):

Add 1g of sediment/tailings to 20ml of Ammonium Oxalate Buffer (see recipe below).
Shake mixture at 150rpm for 2 hours.
Spin down mixture and decant.
Repeat 3 times.
Add 20ml TE Buffer to pellet.
Shake mixture at 150rpm for 2 hours.
Spin down and decant.
Repeat 3 times.
Add 20ml ddH_2O pellet and shake at 150rpm for 1 hour.
Spin down and decant.
Repeat three times.
Extract from pellet.

Ammonium Oxalate Buffer:

Mix 56.8g ammonium oxalate ($(NH_4)_2C_2O_4 \cdot H_2O$) to 2L dd$H_2O$.
Mix 50.4g oxalic acid ($H_2C_2O_4 \cdot H_2O$) to 2L ddH_2O.
Mix 4 parts ammonium oxalate solution with 3 parts oxalic acid solution.
Adjust to pH 3.0.

Trials of the Ammonium Oxalate washing procedure showed little increase in genomic DNA yield from extraction, and no noticeable increase in PCR efficiency.

MoBio protocol aleteration:

Step 15 of the MoBio UltraClean protocol is described as an ethanol based wash to remove contaminants from samples. This step (and the subsequent flow-through discarding step) was repeated in DNA extraction trials in an effort to further remove contaminants from the highly contaminated samples.

The result was a noticeable increase in the yield of genomic DNA from the extraction procedure, and an increased efficiency in PCR.

As the repeating of steps 15 and 16 is far less time consuming and proved more helpful, this alteration of the MoBio protocol was used in all DNA extractions.

PCR Optimization:

General PCR optimization was performed using a variety of samples from both sites. The final concentration of $MgCl_2$ in the reactions was altered from 0.05mM up to 6.0mM, while DNA template concentrations from 1x to 1000x were also tried. Generally, low $MgCl_2$ concentrations worked best: 0.5-1.5mM. A DNA template dilution of 1x was most successful.

Additionally, several different types of *Taq* polymerase and PCR Buffer were used during this study:

***Taq* Polymerase**	**PCR Buffer**	**Effectiveness/Use**
NEB Crimson Taq	NEB Crimson PCR Buffer	*****
NEB Standard Taq	NEB Standard 10X Buffer	***
Invitrogen *Taq* Polymerase	Invitrogen 10X CoralLoad PCR Buffer	****
Invitrogen *Taq* Polymerase	Invitrogen 10X PCR Buffer	***
Invitrogen Platinum *Taq* Polymerase	Invitrogen 10X PCR Buffer	**

PCR reactions were often amended with various solutions known to increase efficiency of amplification in difficult samples:

Solution	**Effectiveness/Use**
Invitrogen Q Solution	*****
DMSO	***
Both	***

Despite best efforts and optimization, no one PCR reaction was found to be consistently successful for all samples. This is likely due to the compositional diversity seen in the tailings. As it is not practical to run a full PCR optimization for every sample, certain consistently hard to amplify samples were eventually abandoned for analysis. This could represent an important gap in the data presented and conclusion made here.

The most commonly used PCR recipe was a 50µl reaction which involved the use of NEB Crimson *Taq* and Buffer, and the addition of Invitrogen Solution Q:

ddH_2O	27.75µl
NEB Crimson PCR Buffer	10µl
50mM $MgCl_2$	2.5µl
10mM dNTP	1µl
Forward Primer (25mM)	1µl
Reverse Primer (25mM)	1µl
Solution Q	5µl
NEB Crimson *Taq* Polymerase	0.25µl
DNA Template (1X Dilution)	+ 2µl