

**Geomicrobiological analysis of acid generating rock of
the Meguma Group pertaining to disposal in the
Bedford Basin, Nova Scotia**

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Submitted in Partial Fulfillment of the Requirements
For the Degree of Bachelor of Science, Honours
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April 2004

Geomicrobiological analysis of acid generating rock of the Meguma Group pertaining to disposal in the Bedford Basin, Nova Scotia.

The physical disturbance of the sulphide-bearing rocks of the Meguma Group can lead to oxidation of the sulphides and possibly generate Acid Rock Drainage (ARD). ARD is a well known, and costly problem associated with the Meguma Group in the Halifax region of Nova Scotia, Canada. Finding the ideal site for waste rock disposal is not always easy, and disposing waste acid generating materials into salt water of the Bedford Basin is preferred over higher risk land disposal. This study focuses on the survival and geomicrobiological interactions of *Acidithiobacillus ferrooxidans*, and the behaviour of the Halifax slates and the Goldenville-Halifax transition zone (GHT) in fresh and saline waters. This study examines pH changes in both rock types in fresh and salt water static tests. Rocks from both the GHT and Halifax slate were crushed into several size fractions for the pH. Data collected over a period of 5 weeks indicate that for all size fractions, and for both the fresh and salt water samples, an initial dramatic drop of pH is followed by an apparent levelling off in pH, producing an exponential curve of pH versus time (days). This trend is evident for the Halifax slate with an initial average fresh water pH of 5.6 (range 5.3-6.1), and an average final pH of 3.2 (range 3.1 - 3.5). The salt water samples of Halifax slate had an average initial pH of 6.71 (range 6.3 – 7.0), and an average final pH of 4.03 (range 3.6-5.5). The trend of the GHT data is similar; however the salt water pH drop is not as dramatic as the drop in pH of the fresh water. The average initial pH of GHT fresh water samples is 7.1 (range 6.9 – 7.4), and an average final pH of 4.7 (range 3.8 – 5.5). The GHT salt water average initial pH is 7.23 (range 7.1 – 7.4), and the average final pH is 6.31 (range 5.4 - 7.0). The addition of bacteria to samples of both rock types in fresh and salt water did not effect the final pH. A water flushing experiment, mimicking fresh water infiltration and tidal action resulted in a similar trend in pH: the pH decrease was approximately the same amount at the end of each day before the fresh water change. Microprobe analysis confirms the dominance of pyrrhotite in the GHT and Halifax slates in the Beaverbank area. Acid prediction tests confirm the ability of the rocks to produce ARD. Scanning electron microscopy (SEM) studies examined un-inoculated and inoculated samples of both rock types in salt water to observe possible attachment to the rock surface. SEM confirmed bacterial survival and attachment to the rock surface of both rock types in salt water. Implications from this study show that although the pH change is somewhat small in salt water, the drop is not insignificant, and indicates the continuing oxidization of sulphide minerals. Care should be taken when disposing of waste acid rock in any aquatic environments. Concentration of biological matter does not effect the rate of ARD production in a sample in both fresh and salt water. Further studies are recommended to produce a complete conclusion on the disposal of sulphide bearing material in a salt water environment.

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Acknowledgements

I would like to take the time to thank everyone who has helped me accomplish this project. I wish to thank my advisors, Dr. Armstrong, Anne Marie O'Beirne-Ryan, and Terry Goodwin, for their constant support and excellent editing skills.

I would also like to thank those who have helped out with the collection and understanding of information, Dr. Marcos Zentilli for advice and support. Dr. Martin Gibling, Sandy Grist, Debra Wheeler, Gordon Brown, Patricia Stoffyn, Dr. Ping Li, Adam Layman, and Sonja Gould for donating some time and help in procedures and analysis.

I would like to acknowledge the love of support of my family, and especially thank Jeff for his support and confidence in me and my work. Finally I would like to thank my thesis classmates who were always there to listen and support my efforts in this project.

Chapter 1: Introduction

1.1 General Statement

Combining geological and microbiological studies is the focus of this research into the response of acid generating rocks under salt and fresh water conditions. Acid rock drainage is the lowering in pH, which may result in the release of heavy metals into the environment as a result of the oxidation of sulphides in sulphide-bearing rocks. Meguma Group metagreywakes and slates of the Halifax area generate acid rock drainage when exposed to atmospheric conditions (Fox *et al.* 1997). The questions explored in this thesis are the possible impacts of the disposal of Meguma rocks into a salt water environment, and how the acid producing microorganisms, probably *Acidithiobacillus ferrooxidans*, survive in a salt water environment. This study is a continuation of ongoing research of acid rock drainage at Dalhousie University, and builds on previous studies by Robinson (1996), Fox *et al.* (1997), Jones (1997), Whittaker (1999), and Fox (1999).

Acid rock drainage is associated with the Meguma Group in Nova Scotia because of locally high sulphide mineral content. Exposure of the Meguma Group to atmospheric conditions has resulted in acidic pH levels and associated elevated metal levels in rivers, lakes, streams, and soils, leading to a host of environmental problems including fish kills (Fox 1999). Any construction in the Halifax area potentially results in disturbance of the locally enriched sulphide-bearing rocks. As the city of Halifax continues to grow, this remains an ongoing issue with disposal of the waste material continuing to be a concern. However, past work at the Halifax International airport suggests that disposal in salt water may significantly reduce ARD production therefore disposal in the Bedford Basin,

a part of Halifax Harbour, has been occurring for approximately 13 years, with no identified environmental problems, yet no study conclusively establishes marine disposal benefits and risks (JWEL 14387 2002).

1.2 Definition of Acid Rock Drainage

Acid rock drainage (ARD) is the lowering of water pH with or without the release of heavy metals (acid rock drainage means that acidic run-off may intercept heavy metals in the ground it travels through/over, and may therefore result in extracting them; however, if they are not there to start with, then they will not be scavenged) as a result of oxidation of sulphide minerals such as pyrite (FeS_2), and pyrrhotite ($\text{Fe}_{1-x}\text{S}_x$). Acid rock drainage is synonymous with the term acid mine drainage (AMD), which has the same end result, however the origins are mine tailing piles. Sulphide minerals will oxidize naturally when in contact with oxygen; however, *A. ferrooxidans* catalyses this reaction rate by one to two orders of magnitude, decreasing the pH and releasing soluble iron into the water more rapidly (MEND 1.42.1 1995). Sulphide minerals can however, oxidize with no biological involvement (Table 1.1). Chemical reactions involving sulphides in the environment are not a single step process, they occur in multiple steps with a number of intermediate products formed (Robinson 1996) (Table 1.2). The bacterial catalysis of the reaction in Table 1.2 results in the production of 16 moles of acid. *A. ferrooxidans* requires a carbon source, in the form of CO_2 , and an oxygen source for survival. If these conditions for survival are present it takes only a matter of days to lower the pH of the surrounding water.

Table 1.1 Chemical reactions of pyrite, pyrrhotite, and chalcopyrite oxidation. After Silver (1997) and Robinson (1996).

| | <u>Reaction</u> |
|--------------|--|
| Pyrite | $2\text{FeS}_2 + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{FeSO}_4 + 2\text{H}_2\text{SO}_4$ $\text{FeS}_2 + \text{Fe}_2(\text{SO}_4)_3 \rightarrow 3\text{FeSO}_4 + 2\text{S}^0$ |
| Pyrrhotite | $\text{Fe}_{(1-x)}\text{S} + (2-x/2)\text{O}_2 + (x)\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{SO}_4^{2-} + (2x)\text{H}^+$ |
| Chalcopyrite | $4\text{CuFeS}_2 + 17\text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{CuSO}_4 + 2\text{Fe}_2(\text{SO}_4)_3 + 2\text{H}_2\text{O}$ $\text{CuFeS}_2 + 2\text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{CuSO}_4 + 2\text{FeSO}_4 + 2\text{S}^0$ |

Table 1.2 Stepwise oxidation of pyrite with intermediate products. After Robinson (1996).

| <u>Reactions in order:</u> | |
|----------------------------|--|
| (Step 1) | $\text{FeS}_2(\text{s}) + \frac{7}{2}\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + 2\text{SO}_4^{2-} + 2\text{H}^+$ |
| (Step 2) | $\text{Fe}^{2+} + \frac{1}{2}\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O}$ |
| (Step 3) | $\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe}(\text{OH})_3(\text{s}) + 3\text{H}^+$ |
| (Step 4) | $\text{FeS}_2(\text{s}) + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$ |

1.3 Aqueous Dumping Policy

Canadian policy on the disposal of materials into aqueous environments begins with the Constitution Act of 1867. The Constitution states that the provinces are ‘owners’ of their respective watersheds, and that the responsibility for the handling of hazardous materials lies with the provincial governments. The Federal Water Policy of 1987 and the Canada Water Act of 1970 provide the structure for provincial action (www.gov.ns.ca) (Canada Water Act, R.S.,c. 5 (1st Supp.), s. 1.). Exceptions to these regulations are granted if applications are filed in advance and the required fees paid. The provincial Minister then approves the method and site of disposal (www.gov.ns.ca). Acid rock drainage is a significant problem in Nova Scotia, and because of this the Sulphide Bearing Material Disposal Guidelines were established in 1991 and incorporated into the Nova Scotia Environmental Act (1994, 1995, 1999) (www.gov.ns.ca). Information on the details of the policy for aqueous dumping of materials can be obtained from Whittaker (1999) and (www.gov.ns.ca). Disposal of materials in marine waters is even more restricted as a result of the Ocean Dumping Regulations of 1988 and the Environmental Protection Act of 1988 and 1996, and most recently superseded by the Canadian Environmental Protection Act of 1999.

1.4 Purpose and Scope

The purpose of this study is to explore the effects of the disposal of waste acid rock into the Bedford Basin, Nova Scotia by examining acid production in the different environments of the basin (i.e. fresh and salt water). The Bedford Basin is part of the

Halifax Harbour, so it is a saltwater environment, however, there is also a fresh water source, and it is in this mixing area where waste rock is being dumped. Samples of rock from the GHT and from the Halifax slate were taken from the Beaverbank Road, an area of known geology and geochemistry from work by Feetham (1996). Duncan method acid prediction tests and petrographic descriptions, and sulphide mineralogy were carried out on bulk rock samples of the two rock types, as well as the sulphide distribution for different size fractions. The size fractions used in the experiments are typically much smaller than most of those disposed into the Bedford Basin; smaller samples were used to facilitate sample collection and experimental techniques applied.

1.5 Methodology

The rock samples of known geochemistry were collected in the field on October 9th, 2003 from specific locations along the Beaverbank Road, Sackville, Nova Scotia (Figure 1.1). One sample of GHT and one of Halifax slate were selected for geochemical analysis. Polished thin sections were made and used for microprobe analysis for mineral identification, and for complete petrographic descriptions.

Geochemical analyses were completed by the Dal Tech Minerals Engineering Centre and I was present for the experimental procedure. In addition to geochemical work, pH water experiments were carried out, and bacterial control experiments were carried out by Armstrong (supervisor). Samples from the controlled experiments were also chosen for SEM work to observe possible bacterial survival and attachment to the rocks.

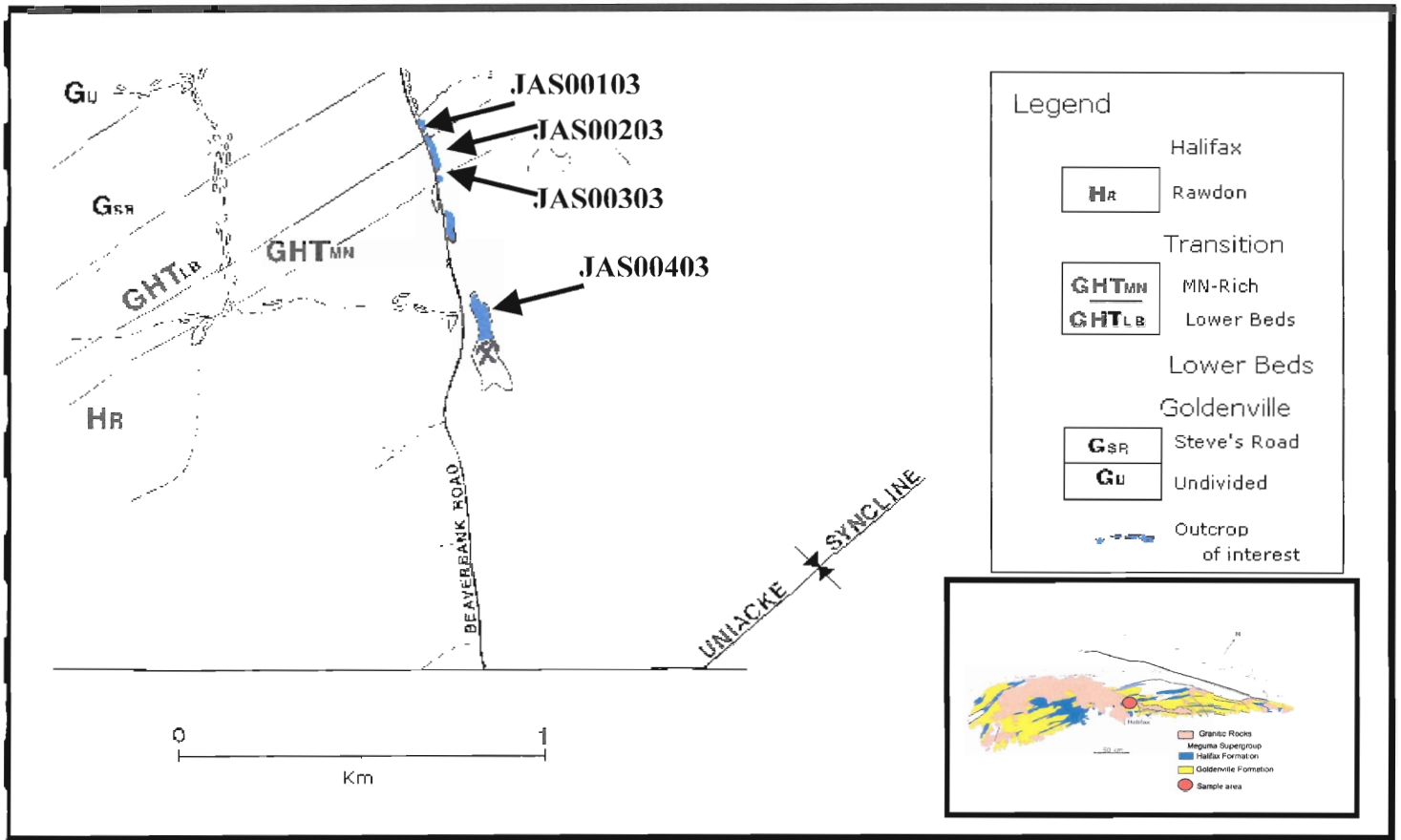


Figure 1.1 Map of the Beaverbank Rd area in Sackville, Nova Scotia. Sample locations are marked with arrows. (Modified after Feetham 1996).

1.6 Organization

Chapter 2 of this thesis will discuss the geology of the study area; chapter 3 the microbiological consideration of this study, specifically acid producing bacteria, such as *Acidithiobacillus ferrooxidans*, and its role in ARD; and chapter 4 presents the detailed methodology. The results are presented in chapter 5, and conclusions and future work in chapter 6.

Chapter 2: Acid Rock of the Meguma Group

2.1 Geology of the Meguma Group

The Meguma Group, metasedimentary rocks of Cambrian-Ordovician age, underlies 125,000 km² of southern Nova Scotia (Figure 2.1) (Zentilli and Fox, 1997). The Meguma Group is part of the Appalachian orogen and has a regional strike of ENE (Zentilli and Fox, 1997). The rocks of the Meguma Group are folded into open to tight upright folds with a subhorizontal axis (Waldron 1992). The Meguma Group consists of two formations, the lower Goldenville Formation of thickly bedded metamorphosed sandstones with subordinate interbedded slate, and the upper Halifax Formation consisting of slates with interbedded metasiltsstones (Fox *et al*, 1997). The transition zone between the two formations, informally termed the Goldenville-Halifax transition zone (GHT), is of particular interest to this study as it is high in sulphide minerals. The Goldenville-Halifax transition zone consists of several informally defined members; the New Harbour Member, the Rissers Beach Member, the Tanook Member and the West Dublin Member (Waldron 1991) (Figure 2.2). The rocks of the Meguma Group are thought to have formed in a basin environment; such an environment could account for the formation of sulphides, such as pyrite in the anoxic zone of the sediment water interface (Waldron 1991). The basin environment creates such an anoxic zone, which may explain the local enrichment of sulphides in the rocks of the Meguma Group (Figure 2.3). The rocks of the Meguma Group have been regionally folded into ENE trending open to tight folds during the Acadian orogeny (Waldron 1991).

2.1.1 Goldenville Formation

The Goldenville Formation is composed of massive, poorly sorted, grey to green-grey quartzose sandstones and metagreywackes with interbedded grey to black slates, and represent a sequence of rocks in a turbidity environment (Figure 2.3) (Waldron 1992). The rocks of the Goldenville Formation have been regionally metamorphosed to the greenschist facies, and locally to the amphibolite facies. The Goldenville Formation has a minimum thickness of 5400 meters, with the base of the formation not being exposed (Williams 1985). These turbidite deposits are typically unfossiliferous, although locally trilobite fragments of Middle Cambrian age have been found (Waldron 1992). The Goldenville Formation also has gold bearing quartz veins in numerous locations throughout the region (Williams 1985) (Ryan 1997).

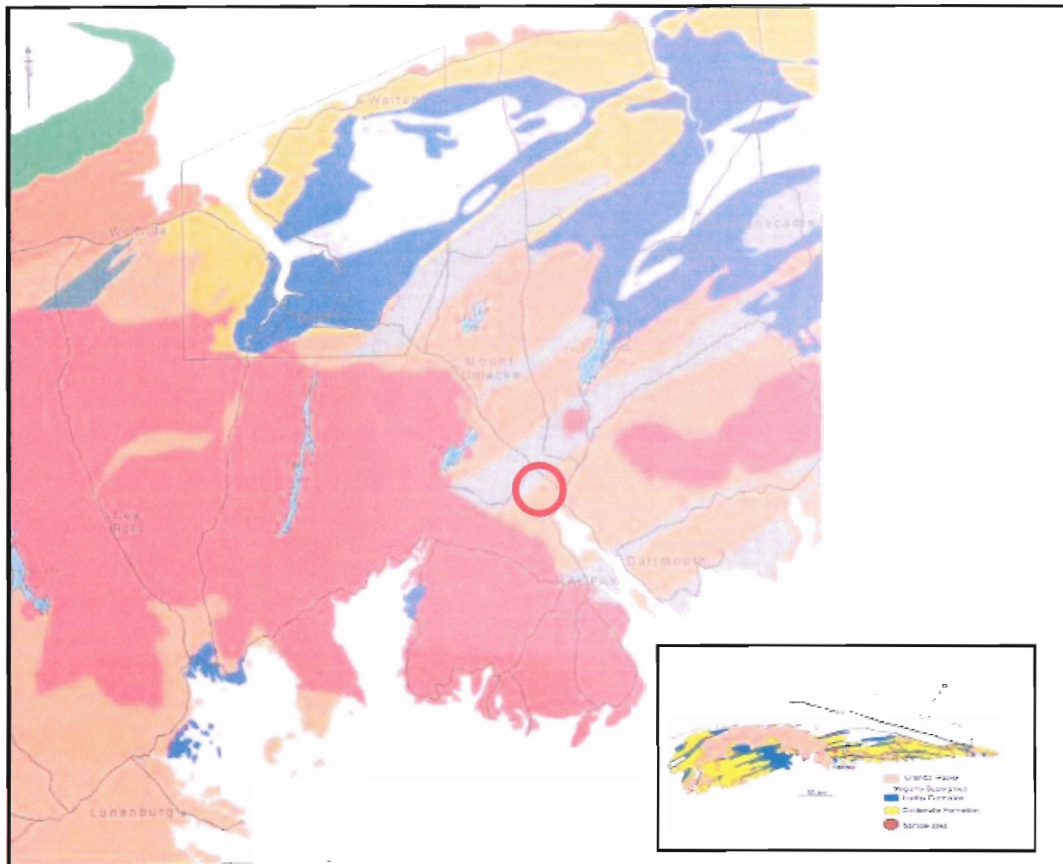


Figure 2.1 Figure representing the Meguma Supergroup of Southern Nova Scotia. The Halifax Formation is represented in grey in this figure and the Goldenville Formation represented in orange. The study area is represented by the red circle.

2.1.2 Goldenville-Halifax Transition Zone (GHT)

The Goldenville-Halifax transition zone (GHT) stratigraphically straddles the older Goldenville Formation and the younger Halifax Formation (Figure 2.1 and 2.2). The GHT is recognised by its rapid changes in physical sedimentology, in chemistry, and in fossil assemblages (Waldron 1992). The GHT is found in alternating bands striking almost parallel to the coastline of southern Nova Scotia as a result of the local folding in the area (Figure 2.1). The GHT is marked by sharp contacts with both the Goldenville and Halifax Formations in the east and gradational contacts in the central and western parts of Nova Scotia. The GHT is of variable thickness and contains thin interbedded manganiferous layers with abundant calcareous nodules and spessartine-garnet rich layers, collectively referred to as the coticule (Schiller & Taylor 1965). The GHT is locally enriched in sulphide minerals. Figure 2.3 presents block diagrams representing the sequence of deposition of the Members of the GHT zone, and how the anoxic zone may have formed; in this way, developing the locally rich sulphide rocks of the Meguma Group we know today (Waldron 1991).

2.1.3 Halifax Formation

The overlying Halifax Formation has an average thickness of 3600 meters (Williams 1985). The Halifax Formation is composed of grey-green to black slates, locally enriched in pyrite and arsenopyrite, with some metasiltsstones and minor metasandstone (Williams 1985).

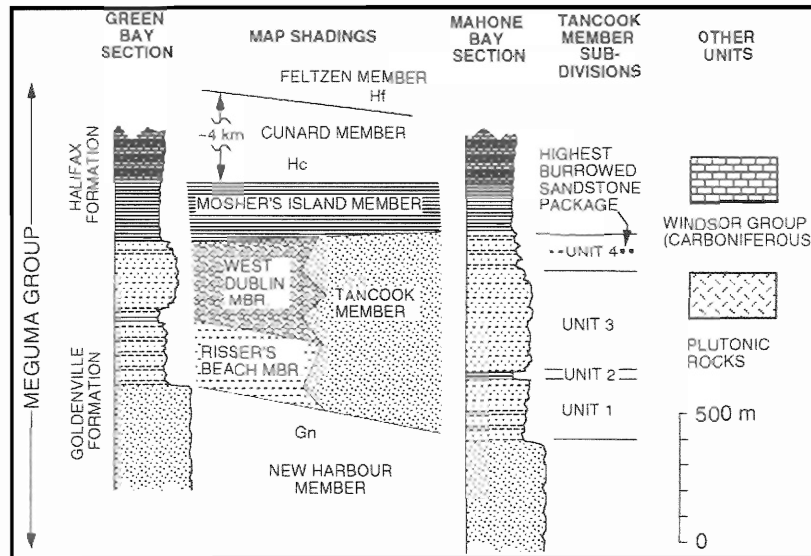


Figure 2.2 A diagram representing the informally defined Members of the Goldenville-Halifax Transition zone (GHT) in the Green Bay and Mahone Bay areas. These members are similar to those found in the Beaverbank Rd. area. After Waldron (1991).

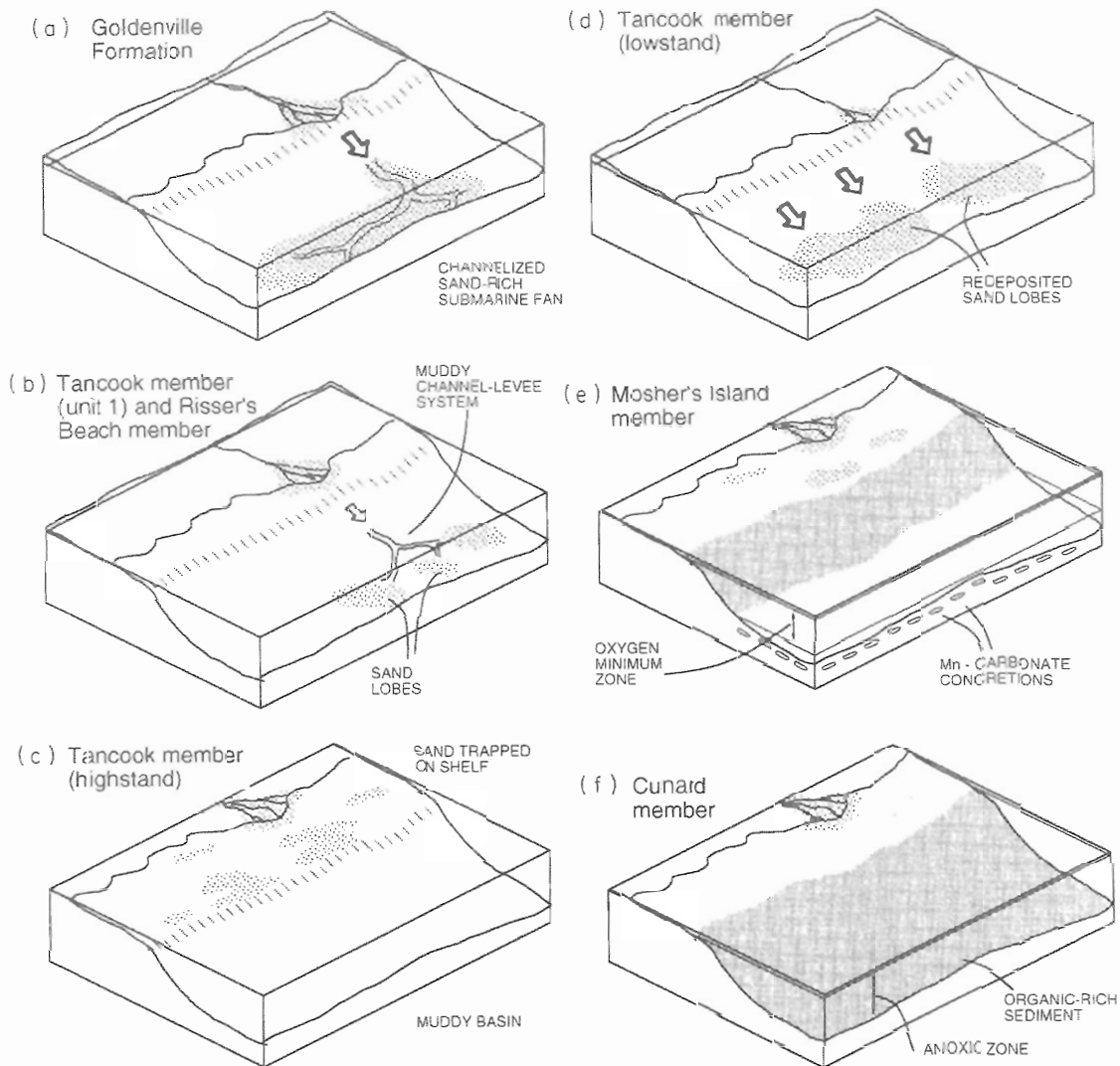


Figure 2.3 Block diagrams representing the formation of the GHT zone overlying the older rocks of the Goldenville Formation:

- (a) Goldenville Formation, sand turbidites as a result of erosion of the continent.
- (b) Tanook Member unit 1 being deposited by marine fans, of sand creating sandstone.
- (c) Tanook Member unit 2 high stand floods blocking supply to the basin.
- (d) Tanook Member units 3 and 4 are composed of sands from a low stand environment.
- (e) Mosher's Island Member of laminated sediments with carbonate concretions deposited in a low oxygen environment.
- (f) The last GHT zone Member, the Cunard Member if silty sediments where the basin is completely anoxic to produce sulphide rich shales and siltstones.
- After Waldron (1991).

Rocks of the Halifax Formation are regionally metamorphosed to greenschist facies and in some places to the amphibolite facies. In areas close to granitic plutons of the South Mountain Batholith, a hornfels facies is also developed (Williams 1985).

2.2 Sample Location

Samples were collected along Highway 354 at North Beaverbank Road approximately 17km north of Lower Sackville, Nova Scotia (Figure 1.1). There is almost continuous exposure in this section from the Goldenville Formation through the GHT to the Halifax slates on the East side of the road for a distance of 550 m. The Halifax slates are well exposed in a nearby abandoned shallow quarry, easily visible from the road (Figure 2.4). The GHT is distinguishable because of the presence of the light coloured cotecule layers (Table 5.1).



Figure 2.4 An abandoned slate quarry in the Steve's road area, which is very similar to that of the Beaverbank area.

Chapter 3: Microbiology of ARD

3.1 Brief Introduction to Microbiology

This study focuses on the bacteria that contribute to the oxidation of sulphides that cause acid rock drainage (ARD). The oxidation of sulphides occurs in nature; causing an initial rapid decrease in pH with the possible release of heavy metals into the surrounding environment. However, bacteria can significantly accelerate the reaction by one to two orders of magnitude (MEND 1.42.1 1995).

Bacteria or prokaryotes are single celled organisms lacking a nucleus and a true membrane. Microbiology involves the study of prokaryotes, some eukaryotes, and viruses. Eukaryotes, animal cells, plant cells, and some single celled organisms have a membrane-enclosed nucleus and are more complex and generally larger than prokaryotes (Table 3.1). Prokaryotes are divided into two domains, the archaeobacteria and the bacteria, based on ribosomal RNA (rRNA) sequences (Figure 3.1) (Prescott 1996). The Archaea and Bacteria domains differ in that bacteria have peptidoglycan and cell membranes with fatty acids while the Archaea have cell membranes with phytanyl groups (Stanley and Reysenbach 2002). Generally the Archaea domain contains bacteria that live in more extreme environments. The metabolism of the bacteria includes all the reactions involved in the maintenance of the cell for growth (Prescott 1996). Even though the acid producing bacteria were not genetically identified by 16S rRNA and physiological techniques, *A. ferrooxidans* is the predominant acid producing bacteria of focus in ARD investigations. *A. ferrooxidans* is associated with ARD and has been studied previously in relation to ARD by Jones (1997). Further information on the study of microbiology may

be found in texts such as Microbiology (Prescott 1996) or Biology of Microorganisms 7th Edition (Brock 1994).

3.2 Introduction to *Acidithiobacillus ferrooxidans*

A. ferrooxidans is an aerobic, Gram negative, obligate chemolithotrophic bacterium found in the Bacteria Domain (Table 3.1, Figure 3.1). *A. ferrooxidans* is a motile rod approximately 0.5 x 1.0 µm in size and is an obligate autotroph which is able to derive energy from the oxidation of ferrous iron as well as from reduced sulfur compounds (Ehrlich 1996). An obligate autotroph is an organism that can only utilize the specific metabolic pathways involving the fixation and usage of inorganic carbon. The term chemolithoautotroph can be applied to *A. ferrooxidans* and refers to the nature of metabolism of the bacteria. By definition a chemolithotroph can be considered as: ‘chemo’, referring to the chemical electron source (instead of photosynthesis); ‘litho’, refers to the use of inorganic electron donors instead of organic or ‘organo’. The suffix ‘trophy’ refers to the metabolism defined by the prefixes. The ability to oxidize Fe²⁺ is the key characteristic that is generally employed in isolation of this species of bacteria; enrichment on Fe²⁺ and a low pH (around 2) can result in almost pure cultures of the *Acidithiobacillus* genus (Balows 1992). There is confidence in the presence of *A. ferrooxidans*, even though the study did not verify this by 16S rRNA ribotype sequencing.

Two main types of metabolism exist in the microbial world with respect to carbon utilization, autotrophic and heterotrophic. Autotrophs are complex bacteria that can utilize light (i.e. photoautotrophs) or inorganic molecules (lithoautotrophs) for energy for

the fixation of CO₂. Heterotrophs are the majority of organisms; they use organic compounds for energy sources and for carbon. There are two types of heterotrophs, chemoheterotrophs that use organic molecules as energy and carbon source, and photoheterotrophs that use light energy and organic molecules for a carbon source. Generally speaking there are two types of carbon utilization (autotrophy and heterotrophy) and three types of energy production (organo-, litho-, and photo-) and various microorganisms are included in the various combinations. *A. ferrooxidans*, by definition, is a chemolithoautotroph. *A. ferrooxidans* has a optimal growth temperature at around 45°C, below 28°C the growth of *A. ferrooxidans* slows, and below 5°C the growth of *A. ferrooxidans* is very slow (JWEL 14387). The optimal growth for *A. ferrooxidans* is determined in a laboratory, actual conditions in nature are almost never optimal however bacteria still flourish. Oxidation of sulphides is an exothermic reaction, creating a favourable micro-environment for bacterial growth, even in temperatures below 5°C (wikipedia.org) (www.biotech.ubc.ca)

Table 3.1 Prokaryotes vs. Eukaryotes. (Modified from Jones 1997)

| | Archaea | Bacteria | Eukaryotes |
|--------------------------|--|--|---|
| Cell Wall | phytanyl groups peptidoglycan | Gram negative and Gram positive (Figure 3.2) Inner and outer membrane Peptidoglycan Muramic acid | True cell wall |
| Organelles | No | No | Yes |
| Microtubules | No | No | Yes |
| Nucleus | No | No | Yes, membrane bound nucleus |
| Genetic material | DNA | Double stranded DNA ring | Membrane bound multiple chromosomes |
| Genetic Recombination | <ul style="list-style-type: none"> • Plasmids • Sex Pili | <ul style="list-style-type: none"> • Plasmids • Transposons • Sex pili | Meiosis |
| Division | Binary fission or budding | Binary fission or budding | Mitosis |
| General Size | R <0.5 μm to approximately 2 μm | <0.5 μm to approximately 2 μm | <5 μm to approximately 10 μm |

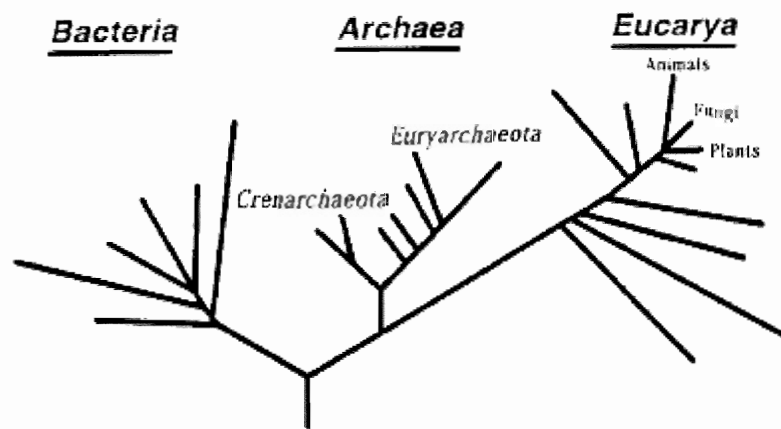


Figure 3.1 A diagram of the Universal Phylogenetic tree determined by rRNA sequence comparisons. After Prescott 1996.

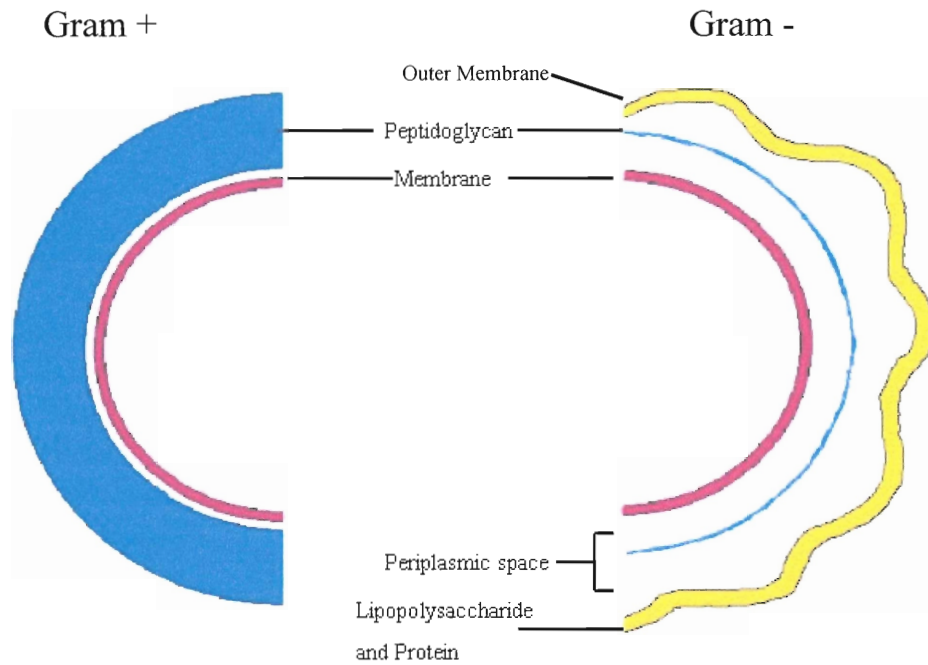


Figure 3.2 The differences between bacterial Gram negative and Gram positive cell walls. *Modified from Brock et. al 1994.*

3.3 *Acidithiobacillus ferrooxidans* and acid rock drainage.

The most widely studied, and common bacteria, associated with ARD is *A. ferrooxidans*. *A. ferrooxidans* is not the only bacteria involved with ARD, however it will be the bacteria of focus in this study. *A. ferrooxidans* catalyses the oxidation of sulphide minerals resulting in a low pH and the release of metals into the surrounding environment, either of which can lead to environmental problems. Sulphide minerals naturally oxidize over time with exposure to oxygen, however in the presence of *A. ferrooxidans* the reaction proceeds more rapidly by one to two orders of magnitude, with a greater initial decrease in pH. The decrease in pH is a result of the enzymes used to catalyze the oxidation reaction.

3.4 Metabolism of *A. ferrooxidans*

The metabolism of *A. ferrooxidans* will only be discussed as it relates to acid rock drainage, or the unique aspects that involve iron and sulphur as major components of survival. Autotrophs assimilate their carbon from CO₂, which requires reducing power; when *A. ferrooxidans* grows on an iron source the reducing power source is Fe²⁺ (Ehrlich 1996). Iron plays two important roles in the growth of *A. ferrooxidans*, that of a nutrition source and that of a source of reducing power. The reduction of fixed carbon is carried out by a process of reverse electron transport via the cytochrome system (Ehrlich 1996). The assimilation of the carbon is then carried out by the Calvin-Bensen cycle, or the minor mechanism involving phosphoenolpyruvate carboxylase, an enzyme also involved in certain amino acid formation (Ehrlich 1996). Energy in the form of ATP (adenosinetriphosphate) is made via an enzyme reaction involving ATP_{ase} and protons

from the proton motive force (PMF) (Figure 3.2). There are two proposed models for the iron oxidizing system in *A. ferrooxidans*; this thesis will use the more current model from 1992 as outlined Ehrlich 1996 which is based on direct observation (Figure 3.2). The second model for the oxidation of Fe^{2+} to Fe^{3+} has an unknown enzyme X, whose interaction with the cytochrome chain is not known (Ehrlich 1996). In direct observation the oxidization of Fe^{2+} to Fe^{3+} is done by the transfer of electrons in the cytochrome chain (Figure 3.2). The cytochrome chain transfers the electrons by a series of oxidizing and reducing steps with the members in the chain, and the end product is water in the cell (Ehrlich 1996). The cytochrome chain also produces energy by the release of protons which form ATP molecules. *A. ferrooxidans* has a unique protein which is involved in the oxidation of iron, called rusticyanin, this protein's exact role however, is not fully understood (Ehrlich 1996).

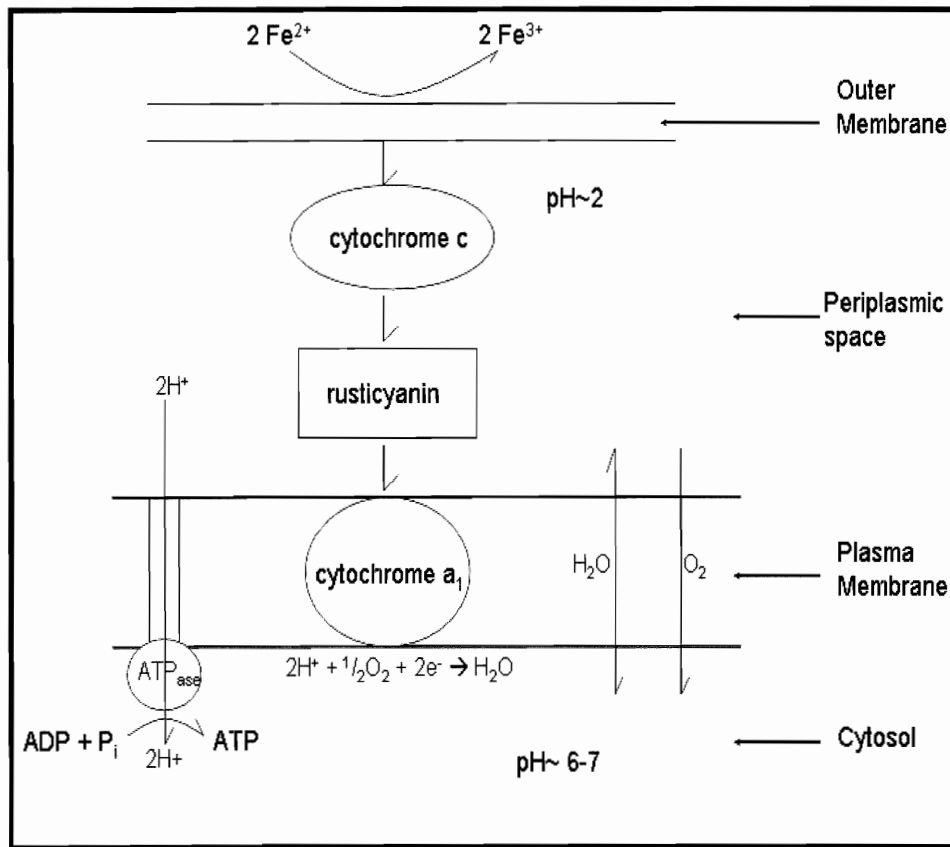
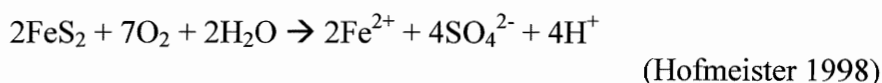


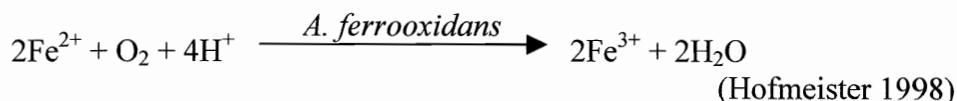
Figure 3.3 A schematic representation of the oxidation of Fe^{2+} to Fe^{3+} by *Acidithiobacillus ferrooxidans* by way of the cytochrome chain and a copper protein rusticyanin. Modified from Ehrlich 1996.

3.5 Steps of Pyrite oxidation by *A. ferrooxidans*

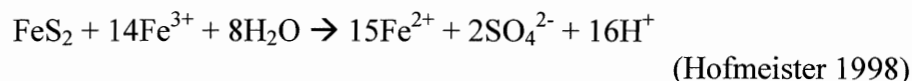
A. ferrooxidans is involved in most sulphide oxidation, however, pyrite is used for the following oxidation reaction as it is more widely studied than the other sulphides in the Meguma Group (McIntosh *et al.* 1997) (Hofmeister 1998). The oxidation of pyrite and other related sulphide minerals involves a combination of chemical and biological reactions. The oxidation of pyrite begins with a slow chemical initiator reaction, which establishes acidic conditions so that ferrous iron, Fe^{2+} , is stable in the presence of oxygen. The chemical initiator reaction is as follows:



The next reaction is the oxidation of the ferrous iron to ferric iron which is catalyzed by *A. ferrooxidans* as follows:



The ferric iron, which is formed by the microbial reactions, can continue to react spontaneously with more pyrite in the following chemical reaction:



The resulting ferrous iron is then oxidized by *A. ferrooxidans* once again producing more ferric iron. The last two reactions result in the oxidation of pyrite in a progressive, propagation cycle, which ultimately results in a significant amount of sulphuric acid of a very low pH, of approximately 3.1 (Hofmeister 1998) (Brock 1994).

Chapter 4: Sampling, Preparation and Analytical Methods

4.1 Sample collection and preparation

Samples of both GHT and Halifax slate were collected along the Beaverbank Rd. in Sackville, NS (Figure 2.1) (Figure 2.2). The sample location was selected based on previous work completed by Mary Feetham in 1996, which indicated that a near complete exposure of the GHT existed at this location. I collected samples of GHT of known geochemistry (Feetham 1996), and samples of Halifax slate, of unknown geochemistry. Fresh surface samples of one representative sample of each rock type were selected and passed through a clean re-enforced steel jaw crusher once. Samples were then sieved using a shaker into 6 separate size fractions. Each size fraction is assigned a letter (a through to F) for recording purposes. The sizes ranged from large gravel (A) to ultra fine silt and clay (F). The sizes A, B, and C were not used in any water experiments as there was not enough reasonably fresh sample for both experimental work and geochemical work.

Table 4.1 The six size fractions used for geochemical analysis and in the microbiological tests.

| | |
|---|-----------------------|
| A | > 19.00 mm |
| B | 19.00 mm - > 11.20 mm |
| C | 11.20 mm - > 4.75 mm |
| D | 4.75 mm - > 1.00 mm |
| E | 1.00 mm - > 0.50 mm |
| F | < 0.5 mm |

Each size fraction was weighed and sealed in a bag to prevent any microbial growth, and stored in a cold room (5⁰ C). The sealed bag would reduce oxygen exposure and the cold room would slow any existing bacterial growth. Salt water was collected from the Bedford Basin, to obtain an initial pH of the water and to use in the pH experiments. Fresh water was collected from Kearney Lake, as it is a fresh water source for the Bedford Basin, the area of interest. The water was filtered through a #1 Whatman filter (11 µm in pore size) using a vacuum. The vacuum filtration removes organics and large debris from the water, but leaves any natural microbes in the water.

4.2 Microbial culture from sample

A liquid media was prepared for *A. ferrooxidans* using Wolfe's mineral media (Appendix A). The media is specific for *A. ferrooxidans* however, it can be used to select for most bacteria in the *Acidithiobacillus* genus. The media has a very low pH, for optimal growth of that particular genus. A sample of Halifax slate from the abandoned quarry (Figure 2.4) had an iron precipitate on the bottom of a flowing stream (Figure 4.1). The slate pebble conglomerate was selected for microbial culture as there was an iron oxide cement, a by-product of sulphide oxidation and thus bacteria could be present. This wet iron precipitate sample JAS00403 A was scraped into an 250 ml Erlenmeyer flask containing 25 ml of liquid media and placed in a 27⁰ C room on a shaker table rotating at 200 rpm's, to aerate the culture. Growth of bacteria typically results in an orange colour of the media (Figure 4.2). The cultures were subcultured to continue bacterial growth and to produce media with no rock fragments remaining (Appendix B).

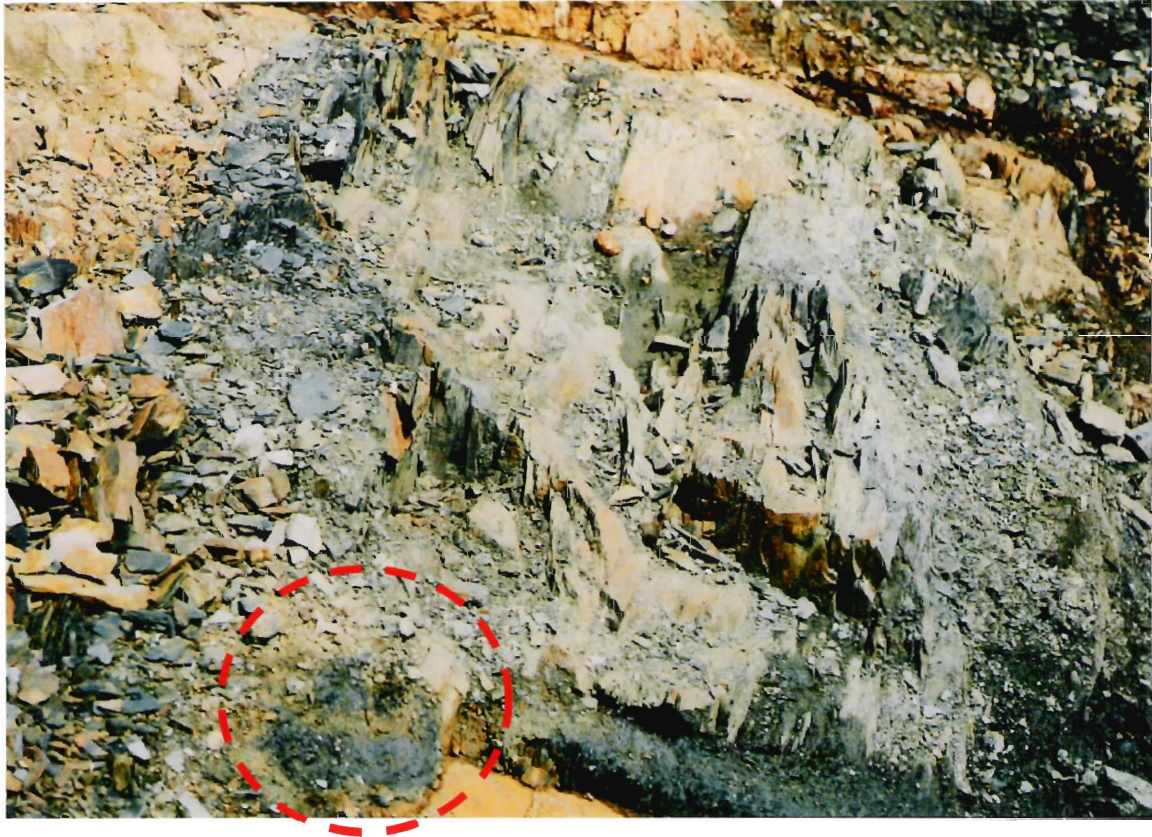


Figure 4.1 Iron conglomerate (red circle) found in the abandoned slate quarry, used for microbial culture. The water run-off has an iron precipitate giving it a rust colour (right of circle).

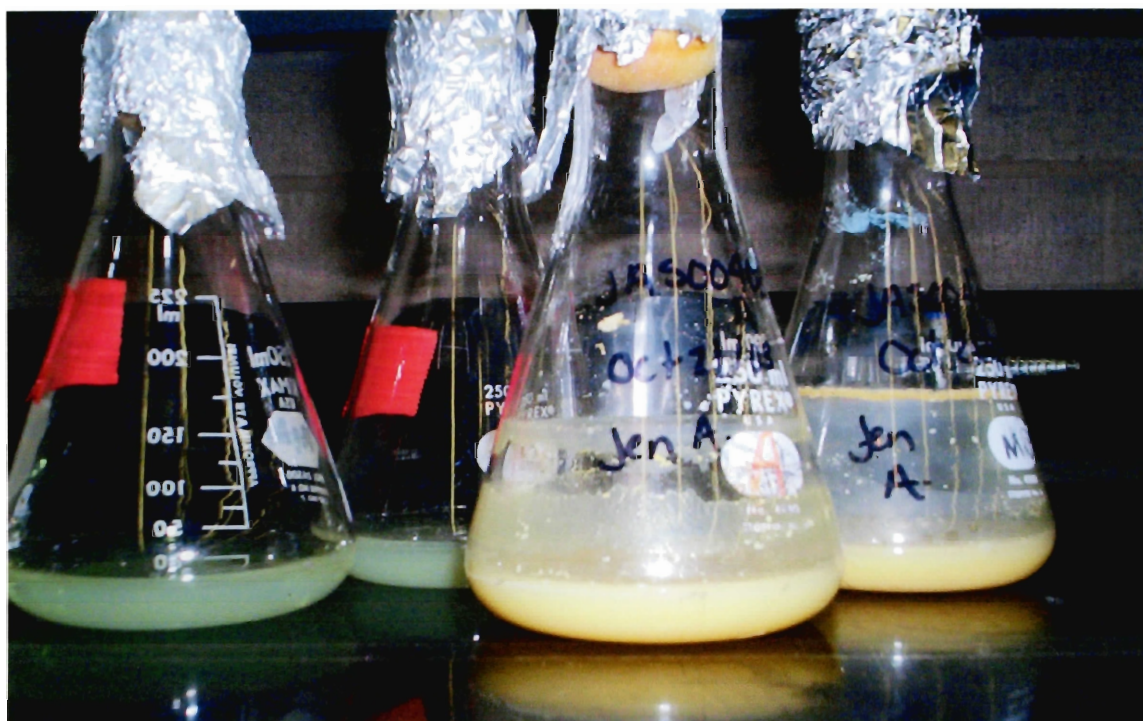


Figure 4.2 Positive growth in *A. ferrooxidans* media on the right, no growth in *A. ferrooxidans* media on the left. There is a definite orange colour observed in the positive growth culture.

Most Probable Number (MPN) technique was used to determine the number of bacteria in each tube, in other words to obtain a bacterial count (Appendix B)

4.3 pH experiments and controls

4.3.1 Water experiments

Testing of pH was carried out on both GHT and Halifax slate, involved using three size fractions D, E, and F (Table 4.1). Ten grams of each sample type of each size fraction was submerged in 50 mL of both filtered fresh and salt water in a 250 mL Erlenmeyer flask. Each flask was duplicated for control, left at room temperature (about 20- 25°C) and covered with parafilm to prevent water loss from evaporation, while allowing for airflow. The pH was recorded every third day, though after three weeks it was only recorded once a week as, at this stage pH reached an apparent plateau. The observed pH readings were then converted to proton concentration for the 35 day data collection period. Also student T tests were conducted on the mean mg/kg/day to identify any significant differences between salt and fresh water samples as well as the different rock types.

4.3.2 Inoculated versus Un-inoculated experiments

Controls were also carried out by Armstrong (supervisor) using 10 g of the D size fraction of both rock types (GHT and Halifax slate) in 100 mL of fresh and salt water. Four jars of each rock type in each water type were prepared, so that 2 of each were a control, and 2 of each were inoculated with the culture obtained from the sample (2.4 x

10^2 cells/ml). The final experiment set up was: 2 jars with GHT and fresh water, 2 jars of GHT in salt water autoclaved, 2 jars of Halifax slate in fresh water un-inoculated, and 2 jars of Halifax slate in salt water un-inoculated. The same number of jars for both rock types in both fresh and salt water were inoculated with bacteria and left at room temperature (about 20-25°C) for a period of 28 days, recording the pH every 7 days.

4.3.3 Water flushing experiment

Removal of water, fresh, salt, and distilled from Halifax slate samples was performed daily for 5 days. This experiment was done to mimic the tidal effects present in the Bedford Basin. Only samples of the Halifax slates were used as these are the dominant rocks being disposed of in the Bedford Basin. The D size fraction was used for the flushing experiment. New water was added every 24 hours, recording the pH before and after the addition of the new water. The flushing experiment used fresh, salt and distilled water.

4.3.4 Water Monitoring

The pH was monitored for all three water types, fresh, salt, and distilled with no rock interaction. Monitoring the water ensures any pH changes recorded were a result of sulphide oxidation.

4.3.5 SEM study

Samples were selected for SEM study, however only the samples of both rock types in salt water were examined to observe bacterial survival in saline waters. The

control samples selected for SEM photography were autoclaved after the pH experiment as the process of autoclaving could affect the rocks integrity during the experiment. Autoclaving kills bacteria, however it does not remove them from the rock surface. Bacterial survival in fresh water is documented elsewhere however, it was previously thought that the microbes could not proliferate in salt water (Temple 1951). Salt water samples of both rock types, both control and inoculated, were chosen for photography for comparison. Two methods of sample preparation were used to prepare the samples for SEM imaging. This first method is the standard drying procedure for biological samples (Appendix C). The second preparation method used for SEM imaging was to let the samples air dry overnight in sterile glass containers, to prevent washing the bacteria off the rock, which can happen during the standard biological sample preparation. SEM images were obtained from both preparation methods.

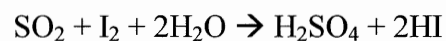
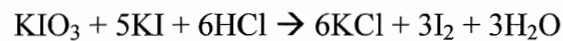
4.4 Mineralogical studies

Thin sections of the whole rock of both the GHT (JAS00303) and the Halifax slate (JAS00403) were selected for petrographic description and compared to samples previously studied by Fox (1999). The JEOL(♯) microprobe was used for identification of the sulphides in the rocks using EDS analysis.

4.5 Geochemistry

One representative sample of GHT (JAS00303) and one from the Halifax Formation was selected (JAS00403) for geochemical analysis. Geochemical analysis was carried out at the DalTech Mineral Engineering Centre laboratory. Whole rock

geochemistry was obtained for the Halifax slate sample only (JAS00403 A). For both rock types the total iron of the water of the pH experiment was determined using atomic absorption (AA) using the Varian 55-B machine. In a typical element analysis nitric acid is added to the water to keep the elements in solution, however, in my samples none was added, as there was sediment build up on the bottom of each container. The addition of nitric acid to my sample would have put more iron or additional elements in solution that were not already in solution, thus altering the results of AA. An acid production potential and acid consuming potential is calculated to find the net acid production of each rock type. Acid production potential is determined by the total sulphur content in the whole rock, the Duncan method (BCRIT research test) (Appendix D). A positive value indicates acid producing rock, and therefore the sample has potential for ARD. Samples were powdered using a steel jaw crusher and a shatter box, a ring of hardened steel. The natural pH of the sample is recorded, and then the sample is titrated to a pH of 3.5 using 1.0 N sulphuric acid using an automatic titrator (Appendix D). The amount of the iodate consumed during the combustion of the sample is a measure of the sulphur (S) in the sample, shown in equation 4.1.



Eq. 4.1

Sulphate/ Sulphur analysis was carried out on the calculated bulk sample by a standard method (Appendix D).

4.6 QA/ QC Standards

For all the analytical work standards were used to ensure data was precise and accurate. For the MPN a standard chart was used to get accurate numbers with a 95% confidence interval. Control experiments were done for pH analysis, of both rock types in both fresh and salt water, was carried out by Armstrong (pers. comm.). The pH meter was calibrated with a buffer of known pH before each reading was taken to ensure accuracy. Chalcopyrite and pyrrhotite standards were used in the microprobe. For the sulphide/ sulphur analysis regulated standard NBM-1 was used to make sure the values were within the accepted limits.

Chapter 5: Results

5.1 Introduction

This chapter presents the results for all data collected during this research. All the data for the GHT are colour coded in yellow and all the data for the Halifax slates are colour coded blue. For size fraction data, letters have been assigned to each size fraction (Table 4.1) for ease of plotting on graphs.

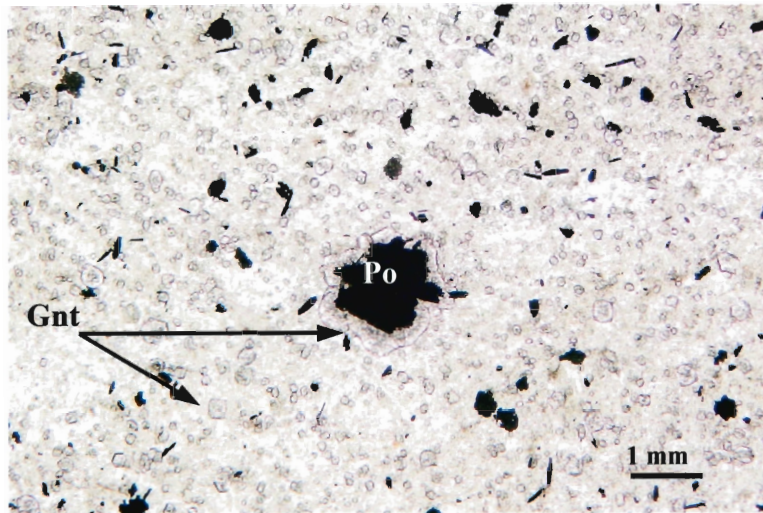
5.2 Rock Descriptions

The sample locations are indicated in Figure 1.1. Four samples of GHT were collected along the Beaverbank Rd; JAS00103; JAS00203; JAS00303 A and B. The samples chosen for analysis in this study were JAS00303 A to represent the GHT and JAS00403 B as typical of the Halifax slate. Sample JAS00403 A, a weakly consolidated slate pebble conglomerate, formed in situ from accumulation and cementation of slate fragments, was also used for microbial culture as it was collected in a flowing stream, and the iron oxide cement was a possible host for microbial life. The GHT sample JAS00303 A was a compact crystalline quartzite. The GHT had well defined cleavage and lighter coloured layers, the cotecule, with visible sulphide crystals. The Halifax slate sample JAS00403 B was a very fissile slate with visible sulphide crystals.

A thin section of JAS00303 A revealed 15 % to 25 % garnet and 5 % to 8 % opaque minerals. Some sulphide porphyroblasts had visible reactions with garnets creating a halo effect (Figure 5.1). A very fine crystalline clay quartz matrix rich in clay minerals of medium to high birefringence formed the bulk of the thin section. A thin section of Halifax slate JAS00403 B was also made and observed to have a well defined

cleavage with very small opaque minerals a part of the matrix. Strain shadows were also visible in the thin section with relatively large porphyroblasts ranging in size from less than 0.5 to 2 mm. The strain shadow porphyroblasts were possibly a chloritized garnet, with undulose extinction (Figure 5.2). The JAS00403 A thin section had a fine crystalline clay quartz matrix with a higher proportion of clay minerals than JAS00303 A.

A)



B)

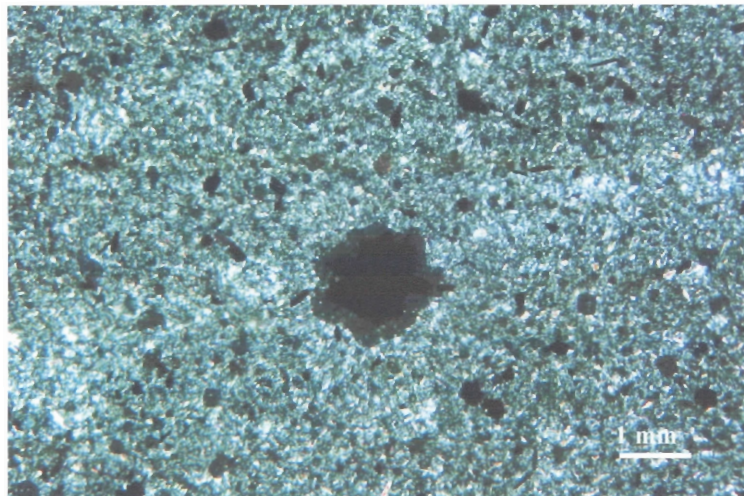


Figure 5.1 GHT whole rock thin (JAS00303 A). The field of view is approximately 10 mm across. The center crystal is pyrrhotite (Po) with a halo of garnet (Gnt) around it **A)** is under plane polarized light (PPL) and **B)** is under crossed Nichols (XN).

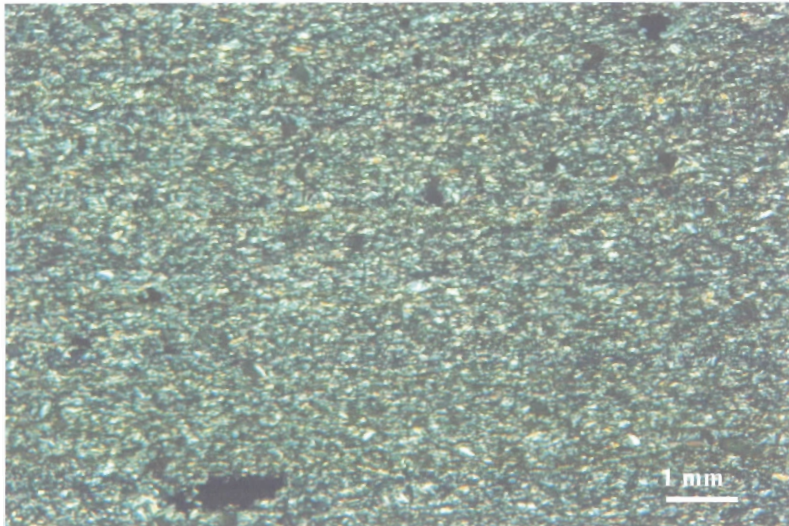
A)**B)**

Figure 5.2 Halifax slate whole rock thin section (JAS00403 B), the field of view is approximately 10 mm. **A** is under PPL and **B** is under XN. A cleavage is visible as well as some strain shadows with unidentified porphyroclast, possibly chloritized garnets.

5.3 Sieve results

Sieving of both the GHT and Halifax slate rocks through 5 different sized screens resulted in a high distribution of the 4.75 mm and 1.00 mm size fraction (Figure 5.1).

This data was collected to compare with the percent sulphur in each size fraction to observe any sulphide partitioning in the different size fractions. These data were collected to determine whether the different size fractions resulted in sulphide partitioning between the size fractions.

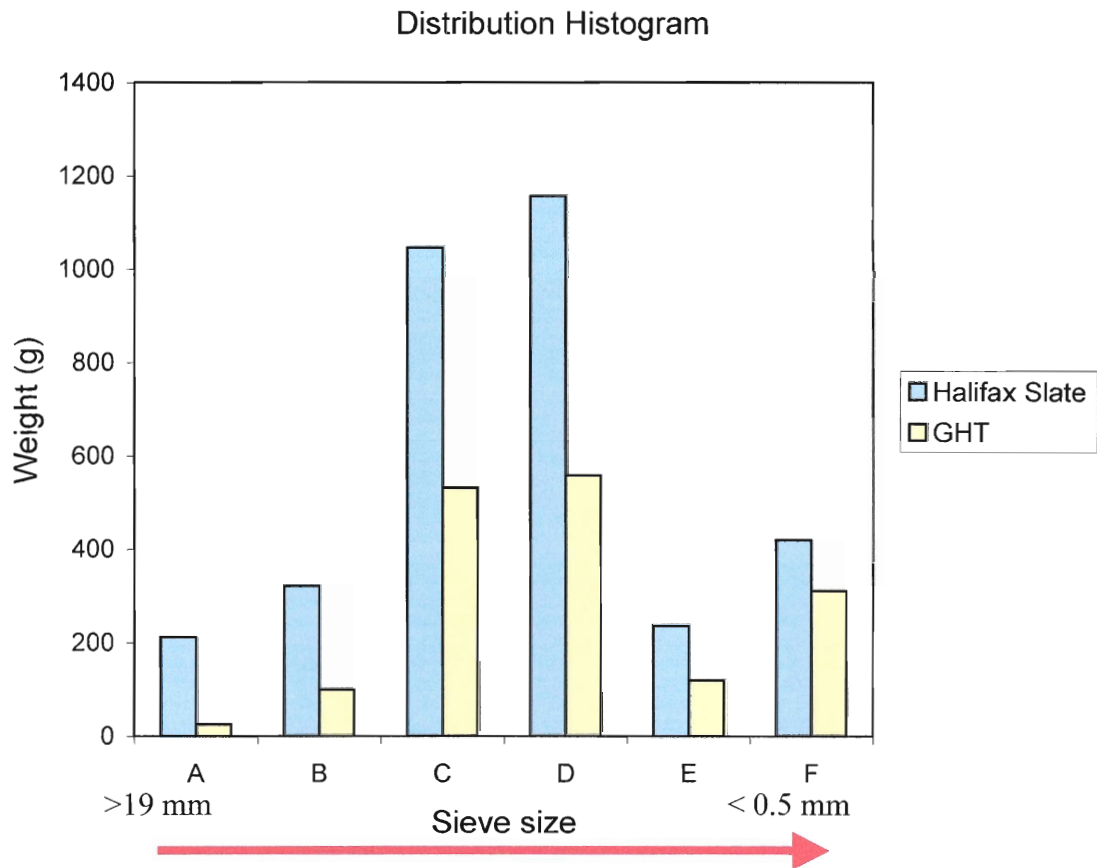


Figure 5.3 Distribution histogram of the weight percent of each size fraction of both rock types; the GHT (yellow) and Halifax slate (blue). Refer to Table 4.1 for the size that each letter represents, the arrow indicated decreasing grain size.

5.4 pH results

5.4.1 Water experiment Results

The average pH results taken over a period of 35 days are presented in Table 5.1. The average pH results were calculated from the duplicates during the 35 day period (Appendix E). Three size fractions (D, E, and F) of each rock type in both fresh and salt water were prepared in duplicate (Table 4.1). Several calculations were done to obtain the proton concentration for each rock type in each water type, however the average between the duplicates was used, not each individual sample (Appendix F). The pH was recorded over a period of 35 days with these three size fractions to: record the pH change over time; observe any difference in pH change between different sizes; and compare with the pH change of inoculated vs. un-inoculated experiment set up by Armstrong (pers. comm.) (Table 5.2) (Figure 5.4 A and B). The Halifax slate samples demonstrated a statistically significant greater decrease in pH than the samples of the GHT. The fresh water samples had a statistically significant greater pH decrease than those of salt water (Appendix F).

5.4.2 Inoculated versus Un-inoculated experiment Results

The control pH experiment was set up using both rock types, GHT and Halifax slate, and both fresh and salt water types. The D (< 4.75 - > 1.00 mm) size fraction of rock was used for this control experiment as the intent was to check for differences in pH in the inoculated versus un-inoculated. Proton calculations of this data set are found in Appendix F and indicate a rise in proton concentration within the first week. The

inoculated and un-inoculated samples had the same decreasing trend in pH decrease, there was no significant difference in the inoculated and un-inoculated samples when comparing the same rock types (Appendix F). The Halifax slate had an overall, greater decrease than the GHT in both inoculated and un-inoculated samples.

5.4.3 Water Flushing experiment Results

The changes in pH for the water flushing experiment mimicking tidal action are presented in Table 5.3. The pH decreases by one unit after 24 hours, however the addition of new water returns the pH to the original value. The pH then decreases again after the following 24 hours, displaying that sulphide oxidation continues after the removal of the reactions products.

5.4.4 Water Monitoring Results

In addition to recording the change in pH of waters with rock, pH was recorded over 28 days in distilled, fresh, and salt waters containing no rocks, to establish that the pH change was due to oxidation of the rocks (Table 5.4). Measurement of the pH in water only samples indicated no change over time.

Table 5.1 The average pH results taken over a period of 35 days in fresh and salt water for both GHT (yellow) and Halifax slate (blue) at room temperature (20- 25°C). Hfw **F** refers to the Halifax slate in fresh water size fraction F, and Gsw **D** refers to the GHT in salt water size fraction D. For size fractions in millimeters refer to Table 4.1.

| | DAYS→ | 0 | 2 | 7 | 9 | 12 | 14 | 16 | 19 | 21 | 28 | 35 |
|-------------|-------|------|------|------|------|------|------|------|------|------|------|------|
| Fresh Water | Hfw F | 5.3 | 4.85 | 3.65 | 3.85 | 3.55 | 3.55 | 3.55 | 3.45 | 3.4 | 3.5 | 3.2 |
| | Hfw E | 5.5 | 4.55 | 3.55 | 3.5 | 3.4 | 3.35 | 3.3 | 3.2 | 3.2 | 3.4 | 3.1 |
| | Hfw D | 6.1 | 5 | 3.85 | 3.8 | 3.7 | 3.7 | 3.7 | 3.6 | 3.6 | 3.7 | 3.5 |
| | Gfw F | 7.05 | 6.9 | 5 | 5.25 | 4.55 | 4.5 | 4.5 | 4.5 | 4.4 | 4.35 | 3.9 |
| | Gfw E | 6.95 | 7 | 5.55 | 5.8 | 5.25 | 5.4 | 5.5 | 5.6 | 5.6 | 5.55 | 5.1 |
| | Gfw D | 7.3 | 6.9 | 5.75 | 5.8 | 5.45 | 5.65 | 5.65 | 5.65 | 5.8 | 5.65 | 5.3 |
| Salt Water | Hsw F | 6.35 | 5.75 | 4.5 | 4.35 | 4 | 4.25 | 4.15 | 4 | 4.05 | 3.9 | 3.55 |
| | Hsw E | 6.8 | 6.35 | 4.65 | 4.45 | 4.1 | 4.05 | 4 | 3.9 | 3.9 | 3.75 | 3.55 |
| | Hsw D | 7 | 6.7 | 6.3 | 6.1 | 6 | 5.95 | 5.95 | 5.9 | 5.85 | 5.4 | 5 |
| | Gsw F | 7.1 | 7.25 | 6.55 | 6.3 | 6.1 | 6.3 | 6.3 | 6.35 | 6.35 | 5.95 | 5.4 |
| | Gsw E | 7.2 | 7.5 | 7.1 | 7 | 6.9 | 7.05 | 7.05 | 7.1 | 7.1 | 6.95 | 6.6 |
| | Gsw D | 7.4 | 7.7 | 7.2 | 7.1 | 7.05 | 7.35 | 7.35 | 7.35 | 7.4 | 7.25 | 6.95 |

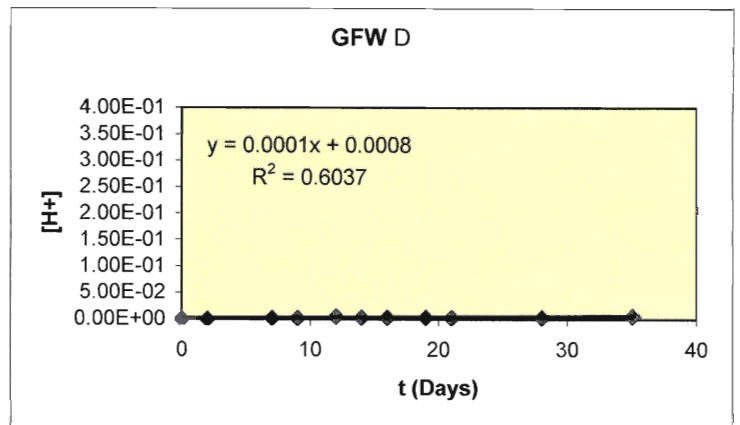
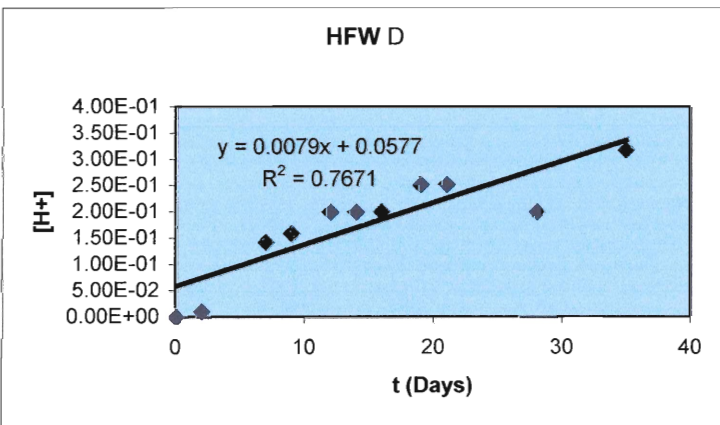
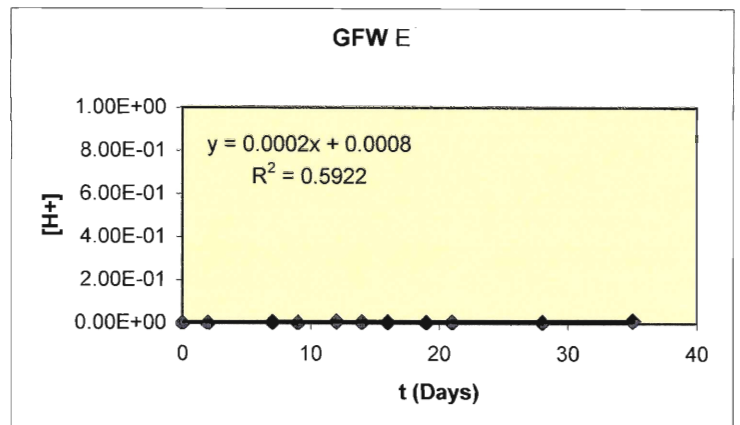
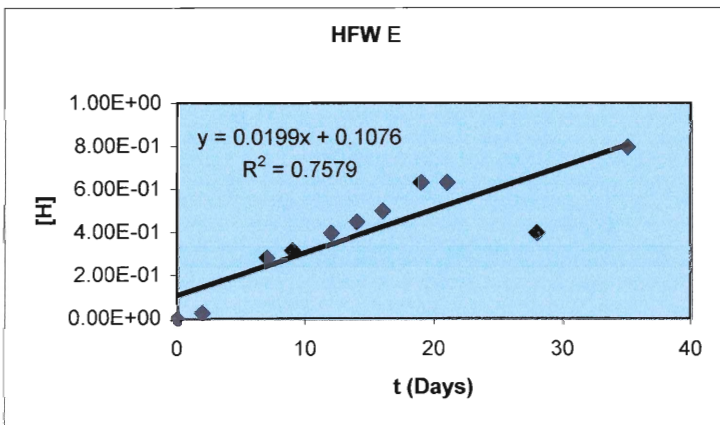
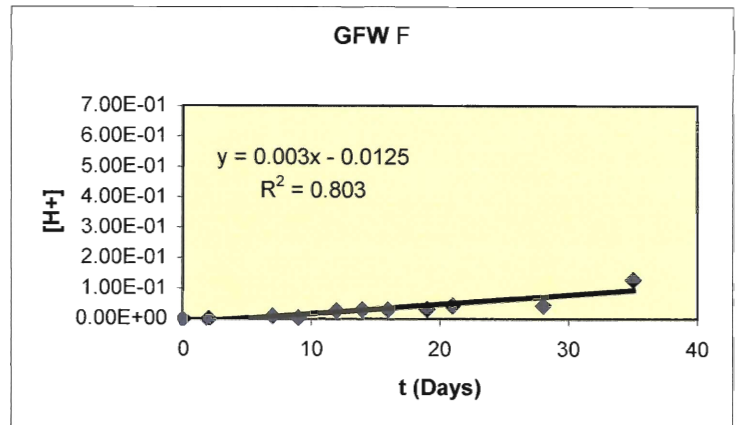
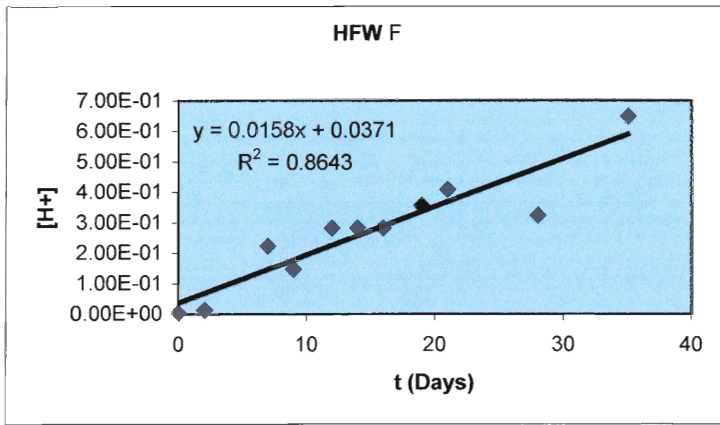


Figure 5.4 A Comparison of proton concentration for all three size fractions for Halifax slate (in blue) and the GHT (in yellow) samples in fresh water.

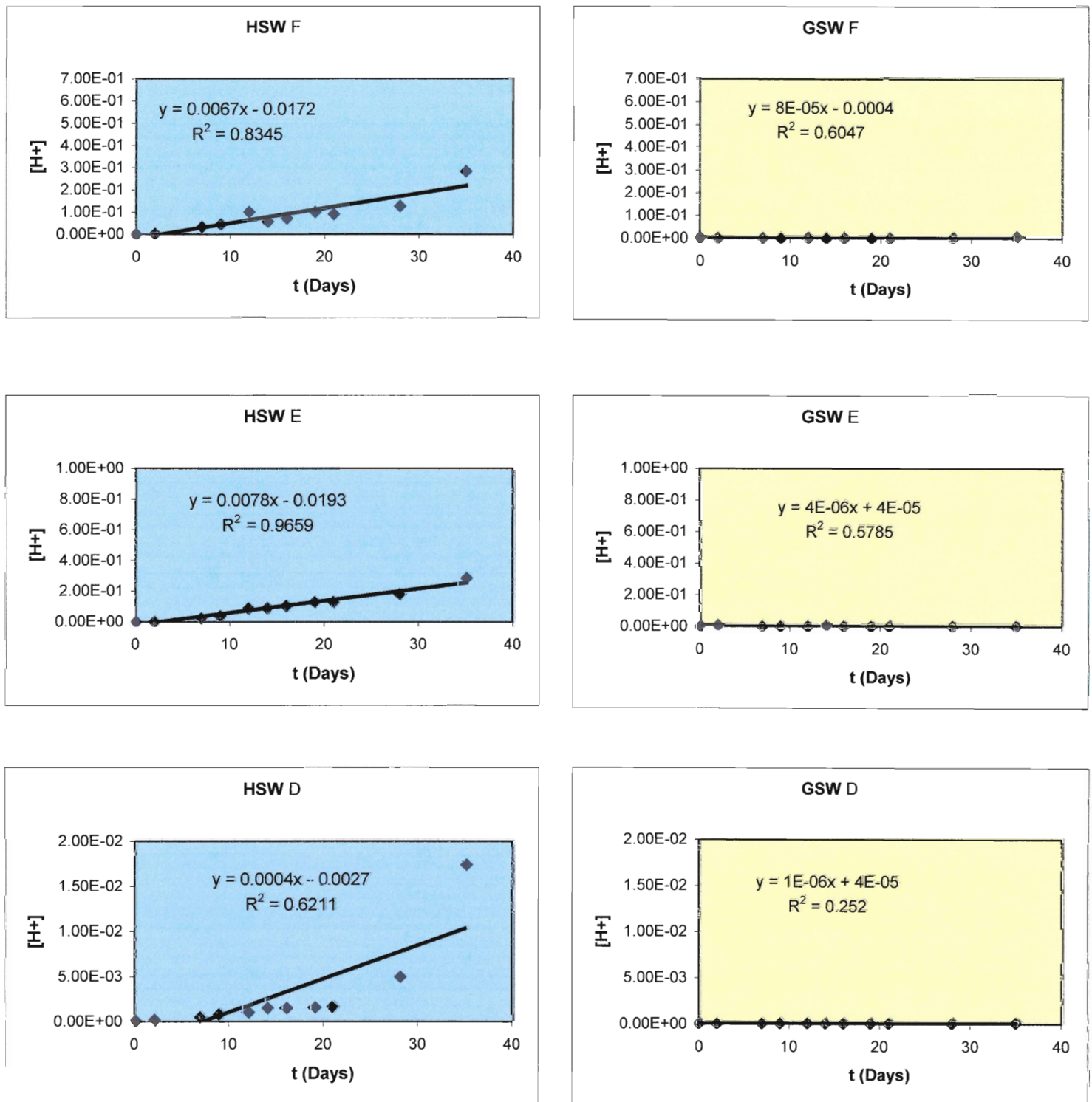


Figure 5.4 B Comparison of proton concentration for all three size fractions for Halifax slate (in blue) and GHT (in yellow) samples in salt water.

Table 5.2 The pH results observed over a 28 day period in, room temperature (20- 25°C), of not inoculated versus inoculated with acid producing bacteria. G and H refer to the two rock types, GHT (yellow) and Halifax slate (blue). Dw, fw, and sw refer to the different water types used, distilled, fresh, and salt respectively.

| | DAYS→ | 0 | 1 | 7 | 14 | 21 | 28 |
|-------------------|--------------|----------|----------|----------|-----------|-----------|-----------|
| Control | Hdw | 5 | 4.7 | 4.05 | 3.65 | 3.45 | 3.35 |
| | Hfw | 6.25 | 4.9 | 3.65 | 3.45 | 3.25 | 3.15 |
| | Hsw | 7 | 6.15 | 4.4 | 3.55 | 3.45 | 3.35 |
| | Gdw | 5.3 | 5.25 | 5.25 | 5.25 | 5.3 | 5.3 |
| | Gfw | 6.85 | 6.25 | 5.8 | 5.5 | 5.45 | 5.4 |
| | Gsw | 7.05 | 7 | 6.85 | 6.55 | 6.5 | 6.4 |
| Inoculated | Hdw | 4.8 | 4.75 | 4.05 | 3.7 | 3.55 | 3.4 |
| | Hfw | 5.85 | 4.8 | 3.65 | 3.65 | 3.45 | 3.35 |
| | Hsw | 6.95 | 6.15 | 4.65 | 3.85 | 3.6 | 3.45 |
| | Gdw | 5.35 | 5.35 | 5.35 | 5.35 | 5.4 | 5.3 |
| | Gfw | 6.6 | 6.35 | 6 | 5.5 | 5.35 | 5.25 |
| | Gsw | 7 | 6.9 | 6.7 | 6.65 | 6.55 | 6.4 |

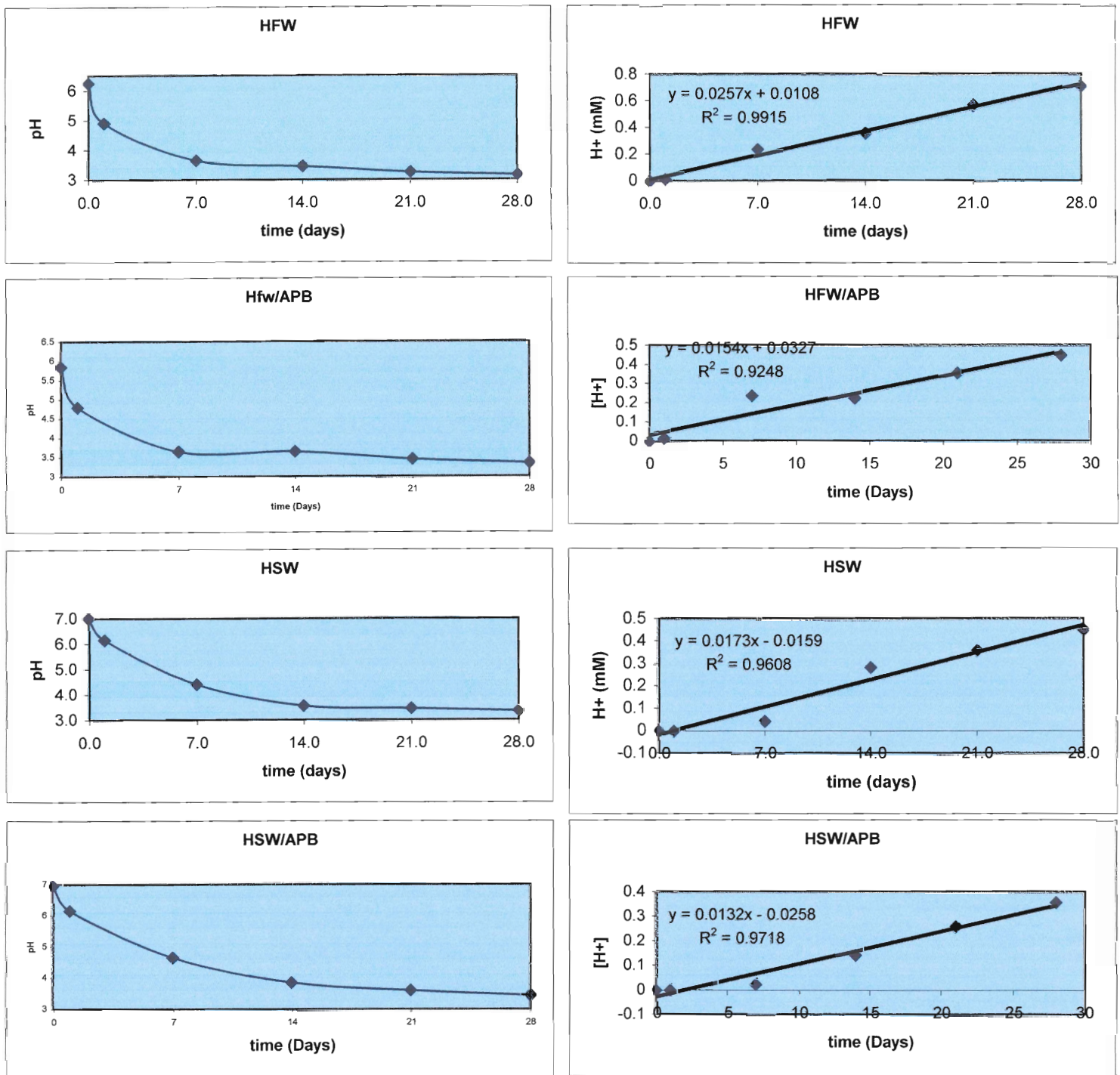


Figure 5.5 A Comparison of logarithmic pH curves and proton concentrations for the inoculated (APB) and un-inoculated Halifax slate samples.

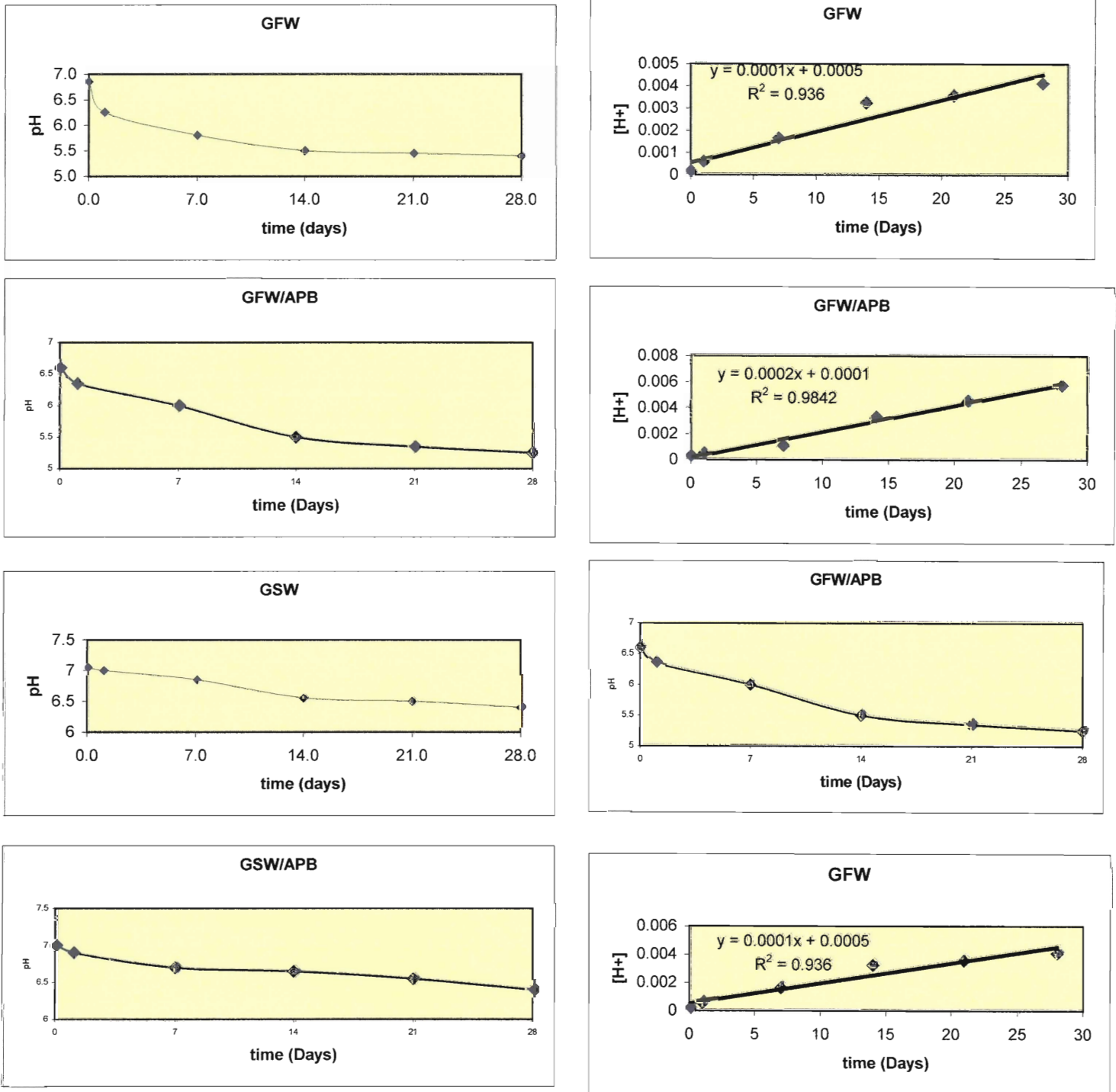


Figure 5.5 B Comparison of logarithmic pH curves to proton concentrations of the inoculated (APB) and un-inoculated GHT samples.

Table 5.3 Changes in pH with acid rock experiments to mimic freshwater infiltration and tidal flow recorded over 5 days. Only Halifax slate samples were used to demonstrate this open system. The rocks were exposed to the waters for 24 hours then replenished with new waters.

| DAYS | Hdw | Hfw | Hsw |
|-------------|------------|------------|------------|
| 0 | 5.0 | 6.3 | 7.0 |
| (before) 1 | 4.7 | 4.9 | 6.2 |
| (after) 1 | 5.0 | 6.2 | 7.0 |
| 2 | 4.6 | 5.0 | 6.4 |
| 2 | 5.1 | 6.3 | 6.9 |
| 3 | 4.7 | 4.9 | 6.1 |
| 3 | 5.0 | 6.0 | 7.1 |
| 4 | 4.8 | 4.7 | 6.4 |
| 4 | 5.0 | 6.3 | 7.0 |
| 5 | 4.7 | 4.8 | 6.0 |

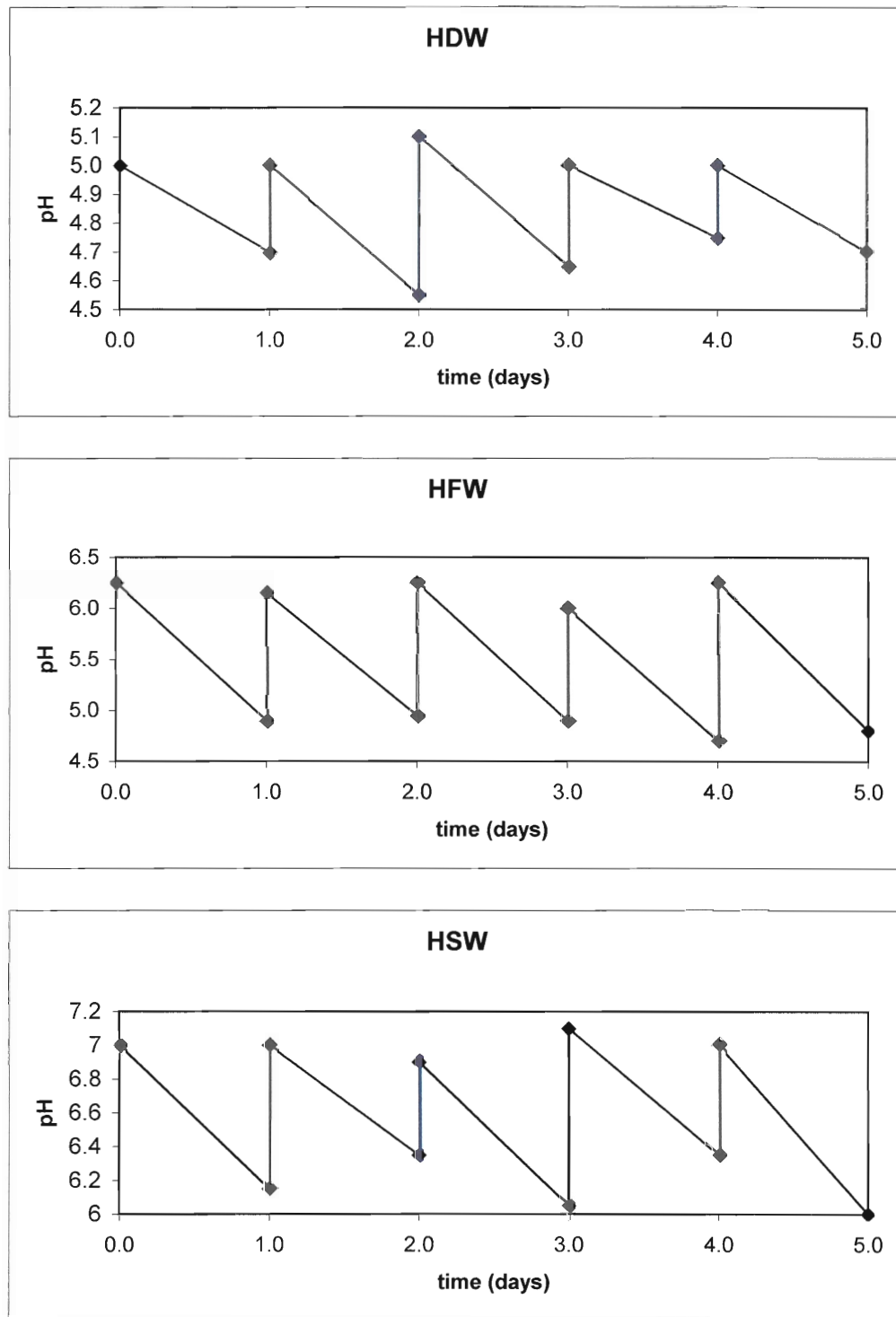


Figure 5.6 pH changes in the water flushing experiment for the Halifax slate in distilled, fresh, and salt water. You can see the pH decrease over 24 hours, then the pH rise after the addition of new water.

Table 5.4 Recorded control pH values over a 28 day period of three water types; fw- fresh water; sw- salt water; and dw- distilled water.

| DAYS→ | 0 | 1 | 7 | 14 | 21 | 28 |
|--------------|----------|----------|----------|-----------|-----------|-----------|
| fw | 6.6 | 6.6 | 6.6 | 6.55 | 6.6 | 6.6 |
| sw | 7.4 | 7.35 | 7.35 | 7.3 | 7.25 | 7.2 |
| dw | 6.15 | 6.1 | 6.1 | 6.05 | 6.05 | 6.05 |

5.5 Probe results

The JEOL microprobe was used to identify the sulphides in both rock types in the area of study. Some totals are very high or low, possibly as a result of the interference from surrounding minerals as the grain sizes were small (Figure 5.5). The averages of the sulphides in each sample analysed are presented in Table 5.5, with the proportions of iron and sulphur calculated from averages. For original microprobe data see Appendix G. Some of the pyrrhotite crystals had a chalcopyrite phase present as well (Figure 5.6, 5.7, and 5.8), and there was little pyrite (Figure 5.10). Also, in both rock types some of the pyrrhotite crystals had a garnet halo around them (Figure 5.1, 5.8, and 5.9)

Table 5.5 Totals and iron sulphur proportions for the sulphide averages in each sample.
(** ratios of Fe if S is set at 8.00)

| Sample # | Mineral | S | Fe | Co | Ni | Cu | Zn | Total | Fe** | S** |
|-----------------|--------------|-------|-------|------|------|-------|------|----------|------|-----|
| JAS00303 4.75 | Chalcopyrite | 36.35 | 31.88 | 0.02 | 0.05 | 36.46 | 0.83 | 105.60 | 4.03 | 8 |
| | Chalcopyrite | 35.98 | 32.33 | 0.05 | 0.00 | 36.64 | 0.39 | 105.41 | 4.13 | 8 |
| | Chalcopyrite | 36.17 | 32.60 | 0.07 | 0.43 | 36.83 | 1.21 | 107.31 | 4.14 | 8 |
| | Pyrrhotite | 44.10 | 57.47 | 0.01 | 0.68 | 0.11 | 0.80 | 103.17 | 5.99 | 8 |
| | Pyrrhotite | 43.98 | 57.98 | 0.00 | 0.84 | 0.05 | 0.16 | 103.01 | 6.06 | 8 |
| | Pyrrhotite | 40.45 | 62.56 | 0.01 | 0.38 | 0.12 | 0.52 | 104.03 | 7.10 | 8 |
| | Pyrrhotite | 40.48 | 62.50 | 0.00 | 0.66 | 0.04 | 0.10 | 103.78 | 7.09 | 8 |
| | | | | | | | | | | |
| JAS00303 (WR) | Pyrrhotite | 38.48 | 60.39 | 0.09 | 0.36 | 0.00 | 0.03 | 99.35 | 7.21 | 8 |
| JAS00303 0.5mm | Chalcopyrite | 35.76 | 31.24 | 0.05 | 0.00 | 36.65 | 0.00 | 103.70 | 4.01 | 8 |
| | Chalcopyrite | 35.60 | 31.17 | 0.06 | 0.00 | 35.81 | 0.00 | 102.63 | 4.02 | 8 |
| | Pyrrhotite | 41.93 | 59.16 | 0.24 | 0.43 | 0.00 | 0.03 | 101.79 | 6.48 | 8 |
| | Pyrrhotite | 40.48 | 59.99 | 0.15 | 0.12 | 0.27 | 0.00 | 101.02 | 6.81 | 8 |
| | Pyrrhotite | 40.07 | 60.75 | 0.12 | 0.20 | 0.00 | 0.00 | 101.14 | 6.96 | 8 |
| JAS00403 0.5mm | Chalcopyrite | 36.52 | 38.69 | 0.16 | 0.14 | 18.47 | 0.31 | 94.28 | 4.87 | 8 |
| | Chalcopyrite | 35.55 | 30.55 | 0.06 | 0.04 | 35.72 | 0.00 | 101.92 | 3.95 | 8 |
| | Pyrrhotite | 41.36 | 60.67 | 0.13 | 0.12 | 0.00 | 0.14 | 102.42 | 6.74 | 8 |
| | Pyrrhotite | 52.45 | 41.03 | 0.10 | 0.05 | 0.00 | 0.00 | 93.62 | 3.59 | 8 |
| | Pyrrhotite | 36.47 | 61.13 | 0.18 | 0.12 | 0.12 | 0.22 | 98.24 | 7.70 | 8 |
| | Pyrrhotite | 35.55 | 30.55 | 0.06 | 0.04 | 35.72 | 0.00 | 101.92 | 3.95 | 8 |
| | Pyrite | 54.44 | 45.24 | 0.10 | 0.11 | 0.00 | 0.00 | 99.89 | 3.82 | 8 |
| | Pyrite | 54.18 | 44.10 | 0.10 | 0.05 | 0.00 | 0.00 | 98.43 | 3.74 | 8 |
| | Pyrite | 52.99 | 40.97 | 0.12 | 0.05 | 0.00 | 0.00 | 94.12 | 3.55 | 8 |
| JAS00403 11.2mm | Chalcopyrite | 35.44 | 30.29 | 0.07 | 0.04 | 35.51 | 0.37 | 101.7122 | 3.93 | 8 |
| | Pyrrhotite | 41.25 | 60.78 | 0.11 | 0.06 | 0.00 | 0.00 | 102.20 | 6.77 | 8 |
| | Pyrrhotite | 40.41 | 60.48 | 0.11 | 0.10 | 0.01 | 0.00 | 101.11 | 6.87 | 8 |
| | Pyrrhotite | 40.51 | 60.88 | 0.10 | 0.08 | 0.01 | 0.00 | 101.56 | 6.90 | 8 |
| | Pyrrhotite | 40.50 | 62.61 | 0.13 | 0.10 | 0.02 | 0.00 | 103.35 | 7.10 | 8 |
| | Pyrrhotite | 40.49 | 60.43 | 0.11 | 0.11 | 0.00 | 0.00 | 101.17 | 6.86 | 8 |
| | Pyrrhotite | 40.31 | 59.43 | 0.13 | 0.12 | 0.00 | 0.09 | 100.07 | 6.77 | 8 |
| | Pyrrhotite | 40.52 | 59.41 | 0.11 | 0.12 | 0.00 | 0.00 | 100.15 | 6.73 | 8 |
| | Pyrrhotite | 40.09 | 58.60 | 0.12 | 0.09 | 0.00 | 0.08 | 98.97 | 6.71 | 8 |
| | Pyrrhotite | 40.08 | 59.76 | 0.11 | 0.09 | 0.00 | 0.19 | 100.22 | 6.85 | 8 |
| | Pyrrhotite | 41.77 | 57.11 | 0.11 | 0.19 | 0.00 | 0.05 | 99.24 | 6.28 | 8 |
| | Pyrrhotite | 40.11 | 59.08 | 0.12 | 0.08 | 0.00 | 0.00 | 99.39 | 6.77 | 8 |
| | Pyrrhotite | 40.36 | 58.47 | 0.11 | 0.08 | 0.00 | 0.00 | 99.02 | 6.66 | 8 |
| | Pyrrhotite | 40.19 | 58.57 | 0.10 | 0.06 | 0.00 | 0.00 | 98.92 | 6.70 | 8 |
| | Pyrrhotite | 40.06 | 58.83 | 0.13 | 0.07 | 0.00 | 0.00 | 99.09 | 6.75 | 8 |
| | Pyrite | 55.22 | 44.86 | 0.08 | 0.01 | 0.00 | 0.14 | 100.31 | 3.73 | 8 |

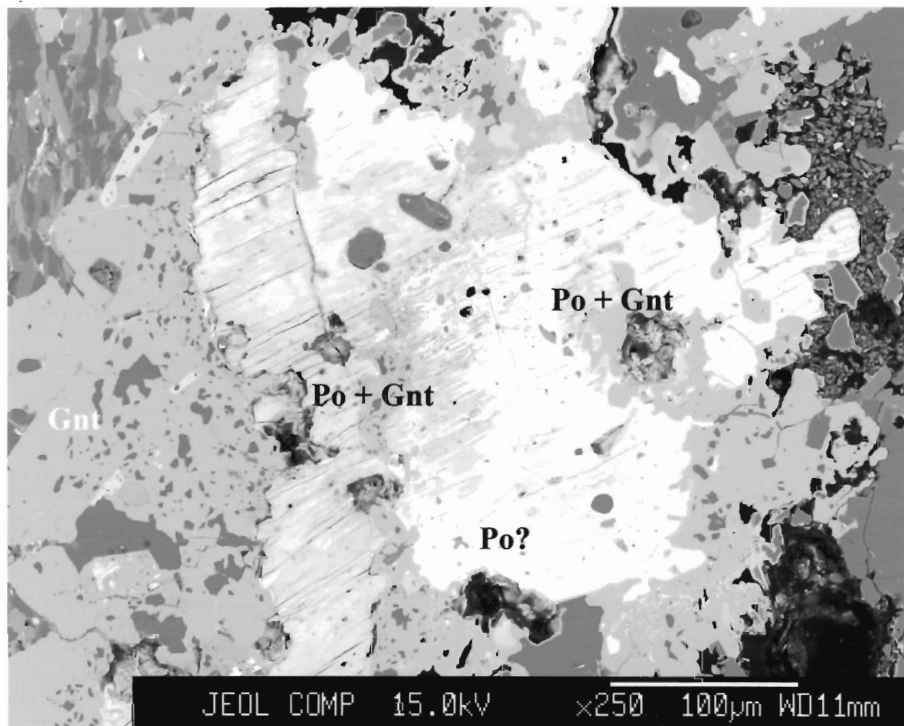


Figure 5.7 Pyrrhotite (Po) crystal surrounded by and reacting (?) with garnet (Gnt). Crystal located in sample JAS00303 whole rock.

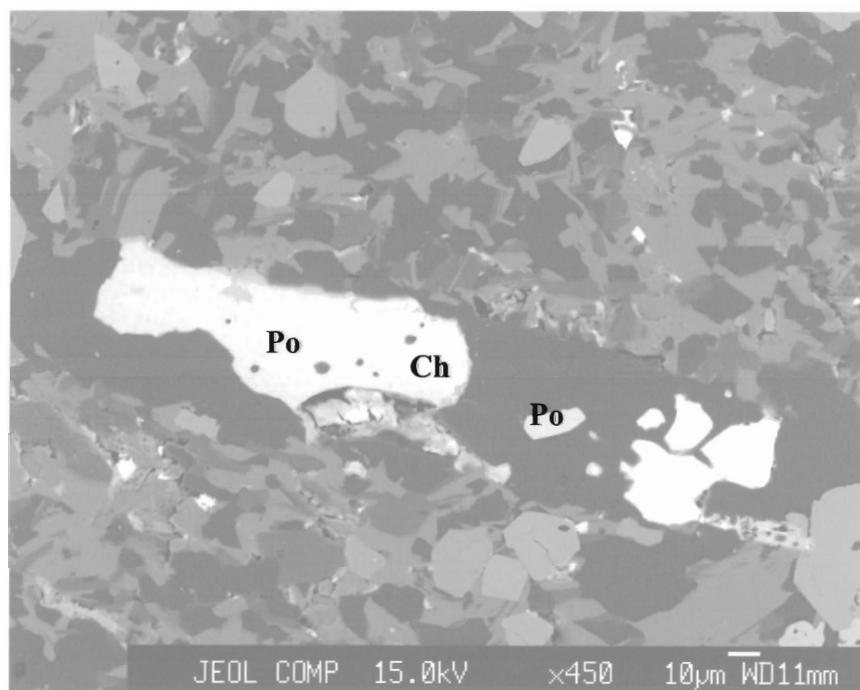


Figure 5.8 Photomicrograph of a pyrrhotite (Po) crystal with a chalcopyrite (Ch) phase in GHT sample JAS00303 of the D size fraction.

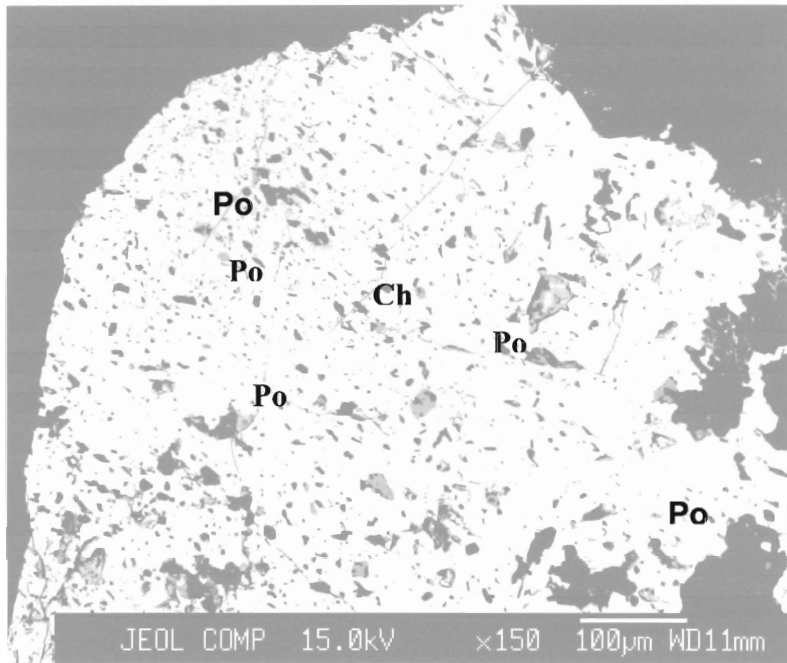


Figure 5.9 A weathered pyrrhotite (Po) crystal from the GHT sample JAS00303 E size fraction.

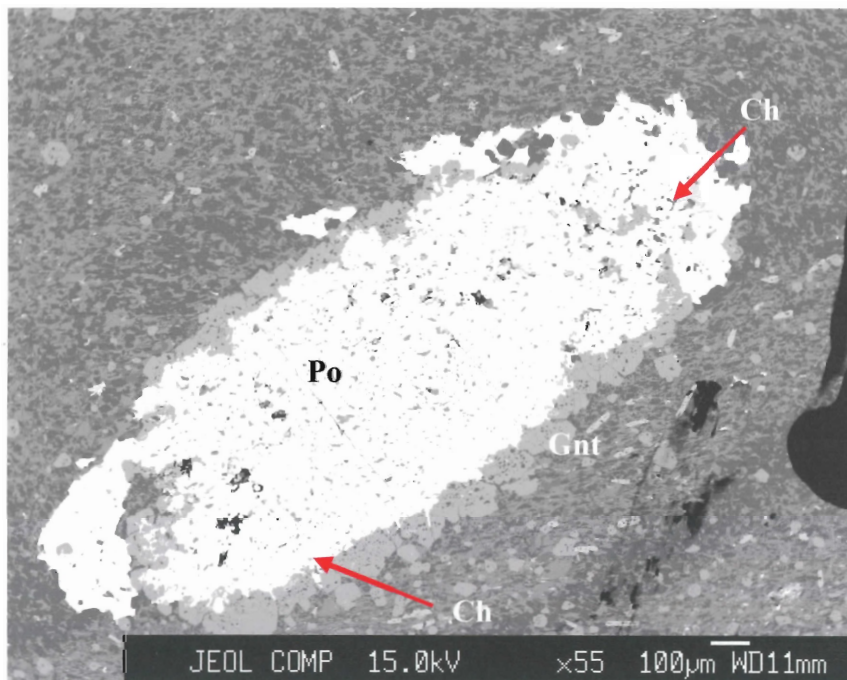


Figure 5.10 Pyrrhotite (Po) crystal with a chalcopyrite (Ch) phase, surrounded by a garnet (Gnt) rim from the GHT sample JAS00303 D size fraction.

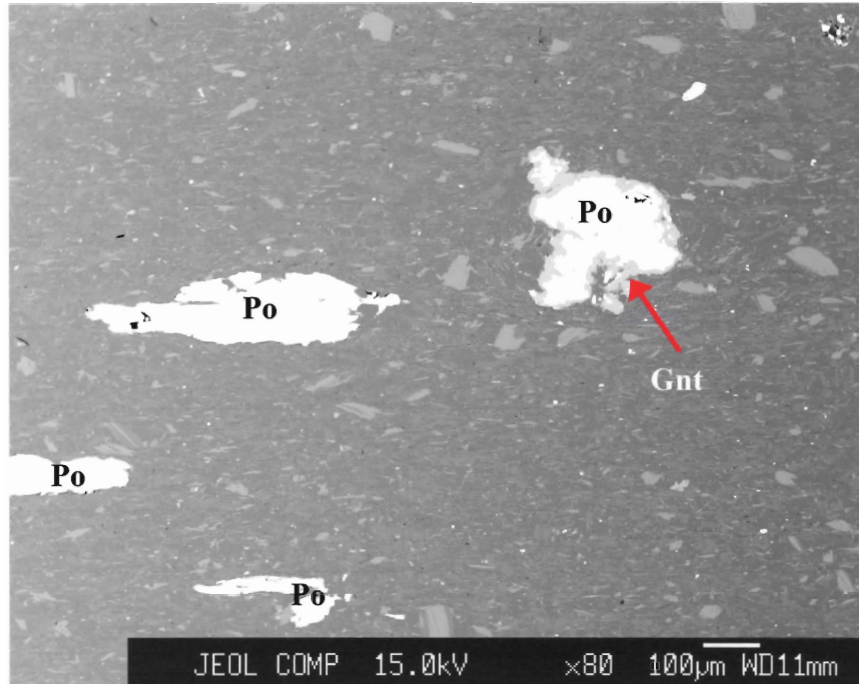


Figure 5.11 Anherdal and linear pyrrhotite crystals (Po) from Halifax slate sample JAS00403 B size fraction. The anhedral grain has a garnet (Gnt) rim.

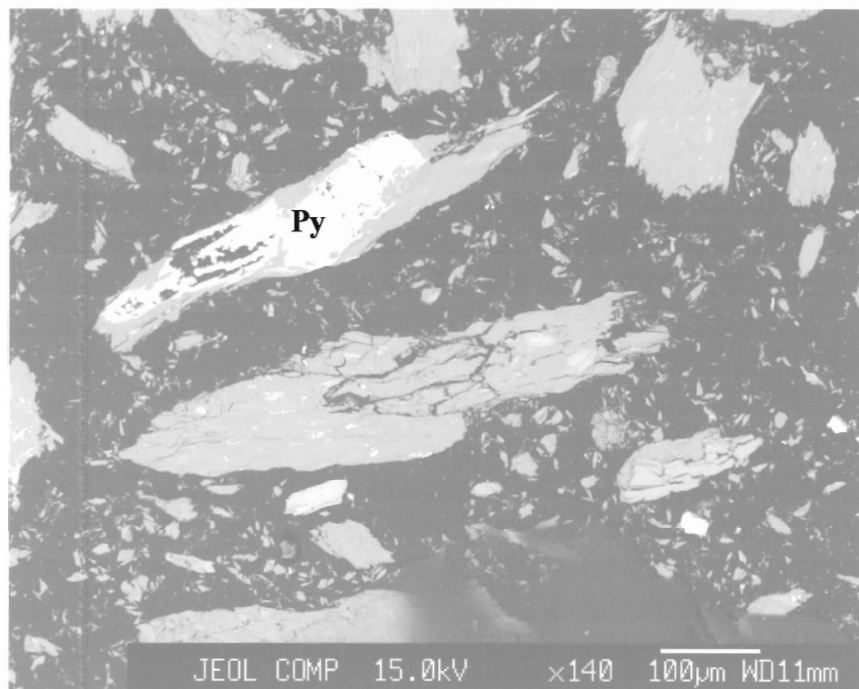


Figure 5.12 A pyrite (Py) crystal in the Halifax slate sample JAS00403 F size fraction.

5.6 SEM micrographs

SEM micrographs were taken to determine whether there was evidence of possible bacterial attachment to the rock surfaces in salt water. SEM micrographs were taken of both rock types, inoculated with bacteria and un-inoculated controls, from Armstrong's experiment (Figure 5.11 to 5.18). Two methods were used to prepare the samples for photography (Appendix C), micrographs were obtained using both methods. Previous work suggests that acid producing bacteria may not survive in salt waters (Temple 1951), and that bacterial attachment is insignificant. In this study, SEM micrographs indicate that bacteria are found on the surface of the GHT control sample as autoclaving does not remove any dead bacteria from the sample. No bacteria were found on the inoculated Halifax slate sample prepared with the standard biological sample method, even though bacteria were observed using light microscopy. A second SEM preparation method was employed to overcome this problem.

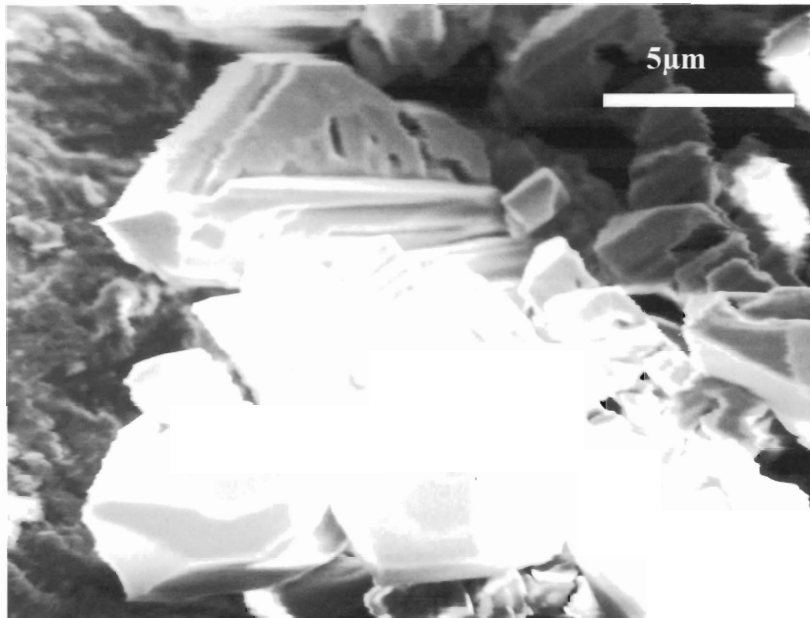


Figure 5.13 A photomicrograph of radiating crystals on the surface of the control Halifax slate sample. This sample was prepared by a simple drying method.

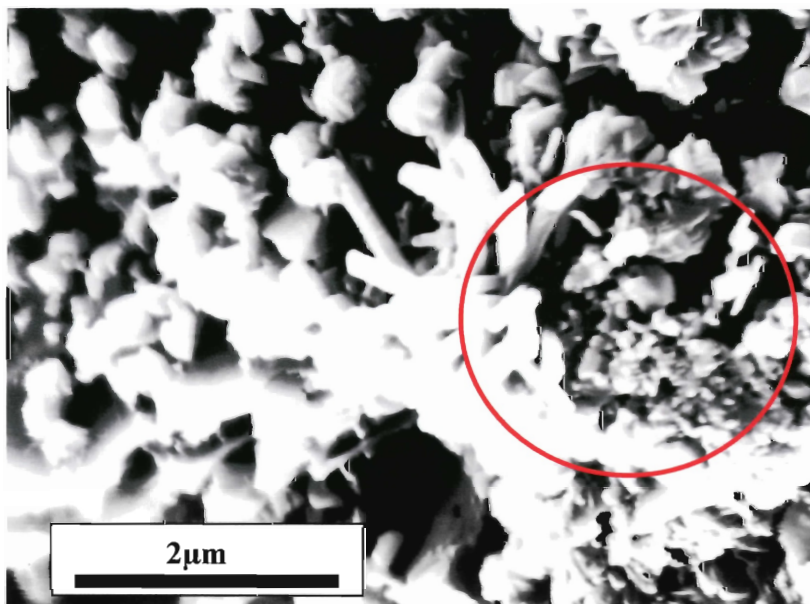


Figure 5.14 Bacterial attachment to the surface (red circle) of the inoculated Halifax slate sample. This sample was prepared by the simple drying method.

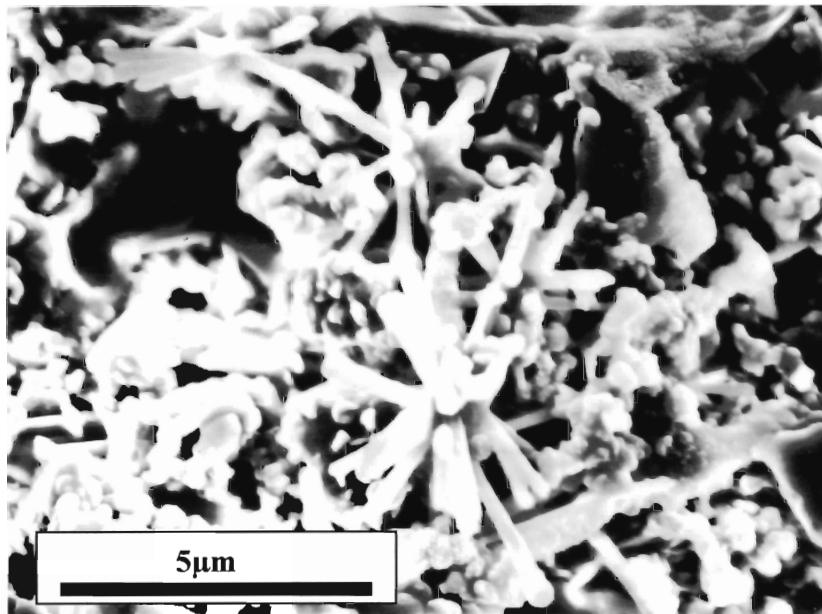


Figure 5.15 Bacteria attached to the surface of the inoculated GHT sample, with an unidentified radiating crystal in the foreground. The sample was prepared using the simple drying method.

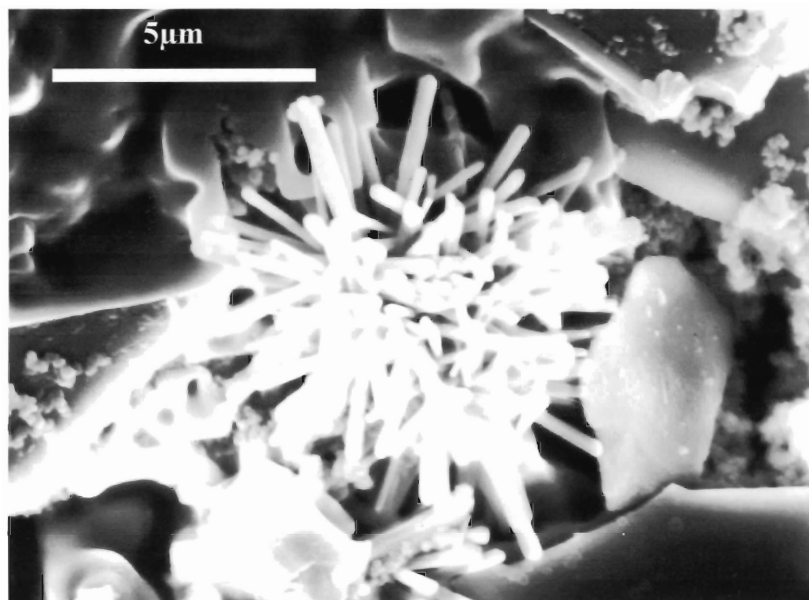


Figure 5.16 Unidentified radiating crystal structure on the surface of the GHT control samples. The samples was prepared using the simple drying method.

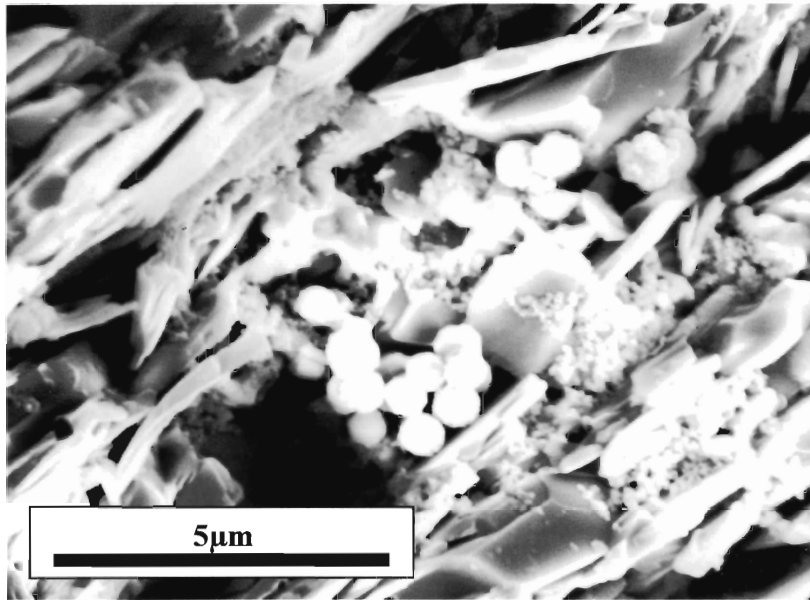


Figure 5.17 Unidentified spherical crystal structures on the surface of the control GHT sample, using the simple drying method for preparation.

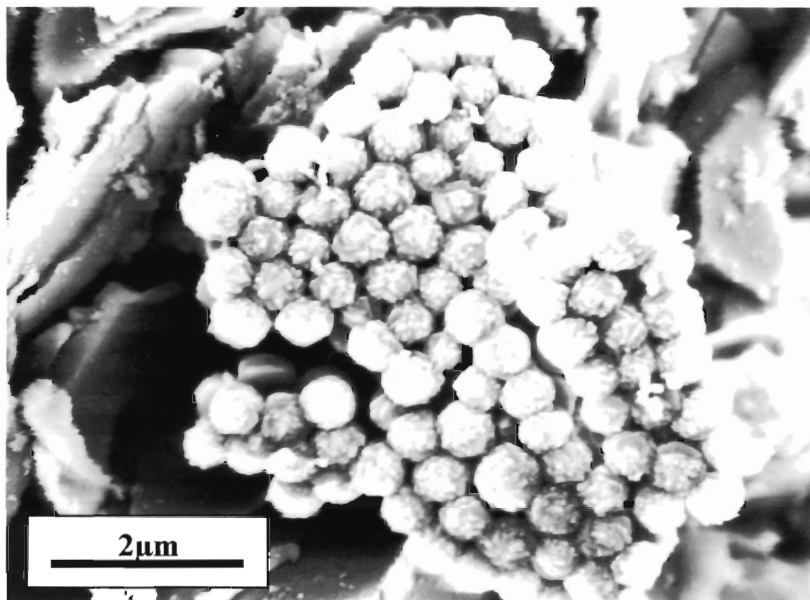


Figure 5.18 Similar unidentified spherical crystal structures in the surface of control GHT rock. This sample was prepared using standard biological sample preparation.

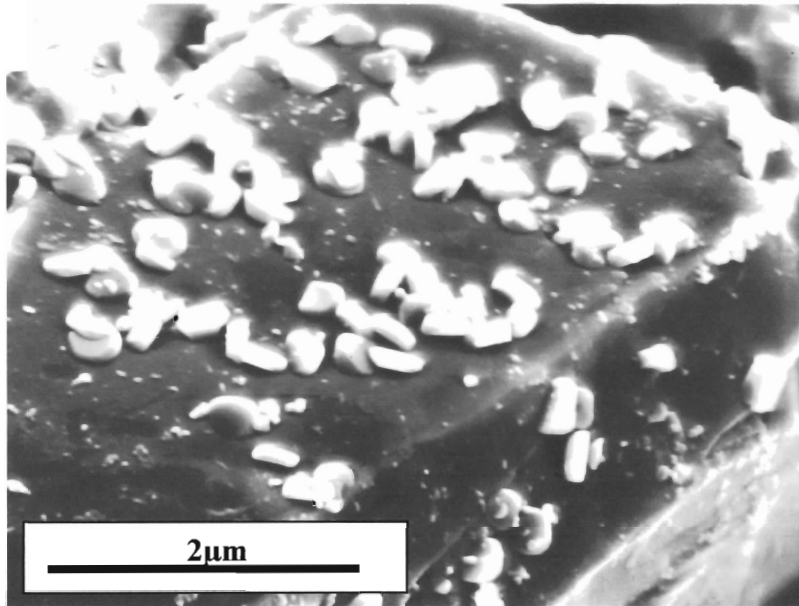


Figure 5.19 Bacterial attachment to the surface of the inoculated GHT sample. This sample was prepared using the standard biological sample preparation technique.

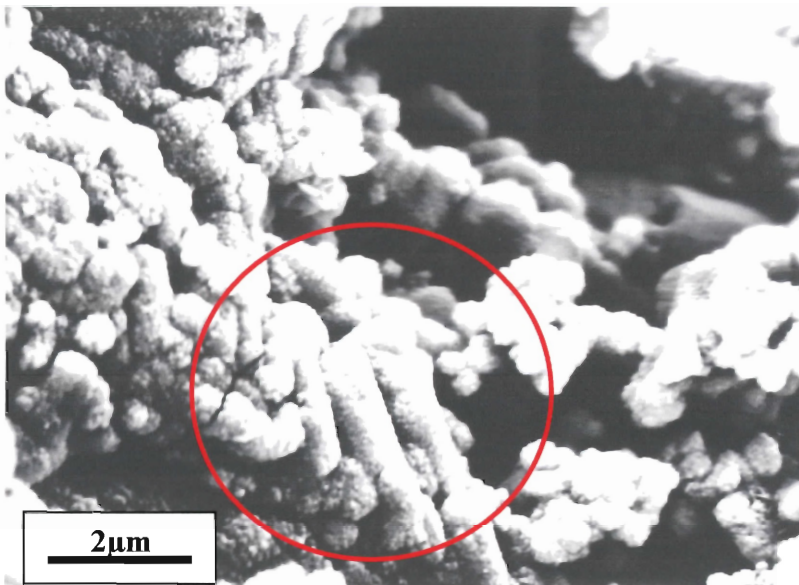


Figure 5.20 Bacteria present (red circle) very sparsely on the control surface of the GHT rocks. The control sample was autoclaved to kill any bacteria, however this technique does not remove the dead bacteria from the sample. This sample was prepared using standard biological sample techniques.

5.7 Geochemical results

The following section presents the results obtained by analysis at the DalTech Minerals Engineering Centre. Acid prediction tests were recorded on calculated bulk rock samples for both the GHT and Halifax slate samples using the Duncan method Table 5.6). Acid prediction/ consumption tests were to determine if the rock was a net acid producer, thus an acid generating rock. The total percent sulphur was observed in each size fraction A to F (Table 4.1) to compare with the weight percentages of each size fraction to observe possible sulphide partitioning to a size fraction (Table 5.7 and Figure 5.11). Current practice in Nova Scotia is that if rock has greater than 0.4 % sulphur, the rocks consuming ability must be three times greater to account for faster acidic reaction rates, or material is at a high risk for acid generation (Albright pers. comm.). Thus, any rock with 0.4 % sulphur is considered acid generating. Whole rock geochemistry was determined for the Halifax slate sample from the Beaverbank Rd. area (Table 5.8).

Table 5.6 Acid Producing and Acid Consuming potentials for both GHT and Halifax slate calculated powdered rock head samples.

| Sample | pH | % | | KG/ TONNE | | NET ACID PRODUCTION** |
|--------------------------|------|-----------|--------------|----------------|----------------|----------------------------|
| | | S (total) | S (sulphide) | Acid Producing | Acid Consuming | Acid Prod.- Acid Consuming |
| JASOO403 (Halifax Slate) | 5 | 0.77 | 0.73 | 22.33 | 2.82 | 19.51 |
| JASOO303 (GHT) | 7.05 | 0.56 | 0.55 | 16.82 | 3.31 | 13.51 |

** Net acid production is the acid consumption values subtracted from acid production values. Acid producing rocks have a positive net acid production value.

Table 5.7 The % Sulphur distribution in each of the six size fractions of both rock types, GHT and Halifax slate.

| JAS00303 (mm) | GHT wt (g) | wt% | %s | wt%/100* %s | %S distribution |
|---|-------------------------|----------|----------------|--------------|-----------------|
| A | 25.15 | 1.53E+00 | 0.32 | 4.89E-03 | 8.62E-01 |
| B | 99.29 | 6.04E+00 | 0.34 | 2.05E-02 | 3.62E+00 |
| C | 531.48 | 3.23E+01 | 0.54 | 1.75E-01 | 3.07E+01 |
| D | 558.14 | 3.39E+01 | 0.73 | 2.48E-01 | 4.36E+01 |
| E | 119.35 | 7.26E+00 | 0.56 | 4.06E-02 | 7.16E+00 |
| F | 311.2 | 1.89E+01 | 0.42 | 7.95E-02 | 1.40E+01 |
| Total | 1644.61 | 100 | | 5.68E-01 | 100 |
| | | | | | |
| JAS00303 (mm) | Halifax Slate wt (g) | wt% | %s | (wt%/100)*%s | %S distribution |
| A | 212.03 | 6.25E+00 | 0.47 | 2.94E-02 | 3.85E+00 |
| B | 321.19 | 9.47E+00 | 0.64 | 6.06E-02 | 7.95E+00 |
| C | 1045.92 | 3.08E+01 | 0.78 | 2.41E-01 | 3.15E+01 |
| D | 1155.96 | 3.41E+01 | 0.66 | 2.25E-01 | 2.95E+01 |
| E | 235.28 | 6.94E+00 | 0.79 | 5.48E-02 | 7.19E+00 |
| F | 420 | 1.24E+01 | 1.23 | 1.52E-01 | 2.00E+01 |
| Total | 3390.38 | 100 | | 7.63E-01 | 100 |
| | | | | | |
| Standard NBM-1 was used (a Government Standard) | | | | | |
| | | %s | accepted value | | (+/- .015) |
| NBM-1 | | 0.29 | 0.30 | | |
| NBM-1 | | 0.30 | 0.30 | | |

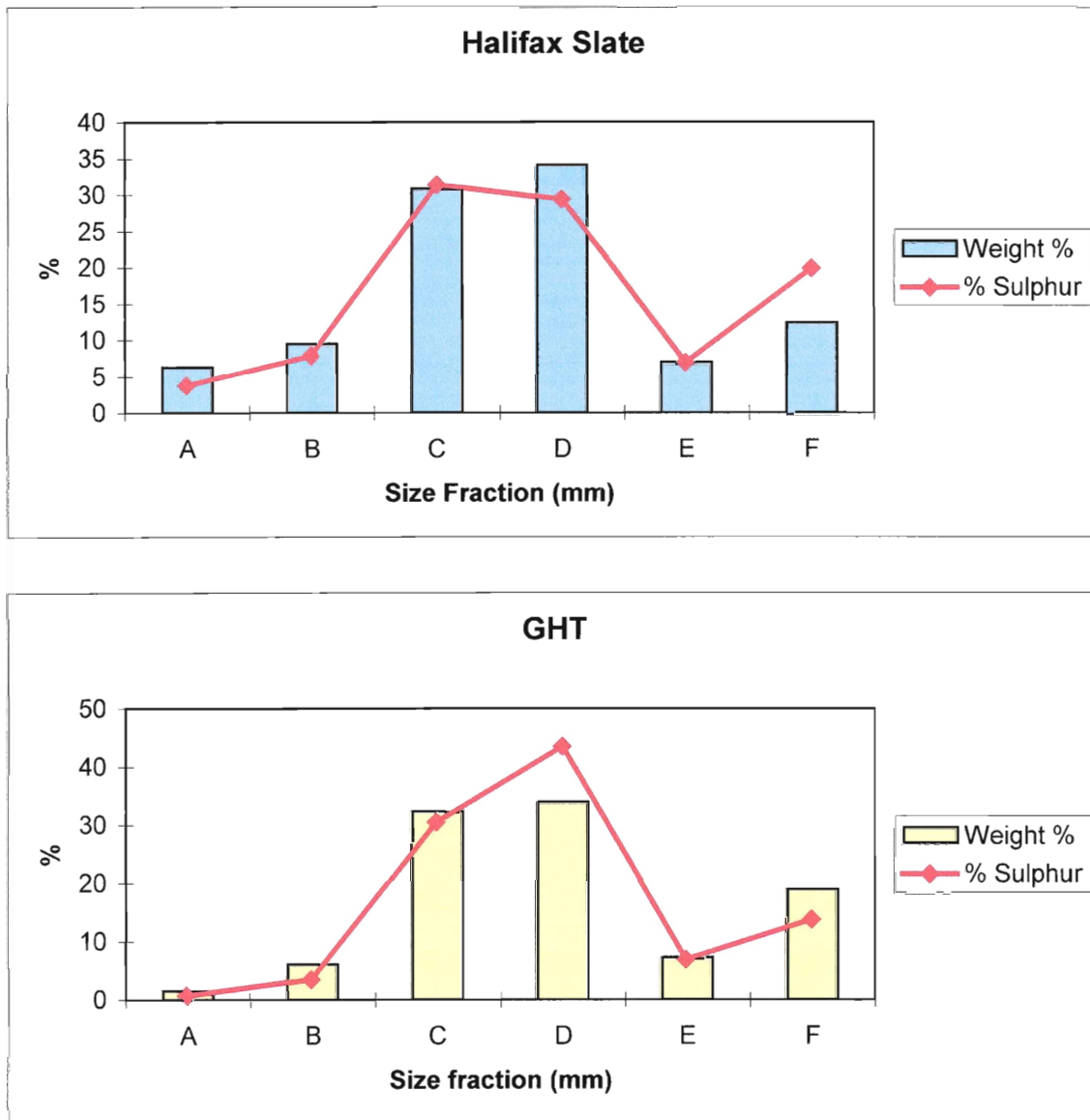


Figure 5.21 The GHT and the Halifax slate, with weight percent of each size fraction with the % Sulphur for each size fraction. Percent sulphur does not seem to concentrate in one size fraction relative to its weight percent.

The geochemistry of the GHT samples in the Beaverbank area was previously documented by Feetham (1996). The Halifax slate samples of the Beaverbank area, however, were of unknown geochemistry. Geochemistry of one Halifax slate sample from the Beaverbank area is presented in Table 5.8 together with the data from Feetham (1996). The GHT sample included in Table 5.8 is from the same location of the GHT samples used in this study.

The high iron and manganese observed in the GHT are different to those in the Halifax slate. Most of the iron and manganese in the GHT is locked in garnet. Total sulphur for the GHT is not available for comparison to the Halifax slate.

Table 5.8 Whole rock geochemistry for the GHT (Feetham 1996) and Halifax slate (This study).

| | Mart Feetham 1996 | This study 2003 |
|------------------------------------|-----------------------|-----------------|
| | MF95-03 (JAS00303) | JAS00403 |
| | GHT | Halifax Slate |
| % | | |
| SiO ₂ | 54.53 | 64.73 |
| TiO ₂ | 0.76 | 1.05 |
| Al ₂ O ₃ | 14.16 | 22.29 |
| Fe ₂ O ₃ (t) | 11.4 | 3.55 |
| MnO | 12.03 | 0.13 |
| MgO | 1.13 | 1.28 |
| CaO | 1.11 | 0.14 |
| Na ₂ O | 0.81 | 1.25 |
| K ₂ O | 0.86 | 3.67 |
| P ₂ O ₅ | 0.09 | N/A |
| S (total) | N/A | 0.39 |
| | | |
| ppm | | |
| Ag | N/A | 0.12 |
| As | N/A | 3 |
| Au | N/A | <0.003 |
| Ba | N/A | 509 |
| Be | N/A | 3 |
| Bi | N/A | 0.14 |
| Cd | N/A | 0.03 |
| Co | 155 | 6 |
| Cr | 46 | 164 |
| Cu | 135 | 9 |
| Ga | N/A | 16 |
| In | N/A | <0.1 |
| Li | N/A | 74 |
| Mo | N/A | 5 |
| Ni | 170 | 19 |
| Pb | N/A | 16 |
| Rb | N/A | 184 |
| Sb | N/A | <0.1 |
| Sr | N/A | 175 |
| Te | N/A | <0.2 |
| V | N/A | 112 |
| Zn | 125 | 78 |

The water used in the pH experiments was tested for total iron by atomic absorption (AA) using the Varion 55-B (Table 5.9). The original waters collected were tested for total Fe to compare the initial and final pH experiment water. All totals are in duplicate as each flask in the pH experiment was done in duplicate for control purposes (Table 5.9).

The finest size fraction F (<0.5 mm) had the highest increase in iron from the initial to the final pH waters in both fresh and salt water. The Halifax slate samples overall, in both water types, had a larger increase in iron after 35 days.

Table 5.9 Total Fe concentrations (ppm) in collected sample water and pH experiment final water for both rock types. Halifax slate (H) represented by blue and GHT (G) represented by yellow.

| Sample | | Sample | |
|-----------------|----------|-----------------|----------|
| | Fe (ppm) | | Fe (ppm) |
| Salt Water | 0.37 | Fresh Water | 0.02 |
| H-SW-<0.5-1 (F) | 189 | H-FW-<0.5-1 (F) | 170 |
| H-SW-<0.5-2 (F) | 224 | H-FW-<0.5-2 (F) | 150 |
| H-SW-1-1 (E) | 3.38 | H-FW-1-1 (E) | 36.3 |
| H-SW-1-2 (E) | 4.43 | H-FW-1-2 (E) | 37.1 |
| H-SW-4-1 (D) | 0.32 | H-FW-4-1 (D) | 0.93 |
| H-SW-4-2 (D) | 0.42 | H-FW-4-2 (D) | 1.52 |
| G-SW-<0.5-1 (F) | 2.96 | G-FW-<0.5-1 (F) | 0.58 |
| G-SW-<0.5-2 (F) | 3.03 | G-FW-<0.5-2 (F) | 0.52 |
| G-SW-1-1 (E) | 0.32 | G-FW-1-1 (E) | 0.11 |
| G-SW-1-2 (E) | 0.34 | G-FW-1-2 (E) | 3.4 |
| G-SW-4-1 (D) | 0.34 | G-FW-4-1 (D) | 0.37 |
| G-SW-4-2 (D) | 0.33 | G-FW-4-2 (D) | 4.86 |

Chapter 6: Discussion, Conclusions and Recommendations

6.1 Introduction

Before any general conclusions can be made, the results of the study will be discussed in an individual nature.

6.1.1 pH experiments

The pH was recorded for both rock types in salt and fresh water for a period of 35 days. The pH change over the period of 35 days was more statistically significant in the Halifax slate samples of all three grain sizes (6.7 to 4.0) than those of the GHT (7.23 to 6.3) in the salt water (Table 5.1). Studies comparing both the Halifax slate and the GHT in fresh and salt water have not been previously done. The pH change in salt water is a significant finding as it is the current rationale for the disposal of acid-generating rock. The material disposed of in the Bedford Basin will be much larger in size than the material used in the experiments for this study, however the findings of this study are significant in that regardless of the size of Halifax slate sample, the sulphide minerals will be exposed along the cleavage, as this is where the rock will preferentially fracture. All experiments were conducted at room temperature (20- 25°C) which is warmer than typical temperatures found in the environment of the Bedford Basin throughout much of the year, however because of the exothermic nature of sulphide oxidation the bacteria will still grow within the cooler environment of the Bedford Basin.

The statistically significant difference in pH of the Halifax slate over the GHT samples could reflect the fact that most of the iron in the GHT is locked away in garnet

crystals, and therefore is not available to the bacteria or atmospheric O₂. The different ratios of clay to quartz in the matrix may have had an effect on the final pH for both rock types in addition to the availability of iron and sulphur. The fresh water samples had a larger decrease in pH, with a difference of 2.3 and 1.8, respectively. Studies of sulphide interaction in both fresh and saltwater have been conducted by Whittaker (1999) using submerged thin sections of Halifax slate. Whittaker found no change in pH over a 40 day period which is contrary to this study's results. This may be a result of the larger relative mass of slate added to our ARD experiments. The ultra fine grain size fraction (F) in both rock types, in both waters, had an overall greater pH decrease. This could be a result of a greater surface area for oxidation of sulphides with the associated increased production of acid. Although larger material is disposed in the Bedford Basin, there will be a percentage of fine material due to the fissile texture of Halifax slate. The fine material will take longer to settle to the bottom, and will be re-agitated into the water upon each addition of new material into the Bedford Basin.

Subsequent pH experiments were also conducted with un-inoculated and inoculated samples in both fresh and salt water for a period of 28 days. These experiments were set up to record if the addition of acid producing bacteria at a concentration of 2.4×10^2 cells/ml would accelerated change in pH. There appears to be no significant difference between the two samples. A study on microbial oxidation of sulphides in fresh water was conducted by Jones (1997) to observe crystal matrix and texture effects on oxidation. However, the pH was not recorded in Jones' study even though oxidation was observed in the unfiltered samples when compared to the filtered (bacteria removed) samples. Jones' findings contradict the findings of this study,

however the un-inoculated samples were not sterilized, so natural bacteria were still present. The change in pH for the control experiment, set up by Armstrong (pers. comm.) was insignificant between the un-inoculated and inoculated samples. The insignificant differences in the pH values between the samples could be resulting from the presence of bacteria in the un-inoculated samples. The un-inoculated samples were not autoclaved since this procedure could have compromised the integrity of the rock causing more unknown chemical reactions.

In contrast some samples for SEM study were autoclaved to kill any bacteria within the sample. However, it is possible that autoclaving the control samples for the SEM study may have introduced some artefacts. Although the final pH difference was undetectable the inoculated samples had a slightly faster initial decrease in pH, even though the final pH was the same.

A water flushing experiment was carried out on the Halifax slate samples to mimic fresh water infiltration and tidal action. Fresh and salt water was changed daily for five days and pH was recorded before and after the addition of new water. This cycling of water modelled an open system, such as those found in the environment of the Bedford Basin. The pH decreased after every water change in both fresh and salt water, exhibiting acid production in an open system environment (Table 5.4). To the best of our knowledge, a laboratory based experiment designed to mimic this tidal flushing in the Bedford Basin has not been conducted previously.

The pH of the collected waters as well as distilled water was monitored for 28 days as well. The pH of all three water types did not change when left undisturbed and not in contact with any rock.

6.1.2 Probe work

The microprobe was used to identify the different sulphides present in the both rock types to determine what sulphides were present. Identification of sulphides for ARD as previous work has indicated that pyrrhotite oxidizes more rapidly than other sulphides (McInnes *et al.* 1994). Previous studies have been conducted by Robinson (1997) to determine pyrrhotite composition effects of ARD and have found that the predominant sulphide in the Halifax Formation is pyrrhotite, which agrees with this study. Previous work by Jones (1997) looked at the mineralogy and texture effects of ARD. Jones found that the mineralogy and texture of the rock does affect the oxidation rates of sulphides, and confirms previous work that pyrrhotite does reacts more rapid than other sulphides. Some sulphide analyses were greater than 100 %: one possible explanation is that the microprobe was not recalibrated each time a pyrite analysis was conducted prior to analysing a pyrrhotite, and this resulted in anomalous totals. Most of the sulphides present in both rock types were pyrrhotite and very little pyrite was found, although there was some chalcopyrite (Table 5.5). Studies have shown that pyrrhotite has a faster rate of oxidation, and therefore it is more harmful to the environment (Robinson 1996) (Jones 1997) (Fox 1999). The probe study confirms that the samples selected are representative of GHT and Halifax slate in terms of their sulphide mineralogy.

6.1.3 SEM micrographs

SEM micrographs were taken of both rock types in salt water to observe possible bacterial attachment to the rock surface. SEM imaging of the salt water samples is

significant as previous study of acid producing bacteria in salt water had not been conducted. However, previous studies of acid producing bacterial attachment to rock surfaces, has been done by Temple (1951) and Jones (1997) in fresh water. Temple and Jones confirm bacterial attachment in fresh water to a rock surface. This study confirms attachment to rock surfaces in salt water and fresh water. The bacteria seen on the GHT and Halifax formation slates had significant development of biofilms (Fig. 5.12, 5.13, and 5.17). Observed on the control GHT samples was a spherical unidentified structure (Figure 5.15 and 5.16), which could possibly be a by-product of autoclaving the samples or the formation of framboidal pyrite. To positively identify this structure, further analysis must be done.

6.1.4 Geochemistry

Geochemical analysis was carried out by the DalTech Minerals Engineering Centre. Whole rock geochemistry was completed on the Halifax slate sample to compare it to the known geochemistry of the GHT rocks from work carried out by Feetham (1996). Acid prediction and consumption, total sulphur, and sulphate analysis was carried out on both rock types. Atomic absorption to determine total iron amounts was carried out on the initial and final waters of the pH experiment.

Based on whole rock composition the GHT has a significantly higher iron and manganese content, though much of this is locked up in garnets of the cotecule. This unavailability of the iron may account for its higher acid consumption and lower acid

production in the BCRIT tests (Table 5.6). In contrast, the iron minerals in the Halifax slates are present in sulphides.

Acid prediction and consumption tests were carried out to determine if the Meguma Group rocks in the Beaverbank area are net acid producers. Total sulphur analysis was carried out to determine the amount of sulphur in each size fraction to observe any partitioning of sulphides to any specific size fraction. A sulphate analysis on the bulk rock samples was carried out to determine if any sulphur was present in sulphate minerals. Acid prediction and consumption tests on samples from the Beaverbank area have determined that the rocks of both the GHT and Halifax slates are net acid producers (Table 5.5). Acid predictive tests carried out by Robinson (1996) concur with the results of this study. All of the sulphur in both rock samples is located in sulphides, not sulphates. The sulphides did not partition to any particular size fraction upon crushing which is of practical importance as large debris, not powdered rock is the typical disposed material (Figure 5.19).

Atomic absorption results for this study determined that the total iron in the final pH experiment water was extremely high for the ultra fine size fractions (F) in both rock types in both salt and fresh water samples (Table 5.8). Although there is no partitioning of sulphides, the ultra fine size fraction (F) has a much larger surface area, thus more sulphide surfaces are exposed to the O₂ in the water, and to bacterial attack. The GHT samples had an overall lower total iron concentration than the Halifax slate in all three sizes fractions. This is possibly because the end pH was not as low in the GHT so more iron precipitated out. The pH may have influenced the overall lower total iron results in the salt water as well, as the iron may have precipitated out because of the slightly higher

pH. A critical pH could be required to keep more iron in solution, and some samples may have been above this critical pH.

6.2 Conclusions and Recommendations

Four important conclusions can be drawn from this study, which are significant in the study of ARD.

1. The pH decreased in both fresh and salt water systems as well the pH continued to decrease in an open (cycling) system.
2. Acid producing bacteria initially decrease pH, attach to rock surfaces, and survive in salt water, which has not been previously published.
3. The Halifax slate has a statistically significant effect on water pH than the rocks of the GHT in salt water. Decrease in pH is very similar between the two rock types in fresh water.
4. The addition of a significant amount of acid producing bacteria does not affect the final pH values. Bacterial presence is well documented to accelerate sulphide oxidation, however adding more bacteria do not affect the final pH.

A number of further studies are recommended to produce a complete conclusion for the disposal of sulphide-bearing rocks in particular as it pertains to disposal in salt water.

1. The acid producing bacteria involved in ARD should be identified. Determining the acid producing bacteria in the culture can be done using 16S ribosomal RNA sequences
2. More control experiments involving the bacteria should be performed. The pH experiments could have been done with more control with equipment of greater sensitivity, as well as taking readings over different times of the day over a longer period of time to determine the rate determining step for pH change in acid production.
3. Field study on the impacts of sulphide-bearing material on the environment.
4. Bacterial survival in the environment.
5. Field study on tidal influences on acid production.
6. Field study on seasonal and temperature influences on acid production in the environment.
7. Studies of oxygen concentration effects in the water column could aid in the complete understanding of ARD and how to dispose of it properly.

In conclusion disposal in salt water may not be the ideal method of waste removal of the sulphide-bearing rocks of the Meguma Group, however at this time it is the only alternative to land disposal. Further studies are needed to determine a more appropriate disposal method for sulphide-bearing materials.

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Appendix A: Microbial media formula

ATCC medium: 2039 *Acidithiobacillus ferrooxidans* mediumSolution A:

| | |
|---|----------|
| (NH ₄) ₂ SO ₄ | 0.8 g |
| MgSO ₄ * 7H ₂ O..... | 2.0 g |
| K ₂ HPO ₄ | 0.4 g |
| Wolfe's Mineral Solution (below)..... | 5.0 ml |
| Distilled water..... | 800.0 ml |

Adjust solution A to pH 2.3 with H₂SO₄. Filter-sterilize.

Solution B:

| | |
|--|----------|
| FeSO ₄ * 7H ₂ O..... | 20.0 g |
| Distilled water..... | 200.0 ml |

Stir solution B to dissolve and quickly filter-sterilize.

Aseptically combine solutions A and B. (A yellow precipitate is normal; it becomes darker as the iron oxidizes.)

Wolfe's Mineral Solution:

Available from ATCC as a sterile read-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS)

| | |
|--|--------|
| Nitrilotriacetic acid..... | 1.5 g |
| MgSO ₄ * 7H ₂ O..... | 3.0 g |
| MnSO ₄ * H ₂ O..... | 0.5 g |
| NaCl..... | 1.0 g |
| FeSO ₄ * 7H ₂ O..... | 0.1 g |
| CoCl ₂ * 6H ₂ O..... | 0.1 g |
| CaCl ₂ | 0.1 g |
| ZnSO ₄ * 7H ₂ O..... | 0.1 g |
| CuSO ₄ * 5H ₂ O..... | 0.01 g |
| AlK(SO ₄) ₂ * 12H ₂ O..... | 0.01 g |
| H ₃ BO ₃ | 0.01 g |
| Na ₂ MoO ₄ * 2H ₂ O..... | 0.01 g |
| Distilled water..... | 1.0 L |

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

Appendix B: Microbial procedure

Subculturing techniques:

Once a positive growth was established, after about 5 days, 1 ml was transferred to new media (subculturing) using a micropipetter (Figure 4.2). One ml of the second culture was transferred to new media after a positive growth was established, again after 5 days. This transfer of 1 ml of media with bacteria, or subculturing, was done 4 times in total.

MPN procedure:

A most probable number (MPN) experiment was set up as well, using the second subculture flask. Five test tubes are used in each dilution series, up to a final dilution of 10^{-7} for a total of 35 tubes. One millilitre of culture from the second subculture flask was added to 9 ml of media, to produce a 10^{-1} dilution. A 10^{-2} dilution is made using 1ml of the 10^{-1} and adding it to 9ml of media. This was continued until a dilution of 10^{-7} series of tubes was made. The 35 tubes were placed on a shaker in the 27°C room and left for five days, a positive growth results in the colour orange. The MPN test tubes were left in the shaker for 5 days, to ensure that positive growth could be seen. To get a MPN number a standard chart is used and the number of positive tubes in each dilution series is used to determine the number of bacteria in each tube. For example, if the following tubes give a positive reading (orange colour):

- 5 tubes with 10 ml of 1:10 dilution – all five are positive
- 5 tubes with 1 ml of 1:10 dilution – 3 are positive
- 5 tubes with 1 ml of 1:100 dilution – 1 is positive

According to the table below a 5-1-0 reading gives a value of 1.1 (highlighted).

Standard MPN chart:

| | | Most probable number for indicated values of p_3 | | | | | |
|-------|-------|--|-------|-------|-------|-------|-------|
| p_1 | p_2 | 0 | 1 | 2 | 3 | 4 | 5 |
| 0 | 0 | — | 0.018 | 0.036 | 0.054 | 0.072 | 0.090 |
| 0 | 1 | 0.018 | 0.036 | 0.055 | 0.073 | 0.091 | 0.11 |
| 0 | 2 | 0.037 | 0.055 | 0.074 | 0.092 | 0.11 | 0.13 |
| 0 | 3 | 0.056 | 0.074 | 0.093 | 0.11 | 0.13 | 0.15 |
| 0 | 4 | 0.075 | 0.094 | 0.11 | 0.13 | 0.15 | 0.17 |
| 0 | 5 | 0.094 | 0.11 | 0.13 | 0.15 | 0.17 | 0.19 |
| 1 | 0 | 0.020 | 0.040 | 0.060 | 0.080 | 0.10 | 0.12 |
| 1 | 1 | 0.040 | 0.061 | 0.081 | 0.10 | 0.12 | 0.14 |
| 1 | 2 | 0.061 | 0.082 | 0.10 | 0.12 | 0.15 | 0.17 |
| 1 | 3 | 0.083 | 0.1 | 0.13 | 0.15 | 0.17 | 0.19 |
| 1 | 4 | 0.11 | 0.13 | 0.15 | 0.17 | 0.19 | 0.22 |
| 1 | 5 | 0.13 | 0.16 | 0.17 | 0.19 | 0.22 | 0.24 |
| 2 | 0 | 0.045 | 0.068 | 0.091 | 0.12 | 0.14 | 0.16 |
| 2 | 1 | 0.068 | 0.092 | 0.12 | 0.14 | 0.17 | 0.19 |
| 2 | 2 | 0.093 | 0.12 | 0.14 | 0.17 | 0.19 | 0.22 |
| 2 | 3 | 0.12 | 0.14 | 0.17 | 0.20 | 0.22 | 0.25 |
| 2 | 4 | 0.15 | 0.17 | 0.20 | 0.23 | 0.25 | 0.28 |
| 2 | 5 | 0.17 | 0.20 | 0.23 | 0.26 | 0.29 | 0.32 |
| 3 | 0 | 0.078 | 0.11 | 0.13 | 0.16 | 0.20 | 0.23 |
| 3 | 1 | 0.11 | 0.14 | 0.17 | 0.20 | 0.23 | 0.27 |
| 3 | 2 | 0.14 | 0.17 | 0.20 | 0.24 | 0.27 | 0.31 |
| 3 | 3 | 0.17 | 0.21 | 0.24 | 0.28 | 0.31 | 0.35 |
| 3 | 4 | 0.21 | 0.24 | 0.28 | 0.32 | 0.36 | 0.40 |
| 3 | 5 | 0.25 | 0.29 | 0.32 | 0.37 | 0.41 | 0.45 |
| 4 | 0 | 0.13 | 0.17 | 0.21 | 0.25 | 0.30 | 0.36 |
| 4 | 1 | 0.17 | 0.21 | 0.26 | 0.31 | 0.36 | 0.42 |
| 4 | 2 | 0.22 | 0.26 | 0.32 | 0.38 | 0.44 | 0.5 |
| 4 | 3 | 0.27 | 0.33 | 0.39 | 0.45 | 0.52 | 0.59 |
| 4 | 4 | 0.34 | 0.40 | 0.47 | 0.54 | 0.62 | 0.69 |
| 4 | 5 | 0.41 | 0.48 | 0.56 | 0.64 | 0.72 | 0.81 |
| 5 | 0 | 0.23 | 0.31 | 0.43 | 0.58 | 0.76 | 0.95 |
| 5 | 1 | 0.33 | 0.46 | 0.64 | 0.84 | 1.1 | 1.3 |
| 5 | 2 | 0.49 | 0.7 | 0.95 | 1.2 | 1.5 | 1.8 |
| 5 | 3 | 0.79 | 1.1 | 1.4 | 1.8 | 2.1 | 2.5 |
| 5 | 4 | 1.3 | 1.7 | 2.2 | 2.8 | 3.5 | 4.3 |
| 5 | 5 | 2.4 | 3.5 | 5.4 | 9.2 | 16 | — |

Table 2.5. Standard MPN chart for 5-tube series with 0, 1, 2, 3, 4, 5 tubes per replicate.

Appendix C: Standard biological SEM sample preparation

The samples were placed in a well using sterile techniques to prevent cross contamination (sterile tweezers and extracting the autoclaved samples before the inoculated). A new pipette was used for each new application of the 90 % ethanol and afterward.

Once the samples were in the wells:

- Add 25 % ethanol to cover the sample using a pipette and bulb. Cover and leave for 5 minutes.
- Extract the 25 % ethanol and add 50 % ethanol. Cover and leave for 5 minutes.
- Repeat the above steps replacing 50 % with 70 %, then 80 %. Cover and leave for 5 minutes between each application.
- After the 80 % ethanol application, replace with 90 % ethanol. Cover and leave for 10 minutes.
- Repeat with 90 % ethanol, cover and leave for 10 minutes.
- After the second application of 90 % ethanol replace with 100 % ethanol. Cover and leave for 10 minutes.
- Repeat with 100 % ethanol, cover and leave for 10 minutes.

The next steps are carried out under the fumehood:

- Extract the 100 % ethanol and replace it with a 1:1 solution of 100 % ethanol and HDS, a biological drying solution. Cover and leave for 10 minutes.
- Replace the 1:1 solution with pure HDS. Leave uncovered under the hood to sit and dry overnight.

Appendix D: Geochemical Analysis procedure

BCRIT research tests:

Ten gram portions of the powdered sample is suspended in 100 ml of distilled water and stirred for 15 minutes. The addition of acid is continued until less than 0.1 mol acid is added in over a 4 hour time span. The total volume of acid is recorded and converted to kg tonne^{-1}

(<http://technology.infomine.com/enviromine/ard/Acid-Base%20Accounting/acidbase.htm#BCRI%20initial>).

Total Sulphur analysis:

Total sulphur was determined by carrying both a sulphide and sulphate analysis to calculate the total sulphur. The sulphide or total sulphur analysis is carried out by way of sulphur titration. The titration method converts the sulphur to SO_2 gas, by combustion in a Leco Hf-20 furnace. The resulting SO_2 gas is then passed into a dilute acid solution (HCl) with potassium iodate (KIO_3), potassium iodide (KI), and a starch indicator. Accelerants of tin and iron are added to the powdered rock sample to help drive combustion. The starch iodate complex is blue, however when the SO_2 is added it bleaches the complex, changing it from blue to clear. As combustion continues the bleaching process, more iodate is added to return the solution to its original blue colour. The furnace used is a Leco HF-20 furnace, which heats the sample to 1500°C and a 532-500 titrator system. The sulphide/sulphur analysis was carried out for both rock types of all six size fractions, as well as a calculated head sample to compare with the standards. The sulphate/sulphur analysis was carried out using a calculated head to see if there were any sulphates in the rock, before carrying it out on all size fractions.

Sulphate analysis:

- 10 g of powdered sample in a beaker with 80 ml of water. (The original method of sulphate analysis uses 1 g of sample but the Mineral Engineering centre uses 10 g for a higher sensitivity)
- Boil for 1 minute,
- Add 20 ml of 1:1 HCl and then boil again for 15 minutes.
- Filter the liquid rock solution through #4** (20-25 μm) filter paper into a 400 ml beaker and then fill to the 200 ml mark
- Add methyl orange until the solution turns pink
- Then add ammonium hydroxide until the solution turns yellow
- Add 1:1 HCl until the solution returns to pink then add 2 ml more (of HCl)
- Bring solution to a boil and add 5 ml of 10% BaCl
- Put on low heat for 15 minutes and let stand overnight
- Filter solution through #42 filter paper (2.5 μm) by gravity and then burn paper at 800°C in muffle furnace for 2 hours
- Remove from the furnace and cool and calculate the % SO₃, % S, and % SO₄.

Appendix E: pH experiment data

Original pH data of the duplicate samples in all pH experiments outlined in chapter 4.

The averages for this table are in Table 5.1.

| | DAYS→ | 0 | 2 | 7 | 9 | 12 | 14 | 16 | 19 | 21 | 28 | 35 |
|-------------|--------------|----------|----------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | Sample | | | | | | | | | | | |
| Fresh water | hfwp | 5.3 | 4.9 | 3.6 | 3.7 | 3.5 | 3.5 | 3.5 | 3.4 | 3.3 | 3.4 | 3.1 |
| | hfwp | 5.3 | 4.8 | 3.7 | 4 | 3.6 | 3.6 | 3.6 | 3.5 | 3.5 | 3.6 | 3.3 |
| | hfw1 | 5.5 | 4.6 | 3.6 | 3.5 | 3.4 | 3.3 | 3.3 | 3.2 | 3.2 | 3.4 | 3.1 |
| | hfw1 | 5.5 | 4.5 | 3.5 | 3.5 | 3.4 | 3.4 | 3.3 | 3.2 | 3.2 | 3.4 | 3.1 |
| | hfw4 | 6.1 | 5.2 | 3.9 | 3.8 | 3.7 | 3.7 | 3.7 | 3.6 | 3.6 | 3.7 | 3.5 |
| | hfw4 | 6.1 | 4.8 | 3.8 | 3.8 | 3.7 | 3.7 | 3.7 | 3.6 | 3.6 | 3.7 | 3.5 |
| | gfwp | 7.1 | 6.8 | 5 | 5.2 | 4.6 | 4.5 | 4.5 | 4.4 | 4.2 | 4.3 | 3.8 |
| | gfwp | 7 | 7 | 5 | 5.3 | 4.5 | 4.5 | 4.5 | 4.6 | 4.6 | 4.4 | 4 |
| | gfw1 | 7 | 7 | 6 | 6.1 | 5.6 | 5.7 | 5.8 | 6 | 6.1 | 6 | 5.5 |
| | gfw1 | 6.9 | 7 | 5.1 | 5.5 | 4.9 | 5.1 | 5.2 | 5.2 | 5.1 | 5.1 | 4.7 |
| | gfw4 | 7.4 | 7 | 6 | 6 | 5.8 | 6 | 6 | 6 | 6.2 | 6 | 5.5 |
| | gfw4 | 7.2 | 6.8 | 5.5 | 5.6 | 5.1 | 5.3 | 5.3 | 5.3 | 5.4 | 5.3 | 5.1 |
| Salt Water | hswp | 6.4 | 5.7 | 4.4 | 4.3 | 4 | 4.2 | 4.1 | 4 | 4 | 3.9 | 3.6 |
| | hswp | 6.3 | 5.8 | 4.6 | 4.4 | 4 | 4.3 | 4.2 | 4 | 4.1 | 3.9 | 3.5 |
| | hsw1 | 6.7 | 6.1 | 4.8 | 4.6 | 4.3 | 4.1 | 4.1 | 4 | 4 | 3.8 | 3.6 |
| | hsw1 | 6.9 | 6.6 | 4.5 | 4.3 | 3.9 | 4 | 3.9 | 3.8 | 3.8 | 3.7 | 3.5 |
| | hsw4 | 7 | 6.7 | 6.3 | 6.2 | 6.1 | 6.3 | 6.3 | 6.2 | 6.1 | 5.7 | 5.5 |
| | hsw4 | 7 | 6.7 | 6.3 | 6 | 5.9 | 5.6 | 5.6 | 5.6 | 5.6 | 5.1 | 4.5 |
| | gswp | 7.1 | 7 | 6.6 | 6.3 | 6.2 | 6.3 | 6.3 | 6.4 | 6.4 | 6 | 5.4 |
| | gswp | 7.1 | 7.5 | 6.5 | 6.3 | 6 | 6.3 | 6.3 | 6.3 | 6.3 | 5.9 | 5.4 |
| | gsw1 | 7.2 | 7.7 | 7.1 | 7 | 6.9 | 7.1 | 7.1 | 7.2 | 7.1 | 7 | 6.7 |
| | gsw1 | 7.2 | 7.3 | 7.1 | 7 | 6.9 | 7 | 7 | 7 | 7.1 | 6.9 | 6.5 |
| | gsw4 | 7.4 | 7.7 | 7.2 | 7.1 | 7 | 7.3 | 7.3 | 7.3 | 7.4 | 7.3 | 7 |
| | gsw4 | 7.4 | 7.7 | 7.2 | 7.1 | 7.1 | 7.4 | 7.4 | 7.4 | 7.4 | 7.2 | 6.9 |

The averages for this table are in Table 5.2.

| | | DAYS→ | 0 | 1 | 7 | 14 | 21 | 28 |
|---------------|-----|--------------|----------|----------|----------|-----------|-----------|-----------|
| | | Sample | | | | | | |
| Un-Inoculated | Hdw | | 4.9 | 4.6 | 3.9 | 3.7 | 3.6 | 3.4 |
| | Hdw | | 5.1 | 4.8 | 4.2 | 3.6 | 3.3 | 3.3 |
| | Hfw | | 6.2 | 5 | 3.5 | 3.5 | 3.2 | 3.2 |
| | Hfw | | 6.3 | 4.8 | 3.8 | 3.4 | 3.3 | 3.1 |
| | Hsw | | 7.1 | 6 | 4.2 | 3.6 | 3.4 | 3.4 |
| | Hsw | | 6.9 | 6.3 | 4.6 | 3.5 | 3.5 | 3.3 |
| | Gdw | | 5.3 | 5.2 | 5.3 | 5.2 | 5.3 | 5.4 |
| | Gdw | | 5.3 | 5.3 | 5.2 | 5.3 | 5.3 | 5.2 |
| | Gfw | | 6.9 | 6.3 | 5.9 | 5.4 | 5.4 | 5.3 |
| | Gfw | | 6.8 | 6.2 | 5.7 | 5.6 | 5.5 | 5.5 |
| | Gsw | | 7.1 | 7 | 6.9 | 6.5 | 6.6 | 6.4 |
| | Gsw | | 7 | 7 | 6.8 | 6.6 | 6.4 | 6.4 |
| | | | | | | | | |
| Inoculated | Hdw | | 4.7 | 4.6 | 4 | 3.8 | 3.6 | 3.4 |
| | Hdw | | 4.9 | 4.9 | 4.1 | 3.6 | 3.5 | 3.4 |
| | Hfw | | 5.8 | 4.7 | 3.5 | 3.6 | 3.5 | 3.4 |
| | Hfw | | 5.9 | 4.9 | 3.8 | 3.7 | 3.4 | 3.3 |
| | Hsw | | 7 | 6.1 | 4.8 | 3.9 | 3.7 | 3.5 |
| | Hsw | | 6.9 | 6.2 | 4.5 | 3.8 | 3.5 | 3.4 |
| | Gdw | | 5.4 | 5.4 | 5.3 | 5.4 | 5.4 | 5.4 |
| | Gdw | | 5.3 | 5.3 | 5.4 | 5.3 | 5.4 | 5.2 |
| | Gfw | | 6.6 | 6.4 | 5.9 | 5.6 | 5.3 | 5.3 |
| | Gfw | | 6.6 | 6.3 | 6.1 | 5.4 | 5.4 | 5.2 |
| | Gsw | | 6.9 | 6.8 | 6.6 | 6.9 | 6.6 | 6.3 |
| | Gsw | | 7.1 | 7 | 6.8 | 6.4 | 6.5 | 6.5 |

The averages for this table are in Table 5.3.

| DAYS→ | 0 | 1 | 7 | 14 | 21 | 28 |
|-------|-----|-----|-----|-----|-----|-----|
| water | | | | | | |
| fw | 6.6 | 6.6 | 6.6 | 6.6 | 6.6 | 6.6 |
| fw | 6.6 | 6.6 | 6.6 | 6.5 | 6.6 | 6.6 |
| sw | 7.4 | 7.3 | 7.3 | 7.2 | 7.2 | 7.2 |
| sw | 7.4 | 7.4 | 7.4 | 7.4 | 7.3 | 7.2 |
| dw | 6.1 | 6 | 6 | 6.1 | 6.1 | 6.1 |
| dw | 6.2 | 6.2 | 6.2 | 6 | 6 | 6 |

Appendix F: pH calculation data

pH calculations to result in proton concentration for both rock types in fresh and salt water from Appendix E.

| FORMULA → | | $10^{-\text{pH}}$ | | | | | | | | | | |
|-------------|------|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| DAYS→ | | 0 | 2 | 7 | 9 | 12 | 14 | 16 | 19 | 21 | 28 | 35 |
| Fresh Water | hfwp | 5.01E-06 | 1.26E-05 | 2.51E-04 | 2.00E-04 | 3.16E-04 | 3.16E-04 | 3.16E-04 | 3.98E-04 | 5.01E-04 | 3.98E-04 | 7.94E-04 |
| | hfwp | 5.01E-06 | 1.58E-05 | 2.00E-04 | 1.00E-04 | 2.51E-04 | 2.51E-04 | 2.51E-04 | 3.16E-04 | 3.16E-04 | 2.51E-04 | 5.01E-04 |
| | hfw1 | 3.16E-06 | 2.51E-05 | 2.51E-04 | 3.16E-04 | 3.98E-04 | 5.01E-04 | 5.01E-04 | 6.31E-04 | 6.31E-04 | 3.98E-04 | 7.94E-04 |
| | hfw1 | 3.16E-06 | 3.16E-05 | 3.16E-04 | 3.16E-04 | 3.98E-04 | 3.98E-04 | 5.01E-04 | 6.31E-04 | 6.31E-04 | 3.98E-04 | 7.94E-04 |
| | hfw4 | 7.94E-07 | 6.31E-06 | 1.26E-04 | 1.58E-04 | 2.00E-04 | 2.00E-04 | 2.00E-04 | 2.51E-04 | 2.51E-04 | 2.00E-04 | 3.16E-04 |
| | hfw4 | 7.94E-07 | 1.58E-05 | 1.58E-04 | 1.58E-04 | 2.00E-04 | 2.00E-04 | 2.00E-04 | 2.51E-04 | 2.51E-04 | 2.00E-04 | 3.16E-04 |
| | gfwp | 7.94E-08 | 1.58E-07 | 1.00E-05 | 6.31E-06 | 2.51E-05 | 3.16E-05 | 3.16E-05 | 3.98E-05 | 6.31E-05 | 5.01E-05 | 1.58E-04 |
| | gfwp | 1.00E-07 | 1.00E-07 | 1.00E-05 | 5.01E-06 | 3.16E-05 | 3.16E-05 | 3.16E-05 | 2.51E-05 | 2.51E-05 | 3.98E-05 | 1.00E-04 |
| | gfw1 | 1.00E-07 | 1.00E-07 | 1.00E-06 | 7.94E-07 | 2.51E-06 | 2.00E-06 | 1.58E-06 | 1.00E-06 | 7.94E-07 | 1.00E-06 | 3.16E-06 |
| | gfw1 | 1.26E-07 | 1.00E-07 | 7.94E-06 | 3.16E-06 | 1.26E-05 | 7.94E-06 | 6.31E-06 | 6.31E-06 | 7.94E-06 | 7.94E-06 | 2.00E-05 |
| | gfw4 | 3.98E-08 | 1.00E-07 | 1.00E-06 | 1.00E-06 | 1.58E-06 | 1.00E-06 | 1.00E-06 | 1.00E-06 | 6.31E-07 | 1.00E-06 | 3.16E-06 |
| | gfw4 | 6.31E-08 | 1.58E-07 | 3.16E-06 | 2.51E-06 | 7.94E-06 | 5.01E-06 | 5.01E-06 | 5.01E-06 | 3.98E-06 | 5.01E-06 | 7.94E-06 |
| Salt Water | hswp | 3.98E-07 | 2.00E-06 | 3.98E-05 | 5.01E-05 | 1.00E-04 | 6.31E-05 | 7.94E-05 | 1.00E-04 | 1.00E-04 | 1.26E-04 | 2.51E-04 |
| | hswp | 5.01E-07 | 1.58E-06 | 2.51E-05 | 3.98E-05 | 1.00E-04 | 5.01E-05 | 6.31E-05 | 1.00E-04 | 7.94E-05 | 1.26E-04 | 3.16E-04 |
| | hsw1 | 2.00E-07 | 7.94E-07 | 1.58E-05 | 2.51E-05 | 5.01E-05 | 7.94E-05 | 7.94E-05 | 1.00E-04 | 1.00E-04 | 1.58E-04 | 2.51E-04 |
| | hsw1 | 1.26E-07 | 2.51E-07 | 3.16E-05 | 5.01E-05 | 1.26E-04 | 1.00E-04 | 1.26E-04 | 1.58E-04 | 1.58E-04 | 2.00E-04 | 3.16E-04 |
| | hsw4 | 1.00E-07 | 2.00E-07 | 5.01E-07 | 6.31E-07 | 7.94E-07 | 5.01E-07 | 5.01E-07 | 6.31E-07 | 7.94E-07 | 2.00E-06 | 3.16E-06 |
| | hsw4 | 1.00E-07 | 2.00E-07 | 5.01E-07 | 1.00E-06 | 1.26E-06 | 2.51E-06 | 2.51E-06 | 2.51E-06 | 2.51E-06 | 7.94E-06 | 3.16E-05 |
| | gswp | 7.94E-08 | 1.00E-07 | 2.51E-07 | 5.01E-07 | 6.31E-07 | 5.01E-07 | 5.01E-07 | 3.98E-07 | 3.98E-07 | 1.00E-06 | 3.98E-06 |
| | gswp | 7.94E-08 | 3.16E-08 | 3.16E-07 | 5.01E-07 | 1.00E-06 | 5.01E-07 | 5.01E-07 | 5.01E-07 | 5.01E-07 | 1.26E-06 | 3.98E-06 |
| | gsw1 | 6.31E-08 | 2.00E-08 | 7.94E-08 | 1.00E-07 | 1.26E-07 | 7.94E-08 | 7.94E-08 | 6.31E-08 | 7.94E-08 | 1.00E-07 | 2.00E-07 |
| | gsw1 | 6.31E-08 | 5.01E-08 | 7.94E-08 | 1.00E-07 | 1.26E-07 | 1.00E-07 | 1.00E-07 | 1.00E-07 | 7.94E-08 | 1.26E-07 | 3.16E-07 |
| | gsw4 | 3.98E-08 | 2.00E-08 | 6.31E-08 | 7.94E-08 | 1.00E-07 | 5.01E-08 | 5.01E-08 | 5.01E-08 | 3.98E-08 | 5.01E-08 | 1.00E-07 |
| | gsw4 | 3.98E-08 | 2.00E-08 | 6.31E-08 | 7.94E-08 | 7.94E-08 | 3.98E-08 | 3.98E-08 | 3.98E-08 | 3.98E-08 | 6.31E-08 | 1.26E-07 |

| FORMULA→ | | $[H^+] \times 1000 = \text{mol/L}$ | | | | | | | | | | |
|-------------|------|------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| DAYS→ | | 0 | 2 | 7 | 9 | 12 | 14 | 16 | 19 | 21 | 28 | 35 |
| Fresh Water | hfwp | 5.01E-03 | 1.26E-02 | 2.51E-01 | 2.00E-01 | 3.16E-01 | 3.16E-01 | 3.16E-01 | 3.98E-01 | 5.01E-01 | 3.98E-01 | 7.94E-01 |
| | hfwp | 5.01E-03 | 1.58E-02 | 2.00E-01 | 1.00E-01 | 2.51E-01 | 2.51E-01 | 2.51E-01 | 3.16E-01 | 3.16E-01 | 2.51E-01 | 5.01E-01 |
| | hfw1 | 3.16E-03 | 2.51E-02 | 2.51E-01 | 3.16E-01 | 3.98E-01 | 5.01E-01 | 5.01E-01 | 6.31E-01 | 6.31E-01 | 3.98E-01 | 7.94E-01 |
| | hfw1 | 3.16E-03 | 3.16E-02 | 3.16E-01 | 3.16E-01 | 3.98E-01 | 3.98E-01 | 5.01E-01 | 6.31E-01 | 6.31E-01 | 3.98E-01 | 7.94E-01 |
| | hfw4 | 7.94E-04 | 6.31E-03 | 1.26E-01 | 1.58E-01 | 2.00E-01 | 2.00E-01 | 2.00E-01 | 2.51E-01 | 2.51E-01 | 2.00E-01 | 3.16E-01 |
| | hfw4 | 7.94E-04 | 1.58E-02 | 1.58E-01 | 1.58E-01 | 2.00E-01 | 2.00E-01 | 2.00E-01 | 2.51E-01 | 2.51E-01 | 2.00E-01 | 3.16E-01 |
| | gfwp | 7.94E-05 | 1.58E-04 | 1.00E-02 | 6.31E-03 | 2.51E-02 | 3.16E-02 | 3.16E-02 | 3.98E-02 | 6.31E-02 | 5.01E-02 | 1.58E-01 |
| | gfwp | 1.00E-04 | 1.00E-04 | 1.00E-02 | 5.01E-03 | 3.16E-02 | 3.16E-02 | 3.16E-02 | 2.51E-02 | 2.51E-02 | 3.98E-02 | 1.00E-01 |
| | gfw1 | 1.00E-04 | 1.00E-04 | 1.00E-03 | 7.94E-04 | 2.51E-03 | 2.00E-03 | 1.58E-03 | 1.00E-03 | 7.94E-04 | 1.00E-03 | 3.16E-03 |
| | gfw1 | 1.26E-04 | 1.00E-04 | 7.94E-03 | 3.16E-03 | 1.26E-02 | 7.94E-03 | 6.31E-03 | 6.31E-03 | 7.94E-03 | 7.94E-03 | 2.00E-02 |
| | gfw4 | 3.98E-05 | 1.00E-04 | 1.00E-03 | 1.00E-03 | 1.58E-03 | 1.00E-03 | 1.00E-03 | 1.00E-03 | 1.00E-03 | 6.31E-04 | 1.00E-03 |
| | gfw4 | 6.31E-05 | 1.58E-04 | 3.16E-03 | 2.51E-03 | 7.94E-03 | 5.01E-03 | 5.01E-03 | 5.01E-03 | 3.98E-03 | 5.01E-03 | 7.94E-03 |
| Salt Water | hswp | 3.98E-04 | 2.00E-03 | 3.98E-02 | 5.01E-02 | 1.00E-01 | 6.31E-02 | 7.94E-02 | 1.00E-01 | 1.00E-01 | 1.26E-01 | 2.51E-01 |
| | hswp | 5.01E-04 | 1.58E-03 | 2.51E-02 | 3.98E-02 | 1.00E-01 | 5.01E-02 | 6.31E-02 | 1.00E-01 | 7.94E-02 | 1.26E-01 | 3.16E-01 |
| | hsw1 | 2.00E-04 | 7.94E-04 | 1.58E-02 | 2.51E-02 | 5.01E-02 | 7.94E-02 | 7.94E-02 | 1.00E-01 | 1.00E-01 | 1.58E-01 | 2.51E-01 |
| | hsw1 | 1.26E-04 | 2.51E-04 | 3.16E-02 | 5.01E-02 | 1.26E-01 | 1.00E-01 | 1.26E-01 | 1.58E-01 | 1.58E-01 | 2.00E-01 | 3.16E-01 |
| | hsw4 | 1.00E-04 | 2.00E-04 | 5.01E-04 | 6.31E-04 | 7.94E-04 | 5.01E-04 | 5.01E-04 | 6.31E-04 | 7.94E-04 | 2.00E-03 | 3.16E-03 |
| | hsw4 | 1.00E-04 | 2.00E-04 | 5.01E-04 | 1.00E-03 | 1.26E-03 | 2.51E-03 | 2.51E-03 | 2.51E-03 | 2.51E-03 | 7.94E-03 | 3.16E-02 |
| | gswp | 7.94E-05 | 1.00E-04 | 2.51E-04 | 5.01E-04 | 6.31E-04 | 5.01E-04 | 5.01E-04 | 3.98E-04 | 3.98E-04 | 1.00E-03 | 3.98E-03 |
| | gswp | 7.94E-05 | 3.16E-05 | 3.16E-04 | 5.01E-04 | 1.00E-03 | 5.01E-04 | 5.01E-04 | 5.01E-04 | 5.01E-04 | 1.26E-03 | 3.98E-03 |
| | gsw1 | 6.31E-05 | 2.00E-05 | 7.94E-05 | 1.00E-04 | 1.26E-04 | 7.94E-05 | 7.94E-05 | 6.31E-05 | 7.94E-05 | 1.00E-04 | 2.00E-04 |
| | gsw1 | 6.31E-05 | 5.01E-05 | 7.94E-05 | 1.00E-04 | 1.26E-04 | 1.00E-04 | 1.00E-04 | 1.00E-04 | 7.94E-05 | 1.26E-04 | 3.16E-04 |
| | gsw4 | 3.98E-05 | 2.00E-05 | 6.31E-05 | 7.94E-05 | 1.00E-04 | 5.01E-05 | 5.01E-05 | 5.01E-05 | 3.98E-05 | 5.01E-05 | 1.00E-04 |
| | gsw4 | 3.98E-05 | 2.00E-05 | 6.31E-05 | 7.94E-05 | 7.94E-05 | 3.98E-05 | 3.98E-05 | 3.98E-05 | 3.98E-05 | 6.31E-05 | 1.26E-04 |

| | Formula→ | $10^{\text{initial-finalpH}}$ | $10^{\text{finalpH}-10^{\text{initialpH}}}$ | M*20 | moles/kg*1000 | (mg/kg)/35 |
|-------------|----------|-------------------------------|---|-------------|---------------|-------------|
| | | ratio | M | moles/kg | mg/kg | mg/kg/day |
| Fresh Water | Hfw F | 158.49 | 0.0008 | 0.015 | 15.79 | 0.45 |
| | Hfw F | 100.00 | 0.0005 | 0.01 | 9.92 | 0.28 |
| | Hfw E | 251.20 | 0.0008 | 0.015 | 15.82 | 0.45 |
| | Hfw E | 251.20 | 0.0008 | 0.015 | 15.82 | 0.45 |
| | Hfw D | 398.11 | 0.0003 | 0.006 | 6.31 | 0.18 |
| | Hfw D | 398.11 | 0.0003 | 0.006 | 6.31 | 0.18 |
| | Gfw F | 1995.26 | 0.0002 | 0.003 | 3.17 | 0.09 |
| | Gfw F | 1000.00 | 0.0001 | 0.002 | 2.00 | 0.06 |
| | Gfw E | 31.62 | 3.06228E-06 | 6.12456E-05 | 0.06 | 0.001 |
| | Gfw E | 158.49 | 1.98267E-05 | 0.0004 | 0.40 | 0.01 |
| | Gfw D | 79.43 | 3.12247E-06 | 6.24493E-05 | 0.06 | 0.001 |
| | Gfw D | 125.89 | 7.88019E-06 | 0.0002 | 0.16 | 0.005 |
| Salt Water | Hsw F | 630.96 | 0.0003 | 0.005 | 5.02 | 0.14 |
| | Hsw F | 630.96 | 0.0003 | 0.006 | 6.31 | 0.18 |
| | Hsw E | 1258.92 | 0.0003 | 0.005 | 5.02 | 0.14 |
| | Hsw E | 2511.89 | 0.0003 | 0.006 | 6.32 | 0.18 |
| | Hsw D | 31.62 | 3.06228E-06 | 6.12456E-05 | 0.06 | 0.002 |
| | Hsw D | 316.22 | 3.15228E-05 | 0.0006 | 0.63 | 0.02 |
| | Gsw F | 50.12 | 3.90164E-06 | 7.80328E-05 | 0.08 | 0.002 |
| | Gsw F | 50.12 | 3.90164E-06 | 7.80328E-05 | 0.08 | 0.002 |
| | Gsw E | 3.16 | 1.3643E-07 | 2.72861E-06 | 0.003 | 7.79603E-05 |
| | Gsw E | 5.01 | 2.53132E-07 | 5.06264E-06 | 0.005 | 0.0001 |
| | Gsw D | 2.51 | 6.01893E-08 | 1.20379E-06 | 0.001 | 3.43939E-05 |
| | Gsw D | 3.16 | 8.60818E-08 | 1.72164E-06 | 0.002 | 4.91896E-05 |

t-Test Two-sample assuming unequal variance

| | hfwp-4 | gfwp-4 |
|------------------------------|----------|-----------------|
| Mean | 0.333209 | 0.027829 |
| Variance | 0.018283 | 0.001392 |
| Observations | 6 | 6 |
| Hypothesized Mean Difference | 0 | |
| df | 6 | |
| T Stat | 5.33285 | |
| P(T<=t) one-tail | 0.000887 | sig diff |
| T Critical one-tail | 1.943181 | |
| P(T<=t) two-tail | 0.001773 | |
| T Critical two-tail | 2.446914 | |

t-Test Two-sample assuming unequal variance

| | hswp-4 | gswp-4 |
|------------------------------|----------|-----------------|
| Mean | 0.111256 | 0.000794 |
| Variance | 0.006469 | 1.24E-06 |
| Observations | 6 | 6 |
| Hypothesized Mean Difference | 0 | |
| df | 5 | |
| t Stat | 3.363868 | |
| P(T<=t) one-tail | 0.010012 | sig diff |
| t Critical one-tail | 2.015049 | |
| P(T<=t) two-tail | 0.020023 | |
| t Critical two-tail | 2.570578 | |

t-Test Two-sample assuming unequal variance

| | fw | Sw |
|------------------------------|----------|-----------------|
| Mean | 0.180519 | 0.056025 |
| Variance | 0.034377 | 0.006269 |
| Observations | 12 | 12 |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | 2.139096 | |
| P(T<=t) one-tail | 0.024641 | sig diff |
| t Critical one-tail | 1.753051 | |
| P(T<=t) two-tail | 0.049282 | |
| t Critical two-tail | 2.131451 | |

pH calculations for the control experiments from Appendix E.

| | FORMULA→ | $10^{-\text{pH}} = [\text{H}^+]$ | | | | | |
|----------------------|----------|----------------------------------|----------|----------|----------|----------|----------|
| | DAYS→ | 0 | 1 | 7 | 14 | 21 | 28 |
| Un-Inoculated | Hdw | 1.26E-05 | 2.51E-05 | 0.000126 | 0.0002 | 0.000251 | 0.000398 |
| | Hdw | 7.94E-06 | 1.58E-05 | 6.31E-05 | 0.000251 | 0.000501 | 0.000501 |
| | Hfw | 6.31E-07 | 0.00001 | 0.000316 | 0.000316 | 0.000631 | 0.000631 |
| | Hfw | 5.01E-07 | 1.58E-05 | 0.000158 | 0.000398 | 0.000501 | 0.000794 |
| | Hsw | 7.94E-08 | 0.000001 | 6.31E-05 | 0.000251 | 0.000398 | 0.000398 |
| | Hsw | 1.26E-07 | 5.01E-07 | 2.51E-05 | 0.000316 | 0.000316 | 0.000501 |
| | Gdw | 5.01E-06 | 6.31E-06 | 5.01E-06 | 6.31E-06 | 5.01E-06 | 3.98E-06 |
| | Gdw | 5.01E-06 | 5.01E-06 | 6.31E-06 | 5.01E-06 | 5.01E-06 | 6.31E-06 |
| | Gfw | 1.26E-07 | 5.01E-07 | 1.26E-06 | 3.98E-06 | 3.98E-06 | 5.01E-06 |
| | Gfw | 1.58E-07 | 6.31E-07 | 2E-06 | 2.51E-06 | 3.16E-06 | 3.16E-06 |
| | Gsw | 7.94E-08 | 1E-07 | 1.26E-07 | 3.16E-07 | 2.51E-07 | 3.98E-07 |
| | Gsw | 1E-07 | 1E-07 | 1.58E-07 | 2.51E-07 | 3.98E-07 | 3.98E-07 |
| Inoculated | Hdw | 2E-05 | 2.51E-05 | 0.0001 | 0.000158 | 0.000251 | 0.000398 |
| | Hdw | 1.26E-05 | 1.26E-05 | 7.94E-05 | 0.000251 | 0.000316 | 0.000398 |
| | Hfw | 1.58E-06 | 2E-05 | 0.000316 | 0.000251 | 0.000316 | 0.000398 |
| | Hfw | 1.26E-06 | 1.26E-05 | 0.000158 | 0.0002 | 0.000398 | 0.000501 |
| | Hsw | 1E-07 | 7.94E-07 | 1.58E-05 | 0.000126 | 0.0002 | 0.000316 |
| | Hsw | 1.26E-07 | 6.31E-07 | 3.16E-05 | 0.000158 | 0.000316 | 0.000398 |
| | Gdw | 3.98E-06 | 3.98E-06 | 5.01E-06 | 3.98E-06 | 3.98E-06 | 3.98E-06 |
| | Gdw | 5.01E-06 | 5.01E-06 | 3.98E-06 | 5.01E-06 | 3.98E-06 | 6.31E-06 |
| | Gfw | 2.51E-07 | 3.98E-07 | 1.26E-06 | 2.51E-06 | 5.01E-06 | 5.01E-06 |
| | Gfw | 2.51E-07 | 5.01E-07 | 7.94E-07 | 3.98E-06 | 3.98E-06 | 6.31E-06 |
| | Gsw | 1.26E-07 | 1.58E-07 | 2.51E-07 | 1.26E-07 | 2.51E-07 | 5.01E-07 |
| | Gsw | 7.94E-08 | 1E-07 | 1.58E-07 | 3.98E-07 | 3.16E-07 | 3.16E-07 |

| | FORMULA→ | $[H^+] * 1000 = \text{mol/L}$ | | | | | |
|----------------------|----------|-------------------------------|----------|----------|----------|----------|----------|
| | DAYS→ | 0 | 1 | 7 | 14 | 21 | 28 |
| Un-Inoculated | Hdw | 1.26E-02 | 2.51E-02 | 1.26E-01 | 2.00E-01 | 2.51E-01 | 3.98E-01 |
| | Hdw | 7.94E-03 | 1.58E-02 | 6.31E-02 | 2.51E-01 | 5.01E-01 | 5.01E-01 |
| | Hfw | 6.31E-04 | 1.00E-02 | 3.16E-01 | 3.16E-01 | 6.31E-01 | 6.31E-01 |
| | Hfw | 5.01E-04 | 1.58E-02 | 1.58E-01 | 3.98E-01 | 5.01E-01 | 7.94E-01 |
| | Hsw | 7.94E-05 | 1.00E-03 | 6.31E-02 | 2.51E-01 | 3.98E-01 | 3.98E-01 |
| | Hsw | 1.26E-04 | 5.01E-04 | 2.51E-02 | 3.16E-01 | 3.16E-01 | 5.01E-01 |
| | Gdw | 5.01E-03 | 6.31E-03 | 5.01E-03 | 6.31E-03 | 5.01E-03 | 3.98E-03 |
| | Gdw | 5.01E-03 | 5.01E-03 | 6.31E-03 | 5.01E-03 | 5.01E-03 | 6.31E-03 |
| | Gfw | 1.26E-04 | 5.01E-04 | 1.26E-03 | 3.98E-03 | 3.98E-03 | 5.01E-03 |
| | Gfw | 1.58E-04 | 6.31E-04 | 2.00E-03 | 2.51E-03 | 3.16E-03 | 3.16E-03 |
| | Gsw | 7.94E-05 | 1.00E-04 | 1.26E-04 | 3.16E-04 | 2.51E-04 | 3.98E-04 |
| | Gsw | 1.00E-04 | 1.00E-04 | 1.58E-04 | 2.51E-04 | 3.98E-04 | 3.98E-04 |
| Inoculated | Hdw | 2.00E-02 | 2.51E-02 | 1.00E-01 | 1.58E-01 | 2.51E-01 | 3.98E-01 |
| | Hdw | 1.26E-02 | 1.26E-02 | 7.94E-02 | 2.51E-01 | 3.16E-01 | 3.98E-01 |
| | Hfw | 1.58E-03 | 2.00E-02 | 3.16E-01 | 2.51E-01 | 3.16E-01 | 3.98E-01 |
| | Hfw | 1.26E-03 | 1.26E-02 | 1.58E-01 | 2.00E-01 | 3.98E-01 | 5.01E-01 |
| | Hsw | 1.00E-04 | 7.94E-04 | 1.58E-02 | 1.26E-01 | 2.00E-01 | 3.16E-01 |
| | Hsw | 1.26E-04 | 6.31E-04 | 3.16E-02 | 1.58E-01 | 3.16E-01 | 3.98E-01 |
| | Gdw | 3.98E-03 | 3.98E-03 | 5.01E-03 | 3.98E-03 | 3.98E-03 | 3.98E-03 |
| | Gdw | 5.01E-03 | 5.01E-03 | 3.98E-03 | 5.01E-03 | 3.98E-03 | 6.31E-03 |
| | Gfw | 2.51E-04 | 3.98E-04 | 1.26E-03 | 2.51E-03 | 5.01E-03 | 5.01E-03 |
| | Gfw | 2.51E-04 | 5.01E-04 | 7.94E-04 | 3.98E-03 | 3.98E-03 | 6.31E-03 |
| | Gsw | 1.26E-04 | 1.58E-04 | 2.51E-04 | 1.26E-04 | 2.51E-04 | 5.01E-04 |
| | Gsw | 7.94E-05 | 1.00E-04 | 1.58E-04 | 3.98E-04 | 3.16E-04 | 3.16E-04 |

| | Formula→ | $10^{\text{initial-finalpH}}$ | $10^{\text{finalpH}} - 10^{\text{initialpH}}$ | M*20 | Moles/kg*1000 | (mg/kg)/28 | |
|---------------|------------|-------------------------------|---|-----------------|---------------|--------------|-------------|
| | | ratio | M | mole/kg or g/Kg | mg/Kg | mg/kg/day | |
| Un-inoculated | Hdw | 31.62 | 0.00039 | 0.00386 | 3.86 | 0.13768497 | |
| | Hdw | 63.10 | 0.00049 | 0.004932 | 4.93 | 0.176158554 | |
| | Hfw | 1000.00 | 0.00063 | 0.006303 | 6.30 | 0.225116567 | |
| | Hfw | 1584.89 | 0.00079 | 0.007938 | 7.94 | 0.28350966 | |
| | Hsw | 5011.87 | 0.00039 | 0.003980 | 3.98 | 0.142152763 | |
| | Hsw | 3981.07 | 0.00050 | 0.005011 | 5.01 | 0.178950479 | |
| | Gdw | 0.79 | -1.0308E-06 | -0.000010 | -0.01 | -0.000368143 | |
| | Gdw | 1.26 | 1.2977E-06 | 0.000013 | 0.01 | 0.000463465 | |
| | Gfw | 39.81 | 4.88598E-06 | 0.000049 | 0.05 | 0.001744993 | |
| | Gfw | 19.95 | 3.00379E-06 | 0.000030 | 0.03 | 0.001072782 | |
| | Gsw | 5.01 | 3.18674E-07 | 0.000003 | 0.00 | 0.000113812 | |
| | Gsw | 3.98 | 2.98107E-07 | 0.000003 | 0.00 | 0.000106467 | |
| | Inoculated | Hdw | 19.95 | 0.00038 | 0.003782 | 3.78 | 0.135055196 |
| | | Hdw | 31.62 | 0.00039 | 0.003855 | 3.86 | 0.13768497 |
| Hfw | | 251.19 | 0.00040 | 0.003965 | 3.97 | 0.141615099 | |
| Hfw | | 398.10 | 0.00050 | 0.004999 | 5.00 | 0.178545824 | |
| Hsw | | 3162.27 | 0.00031 | 0.003161 | 3.16 | 0.112902774 | |
| Hsw | | 3162.27 | 0.00040 | 0.003980 | 3.98 | 0.142136171 | |
| Gdw | | 1.00 | 0.00 | 0.000000 | 0.00 | 0 | |
| Gdw | | 1.26 | 1.2977E-06 | 0.000013 | 0.01 | 0.000463465 | |
| Gfw | | 19.95 | 4.76068E-06 | 0.000048 | 0.05 | 0.001700244 | |
| Gfw | | 25.12 | 6.05838E-06 | 0.000061 | 0.06 | 0.002163709 | |
| Gsw | | 3.98 | 3.75295E-07 | 0.000004 | 0.00 | 0.000134034 | |
| Gsw | | 3.98 | 2.36795E-07 | 0.000002 | 0.00 | 8.45696E-05 | |

t-Test Two-sample assuming equal variance

| | Variable 1 | Variable 2 |
|------------------------------|------------|------------|
| Mean | 1.905955 | 0.005836 |
| Variance | 0.3063 | 4.78E-05 |
| Observations | 6 | 6 |
| Pooled Variance | 0.153174 | |
| Hypothesized Mean Difference | 0 | |
| df | 10 | |
| t Stat | 8.409091 | |
| P(T<=t) one-tail | 3.79E-06 | |
| t Critical one-tail | 1.812462 | |
| P(T<=t) two-tail | 7.59E-06 | |
| t Critical two-tail | 2.228139 | |

t-Test Two-sample assuming equal variance

| | 31.62278 | 19.95262 |
|------------------------------|----------|----------|
| Mean | 1064.723 | 778.5383 |
| Variance | 3201571 | 1843158 |
| Observations | 11 | 9 |
| Pooled Variance | 2597832 | |
| Hypothesized Mean Difference | 0 | |
| df | 18 | |
| t Stat | 0.395042 | |
| P(T<=t) one-tail | 0.348727 | |
| t Critical one-tail | 1.734063 | |
| P(T<=t) two-tail | 0.697454 | |
| t Critical two-tail | 2.100924 | |

t-Test Two-sample assuming unequal variance

| | Variable 1 | Variable 2 |
|------------------------------|------------|------------|
| Mean | 1945.426 | 1162.294 |
| Variance | 4355314 | 2418827 |
| Observations | 6 | 6 |
| Hypothesized Mean Difference | 0 | |
| df | 9 | |
| t Stat | 0.737027 | |
| P(T<=t) one-tail | 0.239939 | |
| t Critical one-tail | 1.833114 | |
| P(T<=t) two-tail | 0.479877 | |
| t Critical two-tail | 2.262159 | |

Appendix G: Microprobe data

Original totals for the sulphides from microprobe analysis, the averages of crystals in each sample analysed are presented in Table 5.4.

| MINERAL | SAMPLE/ SLIDE # | POINT # | S | FE | CO | NI | CU | ZN | TOTAL |
|--------------|--------------------|---------|---------|---------|--------|--------|---------|---------|----------|
| pyrrhotite | JAS0030 3 4.75 | 1 | 40.4572 | 63.6815 | 0 | 0.2746 | 0.2163 | 0.555 | 105.1846 |
| pyrrhotite | | 2 | 47.7479 | 51.2535 | 0.0139 | 1.0783 | 0 | 1.0525 | 101.1461 |
| chalcopyrite | | 3 | 36.4478 | 32.3272 | 0 | 0 | 37.5715 | 0.8406 | 107.1871 |
| chalcopyrite | | 4 | 36.2608 | 31.4397 | 0.0432 | 0.1013 | 35.342 | 0.8236 | 104.0106 |
| pyrrhotite | | 5 | 40.7837 | 62.6441 | 0 | 0.3614 | 0.1062 | 0.3161 | 104.2115 |
| pyrrhotite | | 6 | 47.174 | 53.3171 | 0 | 1.3147 | 0 | 0 | 101.8058 |
| pyrrhotite | | 7 | 40.0798 | 62.423 | 0 | 0.8539 | 0 | 0 | 103.3567 |
| chalcopyrite | | 8 | 35.9245 | 33.2988 | 0 | 0 | 36.9306 | 0.3459 | 106.4998 |
| pyrrhotite | | 9 | 40.3262 | 63.3503 | 0.0579 | 0.5848 | 0.3933 | 0.3483 | 105.0608 |
| | | 10 | 42.2022 | 48.651 | 0.3429 | 0.3493 | 0 | 0 | 91.5454 |
| | | 11 | 34.9552 | 8.2232 | 0 | 0.0955 | 0 | 63.9813 | 107.2552 |
| pyrrhotite | | 12 | 40.332 | 62.7536 | 0 | 0.0659 | 0 | 0.9277 | 104.0792 |
| chalcopyrite | | 13 | 36.0354 | 31.3685 | 0.1097 | 0 | 36.3585 | 0.4401 | 104.3122 |
| pyrrhotite | | 14 | 41.0528 | 61.6986 | 0 | 0 | 0.099 | 0.7867 | 103.6371 |
| pyrrhotite | | 17 | 41.0929 | 61.7805 | 0 | 0.6989 | 0 | 0.56 | 104.1323 |
| | | 18 | 34.245 | 7.5644 | 0.1256 | 0 | 0.0215 | 63.6603 | 105.6168 |
| pyrrhotite | | 19 | 40.519 | 62.9604 | 0 | 0.4039 | 0 | 0 | 103.8833 |
| chalcopyrite | | 20 | 35.9153 | 32.2455 | 0 | 0.4604 | 36.9374 | 0.249 | 105.8076 |
| chalcopyrite | | 21 | 36.4273 | 32.9527 | 0.132 | 0.4006 | 36.7283 | 2.1628 | 108.8037 |
| | | 22 | 34.6988 | 9.9275 | 0 | 0.1889 | 0.0982 | 60.2331 | 105.1465 |
| pyrrhotite | | 23 | 40.6285 | 62.6606 | 0 | 0.3615 | 0 | 0.2941 | 103.9447 |
| pyrrhotite | | 24 | 40.2893 | 61.8712 | 0 | 1.2282 | 0.1257 | 0 | 103.5144 |
| pyrrhotite | | 25 | 40.9459 | 63.0169 | 0 | 0.4329 | 0 | 0.2437 | 104.6394 |
| pyrrhotite | JAS0030 3 0.5 | 31(2nd) | 40.5594 | 61.245 | 0.1946 | 0.2468 | 0 | 0 | 102.2458 |
| pyrrhotite | | 32 | 45.2511 | 56.3563 | 0.205 | 0.3317 | 0 | 0.1347 | 102.2788 |
| | | 33 | 47.2126 | 49.4559 | 0.3948 | 1.1258 | 0 | 0.1714 | 98.3605 |
| pyrrhotite | | 34 | 40.5307 | 62.697 | 0.1981 | 0.1718 | 0.0133 | 0 | 103.6109 |
| | | 35 | 34.0744 | 8.6828 | 0.0464 | 0.0229 | 0 | 55.4534 | 98.2799 |
| pyrrhotite | | 36 | 39.3966 | 62.088 | 0.1214 | 0.4833 | 0 | 0 | 102.0893 |
| pyrrhotite | | 37 | 43.8951 | 53.4262 | 0.4973 | 0.9141 | 0 | 0 | 98.7327 |
| | | 38 | | | | | | | |
| chalcopyrite | | 39 | 35.4514 | 31.401 | 0.0456 | 0 | 36.5743 | 0 | 103.4723 |
| pyrrhotite | | 40 | 40.5052 | 59.9534 | 0.1442 | 0.1546 | 0.7162 | 0 | 101.4736 |
| pyrrhotite | | 41 | 40.6153 | 60.4165 | 0.1481 | 0.085 | 0 | 0 | 101.2649 |
| pyrrhotite | | 42 | 40.3277 | 59.599 | 0.1456 | 0.1345 | 0.1064 | 0 | 100.3132 |
| chalcopyrite | | 43 | 36.0631 | 31.0743 | 0.0513 | 0 | 36.7313 | 0 | 103.92 |
| chalcopyrite | | 44 | 35.5502 | 31.3328 | 0.0667 | 0 | 36.3863 | 0 | 103.336 |
| pyrrhotite | | 45 | 39.822 | 60.8314 | 0.1027 | 0.2404 | 0 | 0 | 100.9965 |

| | | | | | | | | | |
|--------------|-------------------|----|---------|---------|--------|--------|---------|--------|----------|
| pyrrhotite | | 46 | 40.3784 | 61.4013 | 0.145 | 0.2493 | 0 | 0 | 102.174 |
| chalcopyrite | | 47 | 35.6461 | 31.0066 | 0.0452 | 0 | 35.2419 | 0 | 101.9298 |
| pyrrhotite | | 48 | 40.1404 | 60.0137 | 0.1128 | 0.0952 | 0 | 0 | 100.3621 |
| pyrrhotite | | 49 | 39.9303 | 60.7407 | 0.1154 | 0.2313 | 0 | 0 | 101.0177 |
| pyrrhotite | JAS0030 3 WR | 50 | 39.7277 | 60.7443 | 0.0781 | 0.4109 | 0 | 0.0832 | 101.0442 |
| | | 51 | 0.1023 | 52.3054 | 0.0609 | 0.3842 | 0.2724 | 0 | 53.1252 |
| | | 52 | 0.0777 | 51.4784 | 0.0192 | 0.2898 | 0.21 | 0 | 52.0751 |
| pyrrhotite | | 53 | 35.5 | 60.2149 | 0.122 | 0.2449 | 0 | 0 | 96.0818 |
| | | 54 | 0 | 20.9883 | 0 | 0 | 0 | 0 | 20.9883 |
| | | 55 | 0.14 | 50.5343 | 0.016 | 0.1219 | 0.2322 | 0 | 51.0444 |
| | | 56 | 0.1182 | 51.5168 | 0.0128 | 0.1677 | 0.4001 | 0 | 52.2156 |
| pyrrhotite | | 57 | 40.2246 | 60.1983 | 0.0734 | 0.4236 | 0 | 0 | 100.9199 |
| pyrrhotite | | 58 | 54.0341 | 43.4473 | 0.1717 | 0.9138 | 0 | 0 | 98.5669 |
| | | 59 | 42.0322 | 42.0851 | 0.25 | 0.8866 | 0.0114 | 0.0031 | 85.2684 |
| | | 60 | 42.526 | 37.7264 | 2.1942 | 3.248 | 0.0375 | 0 | 85.7321 |
| | | 61 | 41.9861 | 40.4558 | 0.1702 | 0.6184 | 0.0283 | 0 | 83.2588 |
| | | 62 | 31.8129 | 35.3765 | 5.9388 | 3.9798 | 0.0657 | 0.1022 | 77.2759 |
| | | 63 | 39.6505 | 45.1281 | 2.7622 | 3.1601 | 0.0973 | 0 | 90.7982 |
| | | 64 | 5.0449 | 46.3334 | 0.322 | 0.941 | 0 | 0 | 52.6413 |
| | | 65 | 0.5294 | 48.9438 | 0.0538 | 0 | 0 | 0 | 49.527 |
| | | 66 | 34.4789 | 49.5718 | 0.2671 | 0.549 | 0.0065 | 0 | 84.8733 |
| | | 67 | 38.3206 | 37.4871 | 1.1519 | 1.9294 | 0.0212 | 0 | 78.9102 |
| | | 68 | | | | | | | |
| pyrrhotite | JAS0040 3 <0.5 | 69 | 41.5644 | 60.9496 | 0.133 | 0.1122 | 0 | 0.1618 | 102.921 |
| pyrrhotite | | 70 | 41.1496 | 60.3992 | 0.1217 | 0.1203 | 0.0077 | 0.1257 | 101.9242 |
| pyrite | | 71 | 54.3986 | 45.4217 | 0.092 | 0.0971 | 0 | 0 | 100.0094 |
| pyrite | | 72 | 54.4804 | 45.0534 | 0.1032 | 0.1262 | 0 | 0 | 99.7632 |
| pyrite | | 73 | 54.2733 | 43.2222 | 0.1118 | 0.0777 | 0 | 0 | 97.685 |
| pyrite | | 74 | 54.0874 | 44.9739 | 0.0821 | 0.0252 | 0 | 0 | 99.1686 |
| pyrite | | 75 | 53.5286 | 40.9056 | 0.1338 | 0.0572 | 0 | 0 | 94.6252 |
| pyrite | | 76 | 52.4495 | 41.0314 | 0.0963 | 0.0459 | 0 | 0 | 93.6231 |
| pyrrhotite | | 77 | 39.4431 | 54.8968 | 0.1227 | 0.1027 | 0.018 | 0 | 94.5833 |
| | | 78 | 4.5099 | 44.0816 | 0.0003 | 0 | 0 | 0 | 48.5918 |
| | | 79 | 5.874 | 50.9457 | 0.0831 | 0 | 0.0649 | 0 | 56.9677 |
| | | 80 | 5.7912 | 48.6603 | 0.0807 | 0 | 0.0175 | 0.1384 | 54.6881 |
| | | 81 | 5.8293 | 46.0672 | 0.0655 | 0 | 0.051 | 0.0886 | 52.1016 |
| | | 82 | 43.9589 | 46.6915 | 0.1916 | 0.1396 | 0.1941 | 0.5139 | 91.6896 |
| | | 83 | 43.6644 | 46.9033 | 0.186 | 0.1465 | 0.162 | 0.4834 | 91.5456 |
| | | 84 | 43.5301 | 46.2372 | 0.1825 | 0.1659 | 0.1662 | 0.2688 | 90.5507 |
| chalcopyrite | | 85 | 29.5014 | 31.1328 | 0.1392 | 0.124 | 36.7714 | 0.3457 | 98.0145 |
| chalcopyrite | | 86 | 29.4195 | 31.1395 | 0.1546 | 0.1321 | 35.9275 | 0.6221 | 97.3951 |
| | | 87 | 44.2821 | 46.739 | 0.1809 | 0.1314 | 0.1872 | 0.1906 | 91.7112 |
| pyrrhotite | | 88 | 32.4181 | 60.8795 | 0.2132 | 0.1716 | 0.2313 | 0.4452 | 94.3589 |

| | | | | | | | | | |
|--------------|-------------------|-----|---------|---------|--------|--------|---------|--------|-----------|
| pyrrhotite | | 89 | 40.5205 | 61.3746 | 0.1481 | 0.0657 | 0.0082 | 0 | 102.1171 |
| chalcopyrite | | 90 | 35.5496 | 30.5506 | 0.0643 | 0.0371 | 35.7193 | 0 | 101.9209 |
| pyrrhotite | | 91 | 40.3354 | 60.7428 | 0.1203 | 0.1145 | 0.018 | 0.0955 | 101.4265 |
| pyrrhotite | JAS0040 3 11.2 | 92 | 40.623 | 61.5714 | 0.1116 | 0.1107 | 0 | 0 | 102.4167 |
| pyrrhotite | | 93 | 41.8716 | 59.9836 | 0.1069 | 0.0181 | 0 | 0 | 101.9802 |
| pyrrhotite | | 94 | 40.4693 | 60.1388 | 0.1166 | 0.1121 | 0.0186 | 0 | 100.8554 |
| pyrrhotite | | 95 | 40.349 | 60.8183 | 0.1085 | 0.0797 | 0.0077 | 0 | 101.3632 |
| pyrrhotite | | 96 | 40.5056 | 60.8805 | 0.0954 | 0.0774 | 0.0052 | 0 | 101.5641 |
| pyrrhotite | | 97 | 40.4972 | 62.6081 | 0.128 | 0.1008 | 0.0206 | 0 | 103.3547 |
| pyrrhotite | | 98 | 40.4926 | 60.4338 | 0.1097 | 0.1136 | 0 | 0 | 101.1697 |
| pyrrhotite | | 99 | 40.5719 | 59.8789 | 0.1181 | 0.131 | 0 | 0.1222 | 100.8221 |
| pyrrhotite | | 100 | 40.0436 | 58.9759 | 0.1319 | 0.1021 | 0 | 0.0586 | 99.3121 |
| pyrrhotite | | 101 | 40.519 | 59.4071 | 0.1061 | 0.1191 | 0 | 0 | 100.1513 |
| chalcopyrite | | 102 | 35.4351 | 30.2873 | 0.0735 | 0.0383 | 35.5059 | 0.3721 | 101.7122. |
| pyrrhotite | | 103 | 40.0893 | 58.5956 | 0.1167 | 0.0949 | 0 | 0.0782 | 98.9747 |
| pyrrhotite | | 104 | 39.9645 | 59.3804 | 0.1069 | 0.0848 | 0 | 0.1144 | 99.651 |
| pyrrhotite | | 105 | 40.1914 | 60.1332 | 0.1163 | 0.0952 | 0 | 0.2572 | 100.7933 |
| pyrrhotite | | 106 | 41.7739 | 57.1148 | 0.1083 | 0.1931 | 0 | 0.0451 | 99.2352 |
| Pyrite | | 107 | 55.1298 | 45.1492 | 0.079 | 0.009 | 0 | 0.1608 | 100.5278 |
| Pyrite | | 108 | 55.3007 | 44.5673 | 0.0754 | 0.014 | 0 | 0.1262 | 100.0836 |
| pyrrhotite | | 109 | 40.0096 | 58.6534 | 0.1187 | 0.0734 | 0 | 0 | 98.8551 |
| pyrrhotite | | 110 | 40.2068 | 59.5046 | 0.1234 | 0.0861 | 0 | 0 | 99.9209 |
| pyrrhotite | | 111 | 40.5657 | 58.8537 | 0.1234 | 0.0745 | 0 | 0 | 99.6173 |
| pyrrhotite | | 112 | 40.1524 | 58.0904 | 0.0994 | 0.0788 | 0 | 0 | 98.421 |
| pyrrhotite | | 113 | 40.107 | 58.0815 | 0.1115 | 0.0846 | 0 | 0 | 98.3846 |
| pyrrhotite | | 114 | 40.2641 | 59.0612 | 0.0962 | 0.0433 | 0 | 0 | 99.4648 |
| pyrrhotite | | 115 | 40.1632 | 58.7864 | 0.1151 | 0.0638 | 0 | 0 | 99.1285 |
| pyrrhotite | | 116 | 39.9576 | 58.8706 | 0.1485 | 0.0749 | 0 | 0 | 99.0516 |