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METHYLMERCURY FORMATION AND SULFATE-REDUCING BACTERIA IN MINE TAILINGS

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ABSTRACT

Mercury (Hg) is a contaminant of global concern due to its toxicity to humans and other organisms. Methylmercury (MeHg) is the most hazardous form of mercury commonly found in the environment, as it bioaccumulates in aquatic food webs. Its production has been linked with microbial sulfate reduction in soils and sediments. Mine tailings cover vast land areas and release acidity and toxic metals, including Hg, to the environment. Sulfate-reducing bacteria (SRB) are active in mine tailings, indicating the potential for MeHg formation. This study investigated MeHg levels in mine tailings and the chemical and microbiological factors that might lead to significant MeHg contamination. Acidic base-metal tailings from northern Ontario were examined because they host active SRB but Hg and MeHg concentrations were unknown. Gold mine tailings from Nova Scotia were studied because Hg amalgamation was used there around 1860-1940, leaving Hg-rich tailings of unknown MeHg content and microbiology.

MeHg accumulated in organic material in both environments. Acidic tailings contained negligible levels of MeHg, except for the Kidd Metsite tailings, which featured ≤ 12 nmol kg^{-1} MeHg in bulk tailings and 88 pM MeHg in porewaters, corresponding to a zone of sulfate reduction in the surficial layer (pH 3.7). Gold mine tailings featured a wide range of both Hg_T (0.2 - 53.5 μmol kg^{-1}) and MeHg (< detection limit - 56.4 nmol kg^{-1}). Hg_T levels decreased with distance from the stamp mills where ore was pulverized and treated with Hg. MeHg was influenced by multiple factors including Hg_T concentration, hydrological conditions, redox conditions, and demethylation. Seasonal fluctuations in MeHg were observed in one bog-type gold tailings dump.

Analysis of DNA from MeHg-contaminated tailings and cultures detected Gram-positive (*Firmicutes*) and -negative (*Deltaproteobacteria*) SRB in both types of tailings. A *Deltaproteobacteria* related sequence from the Kidd Metsite was unrelated to cultured lineages but 98-99% related to sequences detected in acid mine drainage elsewhere. This organism may be an important contributor to MeHg levels in acidic situations. DNA from gold tailings and cultures revealed a highly biodiverse bacterial community that included at least five *Deltaproteobacteria* spp. and sequences related to Hg-resistant genera.

RÉSUMÉ

Contaminant toxique pour l'homme aussi bien que pour d'autres organismes, le mercure (Hg) est un sujet de préoccupation général. Le méthylmercure (MeHg) est la forme de mercure la plus nocive communément présente dans l'environnement puisqu'il se bioaccumule dans le réseau alimentaire aquatique. Sa production a été reliée à la réduction microbienne du sulfate dans les sols et les sédiments. Les résidus d'extraction minière occupent de vastes régions terrestres et libèrent dans l'environnement, dont ils accroissent la toxicité, des métaux toxiques, y compris le Hg. L'activité des bactéries sulfatoréductrices dans les résidus miniers reflète leur potentiel de formation du MeHg. La présente étude s'est penchée sur les niveaux de MeHg dans les résidus miniers ainsi que sur les facteurs chimiques et microbiologiques pouvant aboutir à une contamination significative au MeHg. Des résidus acides des métaux de base venant du nord de l'Ontario ont été étudiés car bien qu'ils contiennent des bactéries sulfatoréductrices actives, les concentrations de Hg et de MeHg étaient inconnues. Des résidus de l'extraction minière de l'or provenant de la Nouvelle-Écosse ont été examinés puisque l'amalgamation du Hg réalisée dans cette région vers 1860-1940 a laissé des résidus miniers riches en Hg dont la teneur en MeHg et la composition microbiologique demeuraient inconnues.

Sur ces deux sites, le MeHg s'est accumulé dans la matière organique. Les résidus acides recèlent des quantités négligeables de MeHg, exception faite de ceux venant du site de Kidd Metsite qui présentent ≤ 12 nmol kg^{-1} de MeHg dans les résidus volumineux et 88pM MeHg dans les eaux interstitielles, quantités qui correspondent à une zone de réduction du sulfate dans la couche superficielle (pH 3.7). Les résidus de l'extraction minière de l'or présentent une grande étendue de concentrations à la fois pour le Hg_T (0.2 - 53.5 μmol kg^{-1}) et le MeHg (< limite de détection - 56.4 nmol kg^{-1}). Les niveaux de Hg_T diminuent avec la distance du pulvérisateur où le minerai était broyé et traité avec le Hg. Plusieurs facteurs influencent les concentrations de MeHg notamment la concentration de Hg_T, les conditions hydrologiques, les conditions d'oxydo-réduction et la déméthylation. Des fluctuations saisonnières des concentrations de MeHg furent observées dans les résidus de l'extraction minière de l'or d'un dépotoir de type tourbière.

Une analyse d'ADN réalisée à partir de résidus contaminés au MeHg et de cultures a révélé la présence de bactéries sulfatoréductrices Gram-positives (*Firmicutes*) et -négatives (*Deltaproteobacteria*) dans les deux types de minerai. Une séquence reliée aux *Deltaproteobacteria* et venant du site de Kidd Metsite n'était pas apparentée aux lignées cultivées bien qu'elle le soit à 98-99% à des séquences détectées dans des cas de drainage minier acide ailleurs. Cet organisme pourrait contribuer de façon importante aux niveaux de MeHg observés sous des conditions acides. L'ADN issu des résidus miniers et des cultures à révélé une communauté bactérienne d'une grande biodiversité qui comprend au moins cinq espèces de *Deltaproteobacteria* et séquences apparentées au genre résistant au Hg.

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LIST OF ABBREVIATIONS

AMD	acid mine drainage
ASM	artisanal and small-scale miners
aSRB	acidophilic (or acid-tolerant) SRB
CCME	Canadian Council of Ministers of the Environment
CFU	colony forming units
DL	detection limit
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
ELC	East Lake Catcha
FAME	fatty acid methyl esters
FeRB	iron reducing bacteria
FP	First Pond
GC/AFS	gas chromatography/atomic fluorescence spectrometry
Hg_T	total mercury
IDRB	International Development and Reconstruction Bank
ISQG	interim sediment quality guideline
LCL	Lake Catcha Lake (beach tailings)
LOI	loss on ignition
LSH	Lower Seal Harbour
MDL	method detection limit
MeHg	methylmercury

MMER	Metal Mining Effluent Regulations
MPN	most probable number
NCBI	National Center for Biotechnology Information
NOAMI	National Orphaned/Abandoned Mines Initiative
NRCan	Natural Resources Canada
OD	oxidative demethylation
PCR	polymerase chain reaction
PEL	probable effect level
RD	reductive demethylation
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	ribosomal RNA
RSE	relative standard error (SE/mean)
SE	standard error (standard deviation/square root of n)
SRB	sulfate-reducing bacteria
SRR	sulfate reduction rates
SSU	small subunit (of ribosome)
USEPA	US Environmental Protection Agency
USGAO	US General Account Office
USH	Upper Seal Harbour
XRD	X-ray diffraction

CHAPTER 1. INTRODUCTION

1.1 THESIS RATIONALE - MERCURY AND MINING

Humans have used mercury for centuries (Hg) in a wide range of applications, including medicine and dentistry, art, industry, technology and science. Although Hg is naturally present in the environment from sources such as volcanoes and hydrothermal vents, human activity has significantly increased the Hg content over background levels in soil, water and air. One of the most important and well-known uses of Hg is in gold mining, since Hg and Au readily form an amalgam from which Hg can be volatilized at high temperatures. Mercury is itself mined from sulfide minerals, with cinnabar (HgS) being the predominant ore. The two largest and oldest Hg mines in the world are Almaden, Spain (>2000 years old; Grey et al., 2004) and Idrija, Slovenia (> 500 years old; Gosar et al., 1997). Since Hg binds strongly with sulfide, other non-Hg sulfide ores (e.g. sphalerite) may contain Hg at concentrations up to ~ 50 $\mu mol\ kg^{-1}$ (Hannington et al., 1999). Hg is often found in Zn ore, and some Cu-Zn or Pb-Zn mines recover volatile Hg from the roasting process and sell it as a by-product of their operations.

Methylmercury (MeHg) is the most toxic form of Hg commonly found in the environment, as it bioaccumulates in aquatic food webs (Hammerschmidt et al., 2002; Pickardt et al., 2005). Humans and other top predators are the most seriously affected. The first documented large-scale MeHg poisoning happened in Japan in the 1950s and 1960s, when large numbers of fishermen were diagnosed with neurological disorders. Their illness was determined to have been caused by consumption of fish contaminated with high concentrations of MeHg which had been emitted from the Chisso chemical plant into

Minimata Bay (Gochfeld, 2003). Later, a severe outbreak of organomercurial poisoning happened in 1971 in Iraq due to consumption of grain treated with organomercurial fungicide (Myers et al., 2000). These events drew worldwide attention to the danger of mercury poisoning in humans. Numerous studies have since documented the sources, speciation and effects of mercury in the aqueous environment, where MeHg bioaccumulates and biomagnifies with increasing trophic level in food webs due to the solubility of CH_3HgCl in lipid which allows for its efficient transfer from prey to predator (Morel et al., 1998). For example, Hammerschmidt et al. (2002) reported that MeHg decreased reproduction in fathead minnows, and Pickardt et al. (2005) showed that the rate of MeHg bioaccumulation in zooplankton depended on zooplankton species and was inversely related to phytoplankton concentration.

Although numerous studies have described the impact of Hg contamination from Au and Hg mines on downstream waters, sediments and ecosystems (e.g. Biester et al., 2000; Ganguli et al., 2000), few describe Hg and MeHg in mine tailings dumps. This is especially true for sulfidic base-metal tailings. Characterization of tails has become more important over time, as the geographic range of human activity expands ever further, and pressure mounts to make use of the vast land surface areas currently occupied by abandoned mine wastes. Many abandoned mine sites are open to the public, or only minimal barriers have been put in place to keep people out. Over the course of this study, evidence of human contact with mine tailings was abundant. For example: housing developments built on mine tailings; a cottage built on gold tailings and next to a lake where tailings had been deposited; ATV tracks on tailings at all abandoned sites visited; an organized, annual 4x4 rally at a gold tailings dump;

marijuana plants growing in mine tailings; garbage dumps in tailings and down mine shafts; shotgun shells and abandoned cars. Local land owners and officials also outlined plans for re-extraction of old tailings, remediation and relocation efforts, road-building, and plans for a new mine underneath an old tailings deposit at one site. As well, evidence of animal life (moose, deer, bears, rabbits, mice, coyotes, frogs, fish, game and wading birds, etc.) was observed on or around every abandoned mine tailings deposit visited.

At the time that many of the tailings investigated in this study were deposited (up to ~ 100 years ago), little, if any, thought was given to the possibility that these materials could cause harm to humans, much less the natural environment. Had there been any concern regarding their impact on human health, it would have been assuaged by the knowledge that these materials were located in remote areas, far from the reach of most people. Today's reality is very different. Mine wastes have been studied extensively with respect to their potential impact on the well-being of humans and the natural environment. Very little of this work, however, has focused on speciation and cycling of Hg in mine wastes and the geomicrobiological processes that may influence them. As we regard abandoned mine sites with an increasingly speculative eye, it becomes essential to develop our understanding of the geochemistry and microbial ecology of mine waste products.

1.2 MINE TAILINGS

1.2.1 General description

Metal mining involves removing metal-rich rock (ore) from beneath the earth's surface and passing it through a series of crushing processes until it becomes pulverized, exposing as much surface area as possible to reagents introduced in the extraction process. Once the desired metals have been extracted, a slurry of fine (sand-sized or smaller) particles suspended in water called "tails" or "tailings", remains to be disposed of. Although some tailings are used to fill closed mine shafts and drifts (horizontal tunnels), very large quantities are deposited on the land surface near the extraction facility of a mine. In modern times, tailings are chemically treated and physically enclosed with liners, dams or other barriers to prevent drainage or leaching of contaminants into nearby surface and ground waters. Old mine tailings, however, were simply poured into low-lying areas such as wetlands, lakes, rivers and streams.

1.2.2 Quantity of tailings in North America

Data regarding the number of abandoned mine sites and tonnage and types of mine tailings are very fragmented. Nonetheless, the number of abandoned mine sites in the United States has been estimated at 560,000, and roughly 14,400 need extensive work to prevent surface water contamination (USGAO, 1996). Canadian tailings dumps contain at least 350 million tons of sulfidic, acid-producing mine tailings (Fortin et al., 1995), as well as an unknown

quantity of historic gold mine tailings, much of which is left from Hg amalgamation processes.

1.2.3 Types of mine tailings addressed in this study

Different types of mines produce physically and chemically different tailings. This study analyzes two kinds: sulfidic, base-metal tailings from Northern Ontario and pH circumneutral gold mine tailings from Nova Scotia. "Base" metals are those metals which are primarily used for industrial purposes and include Cu, Zn, Pb, Ni, Fe, Cd, Hg and any other metal that is not a "precious" metal, like Ag or Au. Because of their propensity to form metal sulfides, many base metals occur in high concentrations in rocks made of sulfide minerals. Some precious metals, e.g. Ag, also occur in sulfide mineral formations, but since the sulfidic tailings addressed in the present study are primarily base-metal tailings, the terms "base-metal tailings" and "sulfidic tailings" will be used to refer to them.

1.3 GEOCHEMISTRY, MINERALOGY AND MICROBIOLOGY OF TAILINGS

Since bacteria in mine tailings catalyze reactions between solid mineral phases and dissolved porewater constituents, the geochemistry, mineralogy and microbiology of tailings are closely interwoven. This section provides a review of the mineralogy, porewater geochemistry and geomicrobiology of sulfidic, base-metal tailings and pH-neutral gold mine tailings.

1.3.1 Sulfidic mine tailings

1.3.1.1 Mineralogy

Fresh tailings are made up of fine sand to silt-sized grains (Blowes et al., 1991) of sulfide minerals (mainly pyrite (FeS_2) and pyrrhotite ($Fe_{1-x}S$)) that remain after metal recovery, and gangue materials such as chlorite minerals, quartz, muscovite, feldspar, pyroxene (Fortin et al., 1995; Blowes et al., 1991). When tailings are stored under saturated/reducing/anoxic, pH-neutral conditions, minerals undergo little or no diagenesis to secondary-phase formation or dissolution of primary minerals (Boulet and Larocque, 1998; Blowes and Jambor, 1990). Many older tailings dumps, however, were not contained or kept saturated, resulting in exposure of tailings particles to water and oxygen over long periods of time. Particulates have undergone changes brought about by abiotic and biologically-catalized reactions with oxygen and water. As a result, old sulfidic tailings often feature two distinct layers: an orange-coloured surficial layer of unsaturated, oxidized tailings, underlain by saturated, grey, unoxidized tailings (Johnson et al., 2000; Fortin et al., 1995; Gunsinger et al., 2006). In the oxidized layer, primary sulfide minerals typically have been largely or completely eroded through oxidative dissolution resulting in depletion of pyrite, pyrrhotite and other sulfide minerals in the oxidized layer and releases to porewaters Fe, sulfate, protons (acidity) and metals such as Cu, Zn, Pb and others associated with sulfide minerals (Johnson et al., 2000; Blowes & Jambor, 1990). Increased acidity leads to partial to total dissolution of carbonate minerals (calcite, dolomite, siderite), aluminosilicate minerals and/or Fe- and Al-hydroxide minerals through buffering reactions (Al et al., 2000; Blowes and Jambor, 1990;

Fortin and Beveridge, 1997; Moncur et al., 2005; Johnson et al., 2000). Precipitation of secondary minerals results from saturation of porewaters by products of the above reactions. Porewater constituents are subsequently controlled by precipitation-dissolution, surface sorption and co-precipitation reactions with secondary minerals such as gypsum and jarosite (Johnson et al., 2000). For example, the surface abundances of Cu, Ag, Pb, Zn and Cd were lowest and porewater concentrations of the same metals highest in the low-pH, oxidized zone of sulfidic tailings from Kidd Creek, Ontario (Al et al., 1997). Table 1 summarizes the primary sulfide minerals, primary gangue minerals and secondary minerals most often reported in the literature on sulfidic mine tailings.

1.3.1.2 Porewater Geochemistry

Table 2 gives values reported in the literature for pH, Eh, major cations and anions in porewaters of the oxic and anoxic layers of sulfidic mine tailings. In general, oxidized, surficial layers feature lower pH, higher Eh, higher concentrations of metals and sulfate and lower concentrations of dissolved Fe than anoxic layers.

1.3.1.3 Microbiology

Although abiotic dissolution of pyrite and/or pyrrhotite and subsequent reactions involving secondary minerals produce sulfate, Fe and acidity and release some metals into solution, this process is greatly accelerated by the activity of bacteria in the tailings. Production of acidity is catalyzed and pH is lowered substantially in the oxic layer by the activity of

acidophilic chemolithotrophs (e.g. *Acidithiobacillus* – Fortin et al., 1996) and heterotrophs which oxidize iron sulfides producing sulfuric acid and ferric iron (Harrison, 1984; Ingledew, 1982), releasing metals and resulting in the depletion of Fe-pyrite and enrichment in Fe-silicates (Fortin et al., 2002; Fortin et al., 2000). Although carbonate minerals in the tailings can neutralize some portion of the acidity and thereby precipitate metals (Al et al., 2000), when buffering capacity of the tailings is exceeded, the activity of Fe- and S-oxidizers underlies the production of acid mine drainage (AMD), a common mining effluent that is environmentally detrimental due not only to its acidic nature but also to the dissolved heavy metals (e.g. Cu, Zn, Pb, Cd, Fe) that it carries to downstream waters.

The following equations describe the formation of AMD from oxidative dissolution of pyrite:

$$FeS_2 + 7/2\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ SO_4^{2-} + 2\ H^+$$

$$2\ Fe^{2+} + \tfrac{1}{2}\ O_2 + 2\ H^+ \xrightarrow{\text{Fe-oxidizing bacteria}} 2\ Fe^{3+} + H_2O$$

$$14\ Fe^{3+} + FeS_2 + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+$$

(Fortin et al., 1995, modified from Singer & Stumm, 1970).

Examples of AMD contamination of surface and groundwaters downstream of tailings dumps from abandoned mine sites worldwide abound in the literature (Blowes and Jambor, 1990 – Waite Amulet mill, Quebec; Dold and Fontboté, 2002 – Punta del Cobre belt, Chile;

McKnight and Bencala, 1990 – Arctic; Regenspurg et al., 2004 – Germany/lignite mines; Benner et al., 2000 – Nickel Rim mine, Sudbury; Boulet and Larocque, 1998 – Silver City, New Mexico; Holmstrom et al., 2001 – Sweden). Although each site is unique, the common factor in all AMD situations is acidity generated through oxidation of sulfide minerals and mobilization of metals.

For example, Boulet and Larocque (1998) described highly oxidized, sulfidic tailings in New Mexico where secondary minerals (jarosite, goethite, hematite, Fe-oxyhydroxides and Fe-oxyhydroxysulfates) predominated. Stream water pH was as low as 2.15 and maximum metal concentrations in stream water immediately downstream of tailings were: 81.2 mM Zn; 7.1 mM Cu; 5.6 μM Pb; 156 μM Cd; 18.7 μM As; and 0.05 μM Hg.

The depth of the oxic layer is determined primarily by the depth of the water table, which, in turn, is influenced by particle size, rainfall and evapotranspiration rates (Fortin et al., 1995). Below the oxic-anoxic interface, pH typically rises, often to near-neutral, as redox potential falls. These conditions combined with a continuous supply of sulfate produced through sulfide oxidation in surficial layers constitute ideal habitat for sulfate-reducing bacteria (SRB).

In contrast to the acidifying impact of *Acidothiobacillus ferrooxidans* and other acidophilic Fe- and S-oxidizers in the oxic layer, SRB produce alkalinity and decrease redox potential in the anoxic zone by the consumption of organic carbon and sulfate, and simultaneous production of H_2S and bicarbonate:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

As pH rises, metals are precipitated from solution (Gabler, 1997). In recognition of this, SRB have frequently been used in AMD bioremediation efforts, particularly in permeable reactive barriers (e.g. Benner et al., 1999).

Availability of sulfate or organic carbon affects the rate of microbial sulfate reduction. Since sulfate is abundant in tailings porewaters, the limiting factor is probably organic carbon. It was previously thought that insufficient organic carbon would be available to support SRB, but they seem to have adapted to their environment and do not require large amounts of organic carbon in order to survive (Fortin and Beveridge, 1997). SRB are known to use a wide range of organic (e.g. lactate, formate, pyruvate) and inorganic (H_2) electron donors (Widdel and Hansen, 1992). The organic compounds are fermentation products from the anaerobic bacterial degradation of carbohydrates, proteins, and other components of dead biomass (Widdel, 1988). The oxidized zone of Kidd Creek tailings features highest abundance of C on the surface of pyrite minerals, coinciding with abundant populations of chemilithotrophic bacteria (probably Fe- and S-oxidizers) (Al et al., 1997). Dead biomass from these bacteria is the most likely source of electron donors for SRB.

Despite more acidic conditions, loss-on-ignition values were found to be higher in Cu-Zn tailings than in Au tailings, and this was attributed to the microbial biomass responsible for the oxidation of metal sulfides (biomass can easily reach 10^9 CFU g^{-1} sediment (dry wt))

(Fortin et al., 2000). The exact types of organic substrates used by SRB in mine tailings are not yet known, although SRB recovered from sulfidic mine tailings have been shown to use lactate (partial oxidation to acetate), acetate and formate as electron donors (Rioux et al., 2004), and to grow with low concentrations of organic carbon, formate and acetate (<1 mM in porewater), likely excretion products of living biomass or degradation products from dead biomass (Fe-oxidizers) (Fortin et al., 1996).

In the past, it was believed that SRB were only able to survive in anoxic conditions, but more recently it has been shown that some SRB may be able to remove oxygen by active respiration (Krekeler et al., 1998). SRB can survive in the outer layers of microbial mats supersaturated with oxygen (Minz et al., 1999) and aerobic sulfate reduction has been reported in well oxygenated photosynthetic zones of microbial mats (Canfield and Des Marais, 1991).

Recent studies have shown some sulfate- and iron-reducers to be present in acidic (as low as pH 2) and oxic (Eh up to 600 mV) environments (Fortin et al., 2002; Wielinga et al., 1999; Benner et al., 2000; Cummings et al., 2000). Sulfate reduction rates, however, are faster at pH > 5 (Peine et al., 2000) and SRB counts depend strongly on redox gradients (Fortin et al., 2002).

Where SRB are present, they increase the mineral content of mine tailings by forming metal sulfide precipitates. Sulfate depletion and the presence of pyrite minerals suggest that diagenetic Fe sulfides serve as sinks for other dissolved metals (Zn, Cu, Pb, Cd, etc.)

(Pedersen et al., 1993). Sedimentary pyrite formation at low temperature (< 100°C) is due to the microbial reduction of sulfate because abiological reduction of sulfate by ferrous iron or organic matter is believed to occur only above 250°C (Trudinger et al., 1985). Large populations of SRB in acidic mine tailings corresponded to an enrichment of pyrite at coincident depths (Fortin and Beveridge, 1997).

Previous papers have examined the abundance and spatial distribution of SRB in sulfidic mine tailings as well as their role in mineral diagenesis/biomineralization/ formation of authigenic metal sulfides (e.g. Fortin et al., 1995). The present study, however, focuses on another aspect of SRB activity: their ability to methylate inorganic Hg. Sulfate reducers have been shown to methylate Hg in other contexts (Compeau and Bartha, 1984), such as marine and freshwater sediments. No papers have yet been published, however, on the potential for SRB to methylate mercury in acidic, sulfidic mine tailings.

Recently, iron-reducing bacteria (FeRB) were shown to methylate Hg in iron-rich, freshwater environments. Specifically, in incubation experiments where sulfate reduction was inhibited with molybdate, bacteria of the genus *Geobacter* methylated Hg at a rate comparable to Hg methylation rates measured for sulfate reducers of the genus *Desulfovibrio* (Fleming et al., 2006). Interestingly, these FeRB fall into the *Deltaproteobacteria* phylum, along with all Gram-negative sulfate reducers that have been demonstrated to methylate Hg (Gilmour et al., 2006).

1.3.1.4 Mercury contamination

Although cinnabar has not been reported as a common sulfide mineral in Cu-Zn or Pb-Zn ores, the greenstone and metasedimentary belts of Ontario, Manitoba and Saskatchewan are all characterized by elevated Hg levels relative to the granitic or sedimentary rocks surrounding them (Garrett, 1995). Hg content in these ores can be as high as 50 μmol kg^{-1} and often correlates with Zn concentration (Hannington et al., 1999). Hg is often associated with non-cinnabar sulfide minerals, particularly pyrite and sphalerite (Garrett, 1995). Zn is extracted by roasting, and since Hg is volatile, Zn smelters are often the source of large amounts of Hg emissions to the atmosphere (e.g. Flin Flon, Manitoba – Garrett, 1995). In this way, much of the Hg in the original ore is lost to the atmosphere, reducing the amount that would otherwise be deposited in tailings dumps. No published studies, however, have documented the Hg content of sulfidic mine tailings in the Timmins area or at other sulfidic, base-metal mining facilities.

Hg in and downstream of sulfidic tailings has mainly been studied in the context of Hg mining, where the primary ore is cinnabar (HgS). The most extensively studied site is the Idrija Mine in Slovenia, which closed in 1995 after over 500 years of operation (Biester et al., 1999). In 1991, stream sediment concentrations of Hg_T increased from 10 μmol kg^{-1} upstream of the mine site to 1.5-5 mmol kg^{-1} within an approximately 5-km stretch downstream of the mine in the Idrija River, and decreased to 25 μmol kg^{-1} by approximately 50 km downstream (in the Soca River), but high Hg_T values have also been reported in sea bottom sediments in the vicinity of the Soca River inflow to the Adriatic Sea (Gosar et al.,

1997). However, contamination from the cinnabar tailings results from erosion of tailings deposits; no mention is made of AMD, in spite of the sulfidic nature of the cinnabar particles. The lack of AMD can be attributed to the mineralogy of the tailings, which varied among sites due to differences in the composition of ores processed at different times over the mine's history. The main Hg ore processed over the first four centuries of the mine's operation was cinnabar-bearing dolomite, but ores of Carboniferous black schist bearing native Hg became more important in the last years of the mine's operation (Biester et al., 1999). No mention is made of pyrite in either ore, and all tailings consisted primarily of dolomite, calcite, quartz, kaolinite and muscovite. Even if small amounts of pyrite were present in the fresh tailings, any acidity generated by its oxidation would have been neutralized by alkalinity produced by dolomite dissolution. Total Hg in the Idrija River increased > 20-fold downstream of the mine from < 15 pM to > 300 pM, with MeHg accounting for ~ 0.5% (Hines et al., 2000).

By contrast, AMD (pH 3.3 - 4.6) was determined to be the principal means of Hg transport out of sulfidic mercury mine tailings at the New Idria mercury mine in California, USA, where cinnabar (HgS), metacinnabar (HgS), pyrite (FeS_2) and marcasite (FeS_2) were present in the ore (Boctor et al., 1987). Total unfiltered Hg concentrations in AMD ($\leq$ 204 pM) were comparable to concentrations upstream of the mine ($\leq$ 65 pM), except in a pond next to a furnace waste pile where pH dropped from 4.6 to 3.3 and Hg_T increased to 2.1 nM (Ganguli et al., 2000). Hg may, then, be more easily mobilized from sulfide minerals (even insoluble cinnabar) under AMD conditions. Monomethylmercury concentrations of 1.3 pM, almost 20-fold higher than background values, increased further to 7.5 pM in unfiltered streamwater

downstream of the mine. These concentrations were attributed to net microbial methylation and demethylation processes, both within the AMD sediments and downstream.

Kim et al. (2000) reported the mineralogy of mercury mine wastes in five abandoned mine sites in California (Turkey Run Mine, Oat Hill Mine, Corona Mine, Sulfur Bank Mine and Gambonini) to be predominantly quartz with some kaolinite, muscovite, hematite or montmorillonite, depending on the ore. As in the case of the Idrija Mine in Slovenia, although the mercury ore was cinnabar, the host rock consisted of non-sulfide minerals. Although no pyrite was mentioned in any of the Clear Lake studies, AMD was present at least at one of these sites, the Sulfur Bank Mercury Mine, located on the shore of Clear Lake, California. Bulldozing of waste rock into the lake, and erosion of waste rock piles on the lake shore were contributing large amounts of Hg to the lake waters (100 metric tons accumulated since the 1920s). Elevated Hg concentrations were discovered in Clear Lake fish in the 1970s, prompting government action to remediate these problems. After remediation, however, AMD (pH 3) high in Hg and extremely high in sulfate was determined to be continuing to transport Hg from the mine into the lake (Suchanek et al., 2000). Hg content increased in the AMD water as it flowed through waste rock. White floc, formed when AMD water mixed with lake water, was higher in MeHg than lake sediments, indicating that Hg transported in AMD was likely more available for methylation by microorganisms than Hg in waste rock. Further, the high sulfate content of the AMD waters would help stimulate growth of SRB in lake sediments.

The only reported Hg values in waters downstream of a sulfidic, non-Hg mine were in stream water draining the highly oxidized Cu-Zn-Pb tailings of the Cleveland Mill in New Mexico (Boulet and Larocque, 1998). The water had pH $\geq$ 2.15 and contained 50 nM Hg_T, comparable to the highest values in stream water receiving AMD from the New Idria Hg mine in California.

1.3.2 Gold mine tailings

1.3.2.1 Mineralogy

Gold ores commonly consist of quartz-veined greywacke or slate where gold has been deposited as native gold metal (sometimes visible) or in other minerals, especially arsenopyrites, at the boundaries of the quartz veins. Although gold ores contain some sulfide minerals, tailings produced from these ores do not produce much acid because the bulk of the host rock is composed of silicate or carbonate minerals (quartz, quartzite, calcite, adularia) (Carillo-Chavez et al., 2003; Ramirez-Requelme et al., 2003; Nriagu and Wong, 1997). Heavy metals are not mobilized to the same extent as in sulfidic mine wastes and dissolution of most metals is controlled by adsorption to Fe-oxyhydroxides (Carillo-Chavez et al., 2003).

Nova Scotia gold deposits are part of the Meguma Group rocks. "Lode" gold is found in close association with auriferous quartz veins that lie within slate, quartzite or greywacke (Bates, 1987). Arsenopyrite is the most abundant mineral in the ore, occurring as isolated

crystals in quartz, quartzite and slate, as well as massive in quartz; minor amounts of pyrite, pyrrhotite, chalcopyrite, galena, sphalerite, calcite and feldspar are also present (Roach, 1937; MacKenzie, 1907).

Since Au is often associated with arsenopyrites, arsenic is a common contaminant originating in gold mine tailings (Savage et al., 2000; Wong et al., 1999). Other arsenic-bearing minerals that may be found in gold ores and tailings include iron (III) oxyhydroxides, scorodite ($FeAsO_4 \cdot 2H_2O$), ferric arsenates, arsenosiderite ($Ca_2Fe_3(AsO_4)_3O_2 \cdot 3H_2O$), Ca-Fe arsenates, pharmacosiderite $KFe_4(AsO_4)_3(OH)_4 \cdot 6–7H_2O$) and jarosite ($K_2Fe_6(SO_4)_4(OH)_{12}$) (Paktunc et al., 2004).

1.3.2.2 Mercury in gold mining

Unlike Hg mines, the Hg associated with Au mine wastes does not originate in the ore, which usually contains negligible Hg content. For example, the range of bedrock Hg concentrations from three Nova Scotia gold mines was 0.1-4 μmol kg^{-1} (Wong et al., 1999). Early in the history of gold mining in Nova Scotia, gold ores were of high grade and often contained visible native gold, often in association with arsenopyrite minerals (Goodwin, 1935). Native gold could be extracted by mercury amalgamation. Gold ore was crushed and flushed over a surface covered with liquid mercury so that it would form an amalgam, which was collected and heated to volatilize the mercury, leaving behind the gold. Non-gold-bearing particles would be carried to a waste stream. In historical times, much of the Hg used in this process was lost to the environment. Wong et al. (1999) reported 5.5 to 32.4

μmol kg^{-1} Hg in tailings at the Goldenville mine site, Nova Scotia, an estimated 6800 kg Hg in the total mass of tailings. Wayne et al. (1996) reported that approximately 7100 metric tons of metallic Hg were released into the Carson River-Lahontan Reservoir watershed in Nevada over a 30 year period beginning in 1860 from the Comstock Lode Ag-Au mine. Regrettably, liquid Hg continues to be used in modern-day artisanal gold mining in many developing countries. For example, Hruschka (1995) reported that the activity of up to 100,000 artisanal miners in the Ecuadorian Amazon region results in the emission of approximately 50 tonnes of Hg annually, much of which is lost to tailings that contain 0.5-5.0 mmol kg^{-1} Hg_T. Tailings from artisanal mining in the Kenyan Migori Gold Belt were found to contain up to 9.6 mmol kg^{-1} Hg_T (Ogala et al., 2002), and approximately 30% of Hg used in artisanal gold mining in the Andacollo mining district of Chile is lost to the environment (Higueras et al., 2004).

Lower grade ores contain gold in lower concentrations, locked in minerals - primarily arsenopyrite. In order to extract gold from arsenopyrites, these minerals were first separated, or "concentrated", from the bulk ore. Concentrates were then treated with cyanide to dissolve sulfide minerals so that gold could be extracted. Tailings from this process contain very little Hg.

The mineralogy of gold tailings reflects gangue materials, for example, quartz, feldspar, calcite, dolomite, siderite, chlorite, kaolin and small amounts of pyrite (Carillo-Chavez et al., 2003; Praharaj and Fortin, 2004; Blowes et al., 1998). Any acid generated by the oxidation of pyrite is neutralized by buffering reactions with calcite and feldspar. As a result, although

metals may be abundant in the tailings, they are present as solids and not mobilized into porewaters (Carillo-Chavez et al., 2003).

1.3.2.3 Porewater geochemistry

Fortin et al. (2002 and 2000) reported that porewaters from the Hollinger and Delnight Au mine tailings were slightly oxidized to slightly reducing, pH was near neutral (6-8) and porewaters contained ≤ 0.1 mM Fe. Sulfate was 1.0-2.6 mM and DOC & dissolved inorganic carbon (DIC) reached maxima of 4.2 & 2.9 mmol C L^{-1}, respectively. Hollinger porewaters were saturated with respect to various Fe-oxides, including magnetite, hematite, ferrihydrite, lepidocrocite, goethite, schwertmannite and Na-jarosite throughout the depth profile, but undersaturated with respect to sulfate-rich minerals, e.g. melanterite, anhydrite, gypsum. The saturation index of Na-jarosite indicates this mineral was being formed, i.e. mineral precipitation could be responsible for removal of sulfate from porewaters. Gold tailings were much poorer in Fe than sulfidic tailings; pyrite was only slightly depleted and Fe-silicates slightly enriched in the oxic layer (Fortin et al., 2002). The Fe-pyrite fraction was smaller than the Fe-silicate and Fe-reactive fractions in both Hollinger and Delnite gold mine tailings (Fortin et al., 2000).

Some gold tailings contain more sulfide minerals than others, however, therefore although gold tailings are usually pH near-neutral, some can have pH as low as ~ 4 with coincidentally higher concentrations of metals such as Cu, Zn, Pb, etc. in porewaters (Londry and Sherriff, 2005).

1.3.2.4 Downstream mercury contamination

Most often, contamination by Hg and other metals from Au tailings is caused by erosion of pH neutral amalgamation tailings from which Hg is carried downstream on particulates, rather than in dissolved form (e.g. Wayne et al., 1996). The amount of contamination depends on the efficiency of extraction techniques used at the time of processing (Higueras et al., 2004). Lechler et al. (1997) found that the proportion of Hg^0 in river water increased from near zero to over 90% as the water coursed along a channel cut through eroded mine tailings. The proportion of Hg^0 in the eroded tailings was 95%. Hg^0 dissolved slowly and was readsorbed onto clays, organic matter and Fe-and Mn-hydroxides, which were deposited downstream in lower-energy reaches of the river.

Despite high levels (up to 32.4 μmol kg^{-1}) of Hg_T in tailings, Hg_T in streamwater from Goldenville (Nova Scotia) was below 250 pM (Wong et al., 1999). Suspended particulates in the same stream water contained 0.5-4.8 μmol kg^{-1} Hg_T.

1.3.2.5 Microbiology

SRB were recovered from several sites sampled at the Hollinger mine site (Fortin et al., 2002; Fortin et al., 2000) and Delnite gold mine tailings contained up to 10^7 CFU g^{-1} dry wt of sediment of SRB (Fortin et al., 2000).

Londry and Sherriff (2005) investigated microbial biomass, biodiversity and biogeochemistry in three gold mine tailings deposits in Manitoba, Canada. They reported that microbial activity influenced the distribution of C, S (total, sulfide, sulfate) and Fe (total, Fe(II)) in the tailings. In vegetated tailings, biomass and biodiversity were greatest near the surface, whereas in barren tailings, the greatest biomass and biodiversity were observed at ~ 15 cm depth. Water movement and hydrology were determined to influence biomass and biodiversity more than other physicochemical conditions. Geochemical parameters (reduced, dissolved Fe and sulfide) indicated the likely presence of both Fe- and sulfate-reducers. Culturing experiments confirmed the presence of both types of microbes, as well as methanogens. FAME (fatty acid methyl esters) analysis provided evidence of Gram-positive bacteria in the surface layers of all three sites, but not consistently at depth, whereas Gram-negative bacteria, although found at the surface of all three sites, were more abundant in the middle layer at one site (Gunner – partially revegetated).

Blowes et al. (1998) found that the most abundant species of *Thiobacillus* in carbonate-rich gold mine tailings was neutrophilic bacteria of the *Thiobacillus thioparus* type, in spite of the observation of a zone of sulfide mineral oxidation at the Agnico-Eagle gold mine tailings impoundment in Joutel (Quebec).

1.4 LIMITATIONS OF PREVIOUS RESEARCH

The above literature review shows that although Hg can be transported both from well-buffered, pH-neutral tailings (e.g. Idrija mine Hg tailings, pH-neutral gold mine tailings) and

from sulfidic tailings (e.g. New Idria mine Hg tailings), the primary means of transport from pH-neutral tailings is erosion of particulate matter, whereas AMD dissolves minerals containing Hg and other metals. Although particles may partially dissolve as they are carried downstream, they settle out in less energetic environments, and the bioavailability of Hg in particles depends on the conditions where they settle. On the other hand, when metal-laden AMD courses through channels containing mine tailings or waste rock, it appears to continue to accumulate dissolved metals, including Hg, before being discharged into larger streams or water bodies. Acidic conditions, then, may be a greater predictor of Hg bioavailability than high Hg concentrations in tailings. No studies investigating the mineralogy of sulfidic base-metal tailings have mentioned cinnabar, therefore Hg is probably present in association with other minerals that may be more soluble than cinnabar. For example, up to 55 μmol kg^{-1} Hg was present in sphalerite clasts from the Kidd Creek Cu-Zn-Pb mine in Timmins, Ontario (Hannington et al., 1999). Under acidic conditions, these minerals would dissolve, releasing any associated Hg into porewaters and making it bioavailable for methylation.

Since SRB have been recovered from the acidic, oxic layers of sulfidic mine tailings, and SRB are known to be capable of methylating Hg, it is important to determine whether significant amounts of MeHg are produced in these environments, and to identify the specific type or types of bacteria that are the methylating agents. Several studies detected SRB in sulfidic tailings by most-probable-number cell count estimation, but no molecular analysis has been done to positively identify the species present in these environments.

Further, no studies have quantified the amount or distribution of Hg_T or MeHg in sulfidic, base-metal tailings.

Although the microbial ecology of sulfidic tailings has been investigated rather extensively due to the microbial acceleration of sulfide mineral oxidation causing AMD, relatively few studies have been conducted on the microbial ecology of pH-neutral gold mine tailings. Although several studies assess the downstream impacts of gold mines by investigating stream sediment and water MeHg concentrations, only one study examined Hg methylation in gold tailings dumps. Bloom (2000) investigated Hg methylation potential by conducting laboratory studies with Au and Hg mine tailings. Results showed that whatever the initial form of Hg in tailings, significant methylation eventually takes place.

1.5 THESIS ORGANIZATION

1.5.1 Objectives

A. To help further the understanding of mine tailings as potential sources of Hg contamination, by:

- determining concentrations and distribution of total Hg and MeHg in surficial mine tailings;
- investigating the mobility of Hg_T and MeHg into liquid phases (porewaters); and
- measuring Hg_T and MeHg concentrations in receiving waters.

B. To elucidate mechanisms of Hg methylation and accumulation of MeHg in tailings by:

- comparing Hg concentrations with other geochemical parameters;
- measuring SRB abundance and distribution in tailings;
- culturing SRB from tailings; and
- conducting molecular analysis of SRB DNA from field samples and enrichment cultures to identify specific SRB species that may be implicated in Hg methylation in mine tailings.

C. To compare Hg concentrations, distribution and methylation in sulfidic, base-metal tailings and pH-neutral gold mine tailings.

1.5.2 Thesis overview and null hypotheses

This thesis presents three research papers (chapters 2-4) that collectively address the objectives outlined above.

Chapter 2 reports the investigation of total and methyl mercury concentrations and distribution with depth in sulfidic, base-metal tailings from the area surrounding Timmins, Ontario, and compares these data with geochemical parameters, abundance and depth distribution of sulfate-reducing bacteria. Chapter 3 describes a similar study to Chapter 2, but addresses historical gold mine tailings in Nova Scotia. The null hypotheses for chapters 2 and 3 are as follows:

H_{01}: Concentrations of MeHg are not influenced by Hg_T levels in tailings and porewaters.

H_{02}: MeHg distribution in sulfidic, base-metal tailings and Au tailings does not correlate with the distribution or activity of sulfate-reducing bacteria.

H_{03}: MeHg and total Hg are present in higher concentrations (in the solid and aqueous phases) in Au tailings than in sulfidic, base-metal tailings.

H_{04}: Hg and MeHg concentrations in surface waters downstream of tailings are not elevated above those specified in the Canadian Water Quality guidelines (Environment Canada, 2005), i.e. 130 pM Hg_T or 20 pM MeHg.

H_{05}: MeHg distribution does not correlate with the organic carbon content of mine tailings.

Chapter 4 examined the microbial community structures of both pH-neutral gold mine tailings and acidic, sulfidic base-metal tailings by culturing SRB from both environments with different electron donors, and cloning and sequencing DNA from field samples and enrichment cultures. It was hypothesized that sulfate reduction is carried out primarily by Gram-positive sulfate reducers of the phylum *Firmicutes* (e.g. *Desulfotomaculum*) in acidic tailings and by Gram-negative sulfate reducers of the phylum *Deltaproteobacteria* in gold mine tailings.

Table 1-1. Mineralogy of primary and secondary minerals in sulfidic mine tailings as reported in published literature.

Category	Mineral	Formula	Source(s)
Primary minerals			
Sulfides:	Pyrite	FeS_2	a, b, c/e, f, g, h, i
	Pyrrhotite	$Fe_{(1-x)}S$	a, b, c/e, g, h, i
	Chalcopyrite	$CuFeS_2$	a, c/e, f, g, h, i
	Sphalerite	ZnS	c/e, f, i
	Galena	PbS	c/e, f, i
	Arsenopyrite	FeAsS	c, f, i
	Pentlandite	$(Fe,Ni)_9S_8$	a, h
	Tetrahedrite	$Cu_{12}Sb_4S_{13}$	c, f, g
Non-sulfides:	Quartz	SiO_2	a, b, c/e, f, g, h, i
	Chlorite	$(Fe,Mg,Al)_6(Si,Al)_4O_{10}(OH)_8$	a, b, c/e, g, h, i
	Biotite	$KMg_3AlSi_3O_{10}(OH)_2$	a, g, h, i
	Talc	$Mg_3Si_4O_{10}(OH)_2$	a, c/e, h, i
	Feldspar	$(Na,K,Ca)Al_{(1-2)}Si_{(3-2)}$	c/e, g, h, i
	Calcite	$CaCO_3$	a, e, f, g, h
	siderite	FeCO3	d, g
Secondary minerals:	Goethite	γ-FeOOH	a, c/e, d, f, h, i, j
	Jarosite	$KFe_3(SO_4)_2(OH)_6$	a, c, d, f, g, h, i, j
	Gypsum	$CaSO4 \cdot 2H_2O$	a, c/e, d, f, h
	Ferrihydrite	$Fe(OH)_3$ or $5Fe_2O_3 \cdot 9H_2O$	a, c/e, d, f
	Lepidocrocite	α-FeOOH	c/e, f, i
	Schwertmannite	$Fe_8O_8(OH)_6SO_4$	f, g, j
	Melanterite	$FeSO_4 \cdot 7H_2O$	c, f

Sources: a - Johnson et al., 2000; b - Fortin et al., 1995; c - Blowes et al., 1991; d - Al et al., 2000; e - Blowes and Jambor, 1990; f - Boulet and Larocque, 1998; g - Dold and Fontboté, 2001; h - Gunsinger et al., 2006; i - Moncur et al., 2005; j - Sidenko and Sherriff, 2005.

Table 1-2. Porewater geochemistry values in sulfidic mine tailings as reported in the literature.

	Variable	Concentration (source)
OXIC ZONE	pH	≥ 2.1(a); 3.8-4.4(d); 1-2(c); 2.1-3.5(g); <1(i); 2.7-3.2(j)
	Eh (mV)	621(d); 625(c); 550-790(j)
	dissolved Fe_T (mM)	175(a); 12.5-346(d); 170(c); 75(h); 2300(i); ≤16,000(j)
	SO_4^{2-} (mM)	250(a); 219-240(d); ≤219 (c); 100(h); ≤2920(i); ≤142(j)
	Cu (μM)	≤205(a); 27-41(d); 2205(c); 4252(h); 25200(i); ≤362(j)
	Zn (μM)	≤115(a); 22000-62480(d); 7500(c); 3460(h); 84230(i); ≤4180(j)
	Pb (μM)	5.3-11.1(d); 386(c); ≤23.2(h); 8(i)
	Cd (μM)	380-477(d); ≤4.4(h); 863(i)
	Ni (μM)	≤9063(a); 112-130(d); ≤65,930(h); 255(i); ≤2760(j)
	Mn (μM)	≤922(a); 14,770-16,160(d); 4736(i)
	Cr (μM)	≤20(a); ≤15(h); 58(i)
	Co (μM)	≤90(a); ≤2650(h); 1870(i)
	Ca (mM)	11.3-11.5(d); 10-15(e); ≤15(j)
	Mg (mM)	82.3-146(d); ≤100(j)
	Na (mM)	3.0-4.8(d); ≤20(j)
	K (mM)	1.0-1.8(d); ≤1.3(j)
	Si (mM)	1.0-1.4(d)
	As (μM)	≤0.3(d); 534(i)
	Cl (mM)	0.5-0.8(d); ≤4.5(j)
	Al (mM)	≤30 (c/e); ≤90(h); ≤270(i)
ANOXIC ZONE	pH	6.3-7.0(a); 6.6-7.0(d); 7(e); ≤8.4(g); 5.8-6.2(j)
	Eh (mV)	94-217(d); 210-710(j)
	dissolved Fe_T (mM)	0.1-7.0(d); 1086(i); 14.6-29.0(j);
	SO_4^{2-} (mM)	38.5-105(d); 954(i); 63.5-135(j)
	Cu (μM)	<0.8(a); ≤1.1(d); 0.6-1.3(j)
	Zn (μM)	<3.1(a); 2.3-6447(d); 1.2-6.4(j)
	Pb (μM)	0.5(a); ≤1.3(d)
	Cd (μM)	0.4(a); ≤7.0(d)
	Ni (μM)	<8.5(a); ≤18.4(d); 29.0-63.0(j)
	Mn (μM)	22.8-1577(d)
	Cr (μM)	<1.0(a)
	Co (μM)	<0.8(a)
	Ca (mM)	9.2-11.3(d); 7.6-10.7(j)
	Mg (mM)	7.2-73.3(d); 44.4-103(j)
	Na (mM)	4.6-63.5(d); 3.8-27.4(j)
	K (mM)	0.6-2.2(d); 2.3-4.8(j)
	Si (mM)	0.1-0.8(d)
	As (μM)	0.3-0.5(d)
	Cl (mM)	0.5-1.5(d); 0.8-9.0(j)
	Al (mM)	nd(h), nd(i)

Sources: a = Johnson et al., 2000; b= Fortin et al., 1995; c= Blowes et al., 1991; d = Al et al., 2000; e=Blowes and Jambor, 1990; f=Boulet and Larocque, 1998; g=Dold and Fontboté, 2001; h=Gunsinger et al., 2006; i=Moncur et al., 2005; j=Sidenko and Sherriff, 2005.

CHAPTER 2. FACTORS AFFECTING METHYLMERCURY ACCUMULATION IN SURFICIAL, ACIDIC, BASE-METAL MINE TAILINGS*

* Adapted from Winch S., Praharaj T., Fortin D. and Lean D. (in prep.)

2.1 INTRODUCTION

Methylmercury (MeHg) is the most toxic form of Hg commonly found in the environment. It is the form of Hg that is of greatest concern for the environment and human health, as it is highly toxic and bioconcentrates up the food web, eventually accumulating in fish, top predators and humans (USEPA, 2001; Clarkson, 1990; Wolfe et al., 1998). It is produced when a methyl group bonds to Hg^{2+}

$$CH_3^- + Hg^{2+} \rightarrow CH_3Hg^+$$

in a reaction widely believed to be catalyzed by sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985). Zones of sulfate reduction in sediments have frequently coincided with zones of MeHg production in wetlands (Branfireun et al., 1999), estuarine sediments (Compeau and Bartha, 1987), marine sediments (Hammerschmidt and Fitzgerald, 2004) and salt marsh sediments (Langer et al., 2001).

Mining wastes cover vast land areas in North America and throughout the world, but very little information exists regarding Hg methylation in mine tailings. Some studies have documented the downstream environmental impact of Hg originating in tailings from gold and mercury mines (Wong et al., 1999; Wayne et al., 1996, Suchanek et al., 2000), showing significant contamination. The area surrounding Timmins, Ontario, Canada, is one of the most heavily mined parts of Canada for metals such as Cu, Zn, Pb, Cd, Sn, Au and Ag. Hg concentrations over 50 mmol Hg kg^{-1} have been measured in massive sulfide ores (Cu-Zn-Pb) from the Timmins area (Hannington et al., 1999). No information is available regarding total Hg (Hg_T) concentrations in tailings produced from sulfidic base-metal ores. It has

repeatedly been demonstrated, however, that active populations of SRB are present in the tailings produced from these ores, despite the conventional understanding that SRB thrive only under reducing, near neutral-pH conditions (Praharaj and Fortin 2004; Fortin et al., 2000; Fortin et al., 2002).

This study examined MeHg concentrations in tailings, as well as chemical and microbial conditions that could influence MeHg production in sulfide-rich, base-metal tailings, in order to determine the potential for these tailings to be a significant source of Hg and MeHg to downstream ecosystems. Depth profiles of MeHg and Hg_T were compiled for surficial tailings and porewaters at four sites in the Timmins area and compared with organic content, *in situ* abundance and activity of SRB, pH, Eh, SO_4^{2-}, dissolved organic matter (measured as dissolved organic carbon – DOC) and organic content. Total Hg and MeHg concentrations were also measured in nearby surface waters. Our main hypotheses were that concentrations of MeHg would be higher at sites having higher Hg_T in tailings and porewaters, but the depth distribution of MeHg would follow trends in SRB abundance and activity.

2.2 MATERIALS AND METHODS

2.2.1 Environmental Setting

Tailings were sampled from three abandoned tailings dump sites (Kam Kotia, Broulan and Potter), and one operational metallurgical facility (Kidd Metallurgical Division) in May, 2003 and August, 2004 (Table 2-1). Since previous studies on the same mine tailings

(Praharaj and Fortin, 2004) have shown that microbial sulfate reduction is significantly controlled by temperature and, hence, is season-dependent, tailings were sampled in the spring 2003 and summer 2004 to investigate seasonal variations in MeHg production. In 2004, however, conditions prevented sampling in the immediate vicinity of the 2003 pits at three of the four sampling sites, therefore seasonal comparisons could not be made (except at the Potter site). Mercury and MeHg data from the two sampling dates are therefore presented as separate sets of data.

2.2.2 Sampling

Sampling was limited to the surficial layers of the tailings, just above the water table. 50 mL polypropylene Falcon tubes were used to collect tailings from sampling pits at 5 cm intervals up to ~ 40 cm depth. Tailings samples for Hg_T and MeHg analysis were scooped directly into the tubes, which were immediately sealed and placed on ice, frozen within three hours and kept frozen until use. Samples for enumeration of SRB were collected with a sterile metal spatula at 5-cm intervals from the same sampling pit as the Hg_T and MeHg samples. The tailings were immediately packed into sterile glass vials and stored on ice until analysis (1–3 days later). Each glass vial was completely filled with tailings to prevent air infiltration and subsequent oxidation.

In August 2004, plexiglass dialysis chambers called "peepers" (Carignan et al., 1985; Carignan and Lean, 1991) were used to collect porewater for DOC and sulfate analysis. The peepers were acid-washed and fitted with 0.22 μm Gelman HT-200 Tuffryn membrane that

was previously soaked for several days in deionized water. In sealed containers, they were submerged in deionized water and degassed for 5 d with nitrogen, then transported to the field in the sealed containers. The peepers were vertically inserted into visibly saturated tailings and allowed to equilibrate for three weeks. After retrieval, peepers were immediately rinsed with deionized water. Porewater was removed in the field using sterile syringes degassed with nitrogen, injected into previously sterilized, sealed, degassed 20-mL septum vials which were placed on ice, then stored at 4°C. Porewaters were analyzed within 24 hrs for sulfate, and remaining peeper samples were stored at 4°C for DOC analysis.

Since larger quantities of porewater were required for Hg_T and MeHg analysis, acid-washed 250-mL HDPE (Nalgene) bottles were used to collect tailings for extraction of porewater for this purpose. Six bottles of tailings were collected at two to four depths at each sampling site, immediately placed on ice and frozen within three hours. Prior to analysis, samples were thawed and centrifuged at 5000 rpm for 20 min. Porewater was immediately drawn off with a syringe, transferred to a clean 250 mL bottle and re-centrifuged to remove residual solid matter. Water from all samples at a given site and depth was pooled to obtain 10-40 mL for Hg_T and MeHg measurements, then divided into acid-washed 50 mL polypropylene tubes. One tube was acidified with 0.5% trace metal grade HCl for MeHg analysis. A second tube was preserved with 1% BrCl for Hg_T analysis. All samples were immediately stored in the dark at 4°C.

Surface water samples were collected in 2004 only. Those samples to be analyzed for MeHg were collected in duplicate in 1 L HDPE bottles rinsed five times with sample water, and

were acidified immediately in the field with 0.5% concentrated, trace-metal grade HCl. Bottles were sealed inside two ziplock bags and kept at 4°C in the dark until analysed. MeHg samples were not filtered in order to avoid contamination during manipulation. Additional surface water samples were collected in 50 mL acid-washed polypropylene tubes for Hg_T and DOC measurements. Half of the Hg_T samples were filtered (0.45 μm) in the field, then all were preserved with 0.5 mL of bromine chloride (27 g of KBr in 2.5 L of HCl followed by 38 g of $KBrO_3$) as outlined elsewhere (USEPA, 2002). DOC samples were also filtered in the field (0.45 μm Pall Gelman Acrodisk 25 mm syringe filter).

Samples for sulfate reduction rates (SRR) were collected in coring tubes (2 cm internal diameter) as described in Praharaj and Fortin (2004) based on the method of Jorgensen (1978). pH and Eh measurements of tailings and surface waters were taken on site using a VWR portable pH meter with a VWR probe and Corning redox probe, respectively.

2.2.3 Laboratory Analysis

Total Hg and MeHg concentrations in tailings were measured as wet weight and normalized to dry weight based on percent water content, to avoid loss of Hg in the drying process and determine the relative proportions of Hg_T and MeHg present in solid and liquid phases.

Total Hg in tailings was measured by high temperature combustion using an NIC automatic Hg analyzer (Nippon SP-3D; MDL = 0.1 ng). Standards were made from 5 mmol Hg L^{-1} HgCl stock solution in 0.1 M nitric acid diluted to 5 μmol Hg L^{-1} and 0.25 μmol Hg L^{-1} in

0.001% cysteine. All samples were analyzed at least in duplicate with relative standard errors (standard error/mean; RSE) typically between 1 and 15%.

MeHg was extracted from tailings using the solvent extraction-GC/AFS method described elsewhere (Cai et al., 1997). All samples were analyzed at least in duplicate with RSE typically between 5 and 20%. Recoveries of MeHg were determined from duplicate samples from one depth at each sampling site spiked with 0.75 ng MeHg. Mean recoveries of 77% (n = 8; CV = 15%) and 85% (n = 7; CV = 10%) were obtained for 2003 and 2004 samples, respectively.

MeHg analysis of porewater and surface water samples was carried out by GC/AFS after solid-phase extraction onto sulfhydryl cotton columns and acidic potassium bromide elution (Cai et al., 1996). Recoveries were 77% for porewaters and 94% for surface waters. MeHg in bottle blanks and travel blanks was below our detection limit of 0.02 ng L^{-1}.

Total Hg in porewater and surface water samples was measured using a Series 2600 Tekran Hg analysis system according to the dual stage gold pre-concentration method (modified USEPA method 1631, MDL = 0.2 ng L^{-1}). Porewater samples were diluted in DIW containing 1% BrCl at a ratio between 1:2 and 1:400, depending on Hg concentration. Immediately prior to analysis, 5 μL of hydroxylamine (previously bubbled with Ar) was added per mL of sample or standard. Peak areas from blanks of DIW containing 1% BrCl were subtracted from sample and standard peak areas prior to calculation of Hg_T concentration.

Organic content of tailings was estimated by loss on ignition (LOI) after drying and heating to 400°C for 8 hr. Since most of the tailings samples were oxidized and acidic, their carbonate content was assumed to be insignificant (Fortin et al., 2002; Jurjovec et al., 2002), therefore LOI values were used to estimate organic carbon content.

DOC in porewater and surface water samples was measured using an IO Analytical 1010 total organic carbon analyzer (persulfate oxidation method; DL = 0.2 mg L^{-1}), and potassium biphthalate standards. Where necessary, samples were diluted in DIW for sufficient volume, and placed into acid-washed glass vials. Peaks for filtered DIW blanks were subtracted from sample peaks. Porewaters were analysed for sulfate by the $BaSO_4$ turbidimetric method.

SRB were enumerated using the most-probable-number (MPN) method, based on the method set out in Cochran (1950), as described in Praharaj and Fortin (2004). Briefly, ten-fold dilution series of all samples were made in culture tubes, starting with approximately 1 g of wet tailings in 9 ml of reduced water containing 36 mM NaCl. From each dilution of each sample, five culture tubes containing 9 mL of sterile, reduced, modified Postgate's medium with four electron donors (lactate, acetate, formate and pyruvate) received 1 mL of inoculum. All solutions were adjusted to pH 7.5 with 2 M NaOH. Tubes were incubated at room temperature in the dark and growth was determined by the presence of black precipitate in the tubes. MPN values were calculated from statistical tables (Cochran, 1950) and expressed in colony forming units per gram dry weight of sediment (CFU g^{-1} dry wt sed).

SRR were measured based on the method of Jorgensen (1978), as described in Praharaj & Fortin (2004). Cores collected in the field were injected with $^{35}SO_4^{2-}$ and incubated 24 h, then sectioned into 2 cm segments, fixed in Zn-acetate and frozen until use. Sulfide precipitates were distilled with HCl and reduced chromium. Produced H_2S was trapped in Zn-acetate and measured by liquid scintillation. SRR were then back-calculated based on radioactivity, as described in Jorgensen (1978).

2.3 RESULTS AND DISCUSSION

Depth profiles of porewater parameters, SRB cell count estimates and SRR for Potter, Broulan, Kam Kotia and Kidd are presented in Figures 2-1, 2-2, 2-3 and 2-4, respectively. 2003 values for pH, Eh, organic content, sulfide, sulfate, SRB abundance and SRR were previously reported in Praharaj & Fortin (2004), and are presented in Table 2-2.

2.3.1 Porewater chemistry and microbial characteristics

The 2004 Potter tailings results (Figure 2-1) show an increase in pH from 2.9 at 0-5 cm to near-neutral conditions below 20 cm depth, and a corresponding decrease in Eh (565 mV dropping to 58 mV). These trends coincide with increases in SRB abundance (max. 277,200 CFU g^{-1} at 30-35 cm) and SRR (max. 473 nmol cm^{-3} d^{-1} at 17 cm). Sulfate concentrations reached a maximum of 96.4 mM at 0-5 cm and were more depleted at depth, as were concentrations of DOC. Organic content was highest near the surface (4.5% and 4.6% at 0-5

cm and 5-10 cm, respectively), dropping to below detection limits at depths greater than 20 cm. 2003 values were comparable, but organic content did not increase near the surface and SRR were lower than in the summer of 2004 (Praharaj and Fortin, 2004).

At Broulan, the 2004 tailings were strongly oxidizing and acidic in the uppermost layer (pH 4.4, Eh 507 mV), but pH increased to near-neutral ($\geq$ 6.8) below 5 cm, and Eh dropped steadily, reaching 267 mV at 20-25 cm, reflecting the layer of Au tailings underlying the Cu-Zn tailings (Figure 2-2, Table 2-1). Sulfate concentrations were on the same order of magnitude as Potter (max. 44.3 mM). SRB were less abundant at Broulan than at the other sites (592-3433 CFU g^{-1} dry wt.), but SRR were comparable to values for Potter (max. 439 nmol cm^{-3} d^{-1} at 17 cm). In 2003, tailings were strongly oxidized (visibly bright orange) and acidic throughout the depth profile, SRR were 2-3 orders of magnitude lower than in 2004 and SRB were not recoverable except in the surface layer (Praharaj and Fortin, 2004).

At Kam Kotia, 2004 tailings featured pH $\leq$ 3 and Eh > 550 mV throughout the depth profile, and a pronounced organic-rich layer at 10-15 cm (max. 42% - Figure 2-3), which was visible as a brown layer of decaying plant material. SRB cell count estimates were highest at this location and peaked with % organic content. DOC followed an inverse trend compared with SRB abundance. SRR were consistently low, spanning from 1.5 to 12.0 nmol cm^{-3} d^{-1} in the top 11 cm, and dropping below detection levels at 15 cm. The tailings at this site were highly acidic and oxidizing throughout, and sulfate levels were lower than at the other sites. The 2003 Kam Kotia tailings featured lower SRB abundance by at least one order of magnitude, and higher sulfate and slightly higher SRR than in 2004. Organic content was more

consistently distributed in the depth profile, with the richest layer again occurring at 10-15 cm (Praharaj and Fortin, 2004).

In 2004, Kidd tailings were also acidic and oxidizing throughout, but less so than Kam Kotia (pH 3.7-5.0; Eh 307-424 mV - Figure 2-4). The top layers were extremely high in sulfate (max. 1.4 M at 0-5 cm). It is likely that the 2004 sampling site contained a surficial layer of unlimed tailings. In the absence of lime, sulfide oxidation is accelerated. More sulfate would have been produced but not precipitated as sulfate-rich minerals (Jurjovec et al., 1995), resulting in high sulfate levels. The high sulfate concentrations were reflected in high SRR (max. 2,771 nmol cm^{-3} d^{-1} at 0-5 cm). SRB abundance was lower in the uppermost layers than at depth (5,817 CFU g^{-1} dry wt. at 0-5 cm vs. 116,700 CFU g^{-1} dry wt. at 20-25 cm), but second highest overall compared with the top acidic, oxidizing layers of other sites. Organic content was negligible throughout the profile, but DOC concentrations were higher than at the other sites. By comparison, the 2003 tailings were more acidic and oxidizing and featured SRB populations that were very similar in number but much less active than in 2004. Sulfate concentrations were also very much lower, particularly in the top 15 cm (40-100 mM - Praharaj and Fortin, 2004).

2.3.2 Methylmercury and total mercury in bulk tailings and porewaters

Most bulk tailings samples, regardless of sampling location or year, contained negligible concentrations of MeHg, even when the corresponding Hg_T was elevated (e.g. Broulan - Figure 2-5). The only samples that contained > 1 nmol kg^{-1} MeHg in the tailings were those

collected at Kam Kotia (max. MeHg = 2.6 nmol kg^{-1} in 2003, 2.9 nmol kg^{-1} in 2004) and the Kidd Metsite (max. MeHg = 11.3 nmol kg^{-1} in 2004). Since Kam Kotia tailings were higher in organic content than any of the other sites, and the 2004 Kidd tailings had extraordinarily elevated levels of sulfate and very high SRR, MeHg appears to coincide with zones of high organic material or sulfate reduction in these tailings.

In Kam Kotia tailings from both 2003 and 2004, Hg_T and MeHg were closely associated with organic layers. In the most organic-rich layer of the 2004 samples, Eh dropped slightly and SRB abundance was maximized. Although Kam Kotia hosted the greatest abundance of SRB and SRB abundance peaked with % organic content, SRR were negligible at this site, likely due to the low pH.

Organic matter has been identified as a key factor in how much Hg_T and MeHg is present in sediments, but it does not necessarily promote methylation, and may even inhibit it or have no effect if the solid organic material immobilizes inorganic Hg, limiting dissolved Hg_T available for microbial methylation (Hammerschmidt and Fitzgerald, 2004). Further, MeHg has been shown to be scavenged by thiol groups in organic material making it non labile (Karlsson and Skyllberg, 2003; Qian et al., 2002). Either one or both of these processes likely decreased Hg_T bioavailability and concentrated MeHg in the organic-rich layer. With Hg_T in Kam Kotia tailings as high as 3.8 μmol kg^{-1} (Figure 2-6), one would expect there to be more Hg_T in the porewaters at this site than anywhere else, but Broulan tailings (max. 31 umol kg^{-1} Hg_T) had much higher Hg_T values for the dissolved phase (max. 8.5 nM) indicating that organic material at Kam Kotia was likely responsible for immobilizing Hg_T

on solid phases. Kam Kotia porewaters also contained a maximum of 7.5 pM MeHg, less than the porewater concentration from Potter (max. 13.3 pM MeHg), where MeHg levels were much lower in the tailings (max. 0.6 nmol kg^{-1} at the surface in 2004; negligible levels in all other Potter samples) than at Kam Kotia. The higher MeHg concentrations measured in Kam Kotia tailings likely resulted from accumulation in solid phase organic material of MeHg produced within or outside of organic-rich layers over long periods. This is consistent with the findings of Bloom et al. (2003), who showed that whether deposited as Hg^0 or Hg(II), eventually ~ 90% of Hg_T from a chlor-alkali plant became bound to soil humic matter.

Despite low Hg_T concentrations at the Kidd Metsite, 2004 MeHg concentrations in both the bulk tailings and the porewater were extremely elevated within the top 15 cm (12.1 nmol kg^{-1} in tailings; 87.9 pM in porewater) (Figures 2-5, 2-6). By comparison, Cossa and Gobeil (2000) measured 5.1-14.4 pM MeHg in Lower St. Lawrence Estuary porewaters. The high MeHg concentrations in Kidd tailings were measured in the zone of high sulfate and high SRR (~ 0-15 cm), which strongly suggests a link between sulfate reduction and Hg methylation in this environment. Surprisingly, however, this zone was the acidic, oxidized top 15 cm. Unlike the Potter site, where all measured parameters were consistent with the presence of a zone of suflate reduction at depth > 10 cm, and only negligible concentrations of MeHg were detected in the bulk tailings, surficial Kidd tailings featured greater sulfate and SRR than oxidized layers from any other site and SRB abundance second only to Kam Kotia.

High concentrations of sulfide promote the formation of charged Hg-S complexes like $HgHS^{2-}$, which are less available to methylating bacteria than HgS^{o}, which tends to predominate at lower sulfide concentrations and more easily passes through bacterial membranes (Benoit et al., 2001). Given the oxidizing conditions that prevailed in the surface layers of the Kidd tailings in 2004, dissolved sulfides would be rapidly reoxidized to sulfate, keeping sulfide concentrations very low, therefore HgS^{o} would likely be more prevalent than $HgHS^{2-}$. If so, more inorganic Hg may have been available for methylation in the surficial layers, even though Hg_T was < 1 umol kg^{-1}.

Broulan porewaters contained up to 8.5 nM and 0.8 nM Hg_T in 2003 and 2004, respectively. These values are 1-2 orders of magnitude higher than other sedimentary environments, e.g. max. 80 pM in Lower St. Lawrence Estuary porewaters (Cossa and Gobeil, 2000).

2.3.3 Methylmercury and total mercury in surface waters

Total Hg and MeHg data for surface waters are presented in Figure 2-7. Filtered concentrations of Hg_T were somewhat lower than in unfiltered samples. Total Hg concentrations were < 50 pM in the Kidd Metsite and Potter samples and all three samples collected in the vicinity of Kam Kotia (standing water from a pond near the sample pit, inflow to Kam Kotia Lake, and a beach site ~ 1 km away from the inflow). These values are all well below the 130 pM maximum stipulated in the Canadian water quality guidelines (Environment Canada, 2005). The lake at Broulan, however, contained high Hg_T in both filtered (838 pM) and unfiltered (961 pM) samples. Conversely, MeHg concentrations were

< 0.5 pM for all samples except the Kidd Metsite, where surface water contained 3.1 pM MeHg. (MeHg could not be extracted from the Kam Kotia standing water sample due to interference by organic matter.) In comparison, Benoit et al. (1998) measured 0.1-1.1 pM MeHg and up to 27.4 pM Hg_T in the upper reaches of the Patuxent River; Sullivan et al. (1998) found average concentrations of 1.6 pM Hg_T and up to 0.21 pM MeHg in Lake Michigan waters; and Cossa and Gobeil (2000) found that Hg_T concentrations in Lower St. Lawrence Estuary water ranged from 1.8 to 7.8 pM. The Canadian water quality guidelines maximum MeHg concentration in fresh waters is 20 pM. Methymercury concentrations, therefore, are not elevated even in Kidd Metsite surface waters, but Broulan surface waters are significantly contaminated with inorganic Hg.

Surface water chemistry data are presented in Table 2-3. There was no observable correlation between MeHg and DOC, or between Hg_T and DOC. DOC values were highest in Kam Kotia Lake, and concentrations were below detection limits in the lake at Broulan.

2.3.4 Relationship between organic matter, microbial sulfate reduction and MeHg formation

At the outset, we hypothesized that MeHg concentrations would be greater in tailings where more Hg_T was available for methylation, and that the depth distribution of MeHg in tailings would follow SRB and SRR. Neither of these trends was consistently observed, owing to the complexities and uniqueness of each individual site. The Broulan site, for example, featured high Hg_T in surficial tailings and porewater in 2003, but very little MeHg. Potter tailings

clearly hosted abundant and active SRB and contained moderate Hg_T concentrations; although porewater MeHg was slightly elevated, MeHg concentrations in the bulk tailings were negligible. MeHg and Hg_T distribution in Kam Kotia tailings was controlled by a prominent organic-rich layer, which also hosted large numbers of SRB that were inactive, probably due to the low pH conditions. The Kidd tailings, although low to moderate in Hg_T content, featured extremely elevated MeHg in both bulk tailings and porewaters in 2004, coinciding with a surficial zone of high sulfate and high sulfate reduction.

Since MeHg reached non-trivial levels in only two sites (Kam Kotia and Kidd), multiple regression analysis did not elucidate clear cause and effect relationships between MeHg concentrations in tailings and any single factor or combination of factors. Qualitative examination of depth profiles at the sites of the anomalously high MeHg concentrations, however, allows some conclusions to be drawn.

First, SRB were confirmed to be present and active in the oxidizing and acidic layers of some sulfidic tailings, as shown in previous studies (Fortin et al., 1995; Fortin et al., 2000; Fortin et al., 2002; Praharaj and Fortin, 2004), although highly acidic conditions (e.g. Kam Kotia, pH ~ 3) deterred sulfate reduction even when cells were abundant. Second, MeHg concentrations were not correlated with Hg_T in either bulk tailings or porewaters, and under the right conditions, MeHg can be surprisingly elevated even where Hg_T is not. These results are consistent with the findings of other studies that examined MeHg and Hg_T in sediments (e.g. Benoit et al., 1998). Third, organic-rich layers trapped both total and methyl mercury. This has also been observed in historic gold mine tailings (Chapter 3). Fourth, elevated

MeHg levels corresponded to high SRR only in an acidic (pH 3.7-4.4), oxidizing environment where sulfate was extremely high. Most SRB are generally active under pH-neutral, reducing conditions (Postgate, 1984). When Fe-reducing bacteria (FeRB) are present, they often outcompete sulfate-reducers for common electron donors (Peine et al., 2000) in the presence of reactive iron as electron acceptor (i.e., Fe(III)-oxides). The levels of reactive Fe (Fortin et al., 2000) and the 2004 Eh conditions in the Kidd tailings describe ideal conditions for Fe-reducers to thrive, indeed to outcompete SRB. Acidophilic or acid-tolerant SRB have, however, been found in acidic mining environments (Sen and Johnson, 1999; Johnson et al., 1993) and other natural habitats (Koschorreck et al., 2003), and evidence of the presence and activity of SRB has previously been observed under oxidizing, permanently acidic conditions (Praharaj and Fortin, 2004). The findings of this study show that when sufficient quantities of sulfate are present in oxidizing and acidic tailings, some types of sulfate reducers may be able to outcompete iron reducers. More importantly, these acid-tolerant SRB could have the capacity to methylate Hg. Although Hg methylation by FeRB has been observed (Fleming et al., 2006), the coincidence of extremely elevated sulfate and MeHg concentrations and very high SRR in the Kidd 2004 samples strongly suggests that sulfate reducers were primarily responsible for Hg methylation at the Kidd Metsite.

With the exception of Kam Kotia, SRB abundance was relatively low, or even nil, in the top layers of tailings, even when SRR were significant, as at the Kidd Metsite and Broulan. This discrepancy indicates that the MPN method employed in this study underestimated SRB populations in acidic, oxidizing conditions. These enumerations involved incubation of

cultures for three weeks under pH-neutral, reducing conditions. It is well known that only a small portion of naturally-occurring SRB can be grown under laboratory conditions (Fortin et al., 2002). If our samples contained acidophilic or acid-tolerant SRB, it is possible that they did not grow well under the incubation conditions, resulting in apparent low SRB abundance in oxidized, acidic tailings such as those at the Kidd Metsite and Broulan. Further, if sulfate reducers that survive in these conditions grow very slowly, three weeks may not have been sufficient time to observe growth. Additional experiments were later conducted using acidic medium; SRB enrichment cultures indeed took approximately three months to become established (unpublished data).

The elevated MeHg concentrations in the Kidd 2004 tailings raise the question why demethylation rates would not have increased to balance the elevated production of MeHg. Demethylation has been shown to be carried out by Hg-resistant bacteria, which dominate microbial communities in Hg-contaminated sediments (Nazaret et al., 2003). Kidd tailings would not likely have contained sufficient Hg_T for Hg-resistant bacteria to be abundant, allowing MeHg to accumulate to the observed levels.

Although Broulan tailings did not contain much MeHg, the surficial layers contained far more Hg_T than the underlying tailings. This could be interpreted to mean that the Pb-Zn tailings contain more Hg than the underlying Au tailings, but this is not supported by the observations made in 2003, where pH and Eh data show that throughout the depth profile the tailings were oxidized Pb-Zn tailings (Praharaj and Fortin, 2004), but Hg_T levels were elevated only in the top ~10 cm. Hg may have been transported over time from greater

depths to surface tailings. Alternately, tailings deposited at different times may have contained different levels of Hg_T. The latter seems a more plausible explanation, since the Pb-Zn ores processed at the Broulan facility were those extracted from Kidd Creek when it first began operations as an open-pit mine (Ecstall, 1974). These ores contained variable Hg concentrations averaging 14 mmol kg^{-1}Hg and ranging up to 55 mmol kg^{-1} Hg (Hannington et al., 1999).

Broulan Au tailings contained very little Hg or MeHg. Elsewhere, Au tailings have been found to contain much higher levels of Hg (e.g. 5.5-32.4 mmol Hg kg^{-1} – Wong et al., 1999). The low Hg content of the Broulan Au tailings is due to the extraction process used, i.e. flotation and/or cyanidation rather than Hg amalgamation (Dutrizak and Chen, 1979). Au ores from the same deposit produced from the nearby Schumacher, Hollinger and Ross mines and processed at the Pamour Porcupine mill contained approximately 0.10 mmol Hg kg^{-1}(Dutrizak and Chen, 1979).

The presence of 81.8 pM MeHg in porewater near the surface of Kidd tailings is clear evidence that when MeHg levels are sufficiently high in the tailings, environmentally significant quantities of MeHg can be found in mobile, liquid phases, raising the possibility that MeHg is transported to surface waters. Surface water concentrations of Hg_T and MeHg followed the same trends as in the adjacent tailings, with Broulan having highest Hg_T (0.8 nM in surface water in 2004; max. 0.9 nM in porewater) and low MeHg in both surface and porewaters, and the Kidd Metsite having the highest MeHg (2.9 pM in surface water; max. 82 pM in porewater) and low Hg_T.

2.4 CONCLUSIONS

In summary, MeHg concentrations in acidic, oxidizing layers of sulfidic base-metal tailings exceeded 1 nmol kg^{-1} only in organic-rich layers or in the presence of extremely elevated sulfate concentrations (350-1406 mM). SRB are the most likely agents of Hg methylation, but pH-neutral, reducing conditions are not necessary for this process to take place. SRR appeared to be deterred by pH less than ~ 3.5. MeHg concentrations in bulk tailings and porewaters were not correlated with Hg_T, and elevated MeHg levels corresponded to high SRR only in an acidic (pH 3.7-4.4), oxidizing environment where sulfate was extremely high. This suggests the existence of acid-tolerant or acidophilic SRB that methylate Hg and can survive under oxidizing conditions, but further research is required to isolate and identify SRB from this type of environment and test their capacity to methylate Hg. If such an organism were to be identified, it might serve as an indicator for Hg methylation potential in acid mine drainage situations.

Overall, most sulfidic tailings and porewaters sampled were not high in MeHg and therefore could not be considered a source of MeHg to downstream waters. This study showed, however, that some exceptions exist, particularly where organic content or sulfate concentrations are high. In general, organic content can be expected to be greatest in tailings that were deposited on vegetated land surfaces such as forests and wetlands (particularly near the tailings-soil boundary, e.g. Kam Kotia), and lowest where tailings have been poured into lakes or on top of previously-existing tailings piles (e.g. Broulan, Kidd). Sulfate

concentrations are likely to be higher in unlimed tailings. Any plans to disturb, re-extract or relocate sulfidic tailings should take into account the levels of organic material and sulfate in the tailings and the resultant probability of elevated MeHg.

Even where elevated MeHg was detected in the tailings and porewaters, nearby surface water concentrations were within the range of values measured in other environments. Surface and porewaters from the Broulan sample site, however, were highly contaminated with Hg_T. MeHg and Hg_T levels in surface waters reflected patterns of MeHg and Hg_T in nearby tailings, indicating that MeHg and Hg_T in tailings influenced surface water concentrations.

Table 2-1. Sampling sites in the vicinity of Timmins, Ontario, Canada.

Site	Type	Status	Environmental situation	May 2003	August 2004
Kam Kotia	Cu-Zn	Abandoned	- tailings released onto low-lying, bog-type land - currently under remediation (lime addition) to address acid mine drainage problem	- oxidized tailings overlying soil layer at ~ 17 cm; organic layer at ~ 12 cm	different location from May 2003 - oxidized layer to ~ 15 cm; highly organic layer at ~ 17 cm; - visible evidence of liming in un-oxidized layer
Potter	Cu-Zn	Abandoned	- tailings released onto low-lying, bog-type land - plans underway to re-extract tailings	- oxidized surface layer (~ 20 cm thick)	- same location (within 5 m) as May 2003 sampling; similar characteristics
Broulan	Pb-Zn over Au	Abandoned	- tailings released into lake - Pb-Zn tailings from Kidd Creek Mine north ore body processed in pilot plant in 1965, deposited on top of older Au tailings (Ecstall, 1974) - tailings likely to be removed to accommodate future Au mining	- oxidized Pb-Zn tailings; visibly orange coloured throughout depth profile	different location from May 2003 - oxidized (orange) Pb-Zn tailings overlying grey Au tailings (~ 10 cm)
Kidd Met	Cu-Zn and/or Pb-Zn	Operational facility	- processes ore from Kidd Creek Mine - very large tailings area (1200 ha – Hannington et al., 1999) - tailings released onto previously forested land - lime added to tailings	- surface oxidized, acidic layer (≤ 15 cm thick); less acidic tailings underneath - This area used for H_2SO_4 production	- different location from May 2003

Table 2-2. Summary of porewater chemistry and sulfate- reducing bacteria (SRB) abundance in 2003 tailings from Potter, Broulan, Kam Kotia and the Kidd Metsite (Praharaj and Fortin, 2004).

Parameter	**Potter**	**Kidd**	**Kam Kotia**	**Broulan**
pH	2.5-7	2-5	2-3	2-4
Eh (mV)	0-800	200-700	300-600	500-700
HS^- (uM)	1-13	1-12	3-4	1-12
SO_4^{2-} (mM)	10-50	0-600	80-110	0-110
% organics	1-7	< dl - 1.6*	0-80	0-3
SRB (CFU g^{-1} dry wt. sed)	< dl - 10^8	10^2-10^7	< dl - 10^{10}	< dl - 10^6
SRR (nmol cm^{-3} sed d^{-1})	1-58	1-8	2-11	0.4-7

*one additional sample at 9%.

Table 2-3. Chemical parameters of surface waters near the mine tailings dumps.

Sample location	**pH**	**Eh (mV)**	**DOC (mM C)**
Broulan lake water	3.38	303.1	< dl
Potter standing water	2.83	481.3	0.1
Kam Kotia standing water	3.18	486.9	0.5
Kam Kotia lake inflow	6.9	216.7	1.6
Kam Kotia lake beach	7.38	167.8	1.7
Kidd Metsite drainage ditch	7.09	103.2	0.4

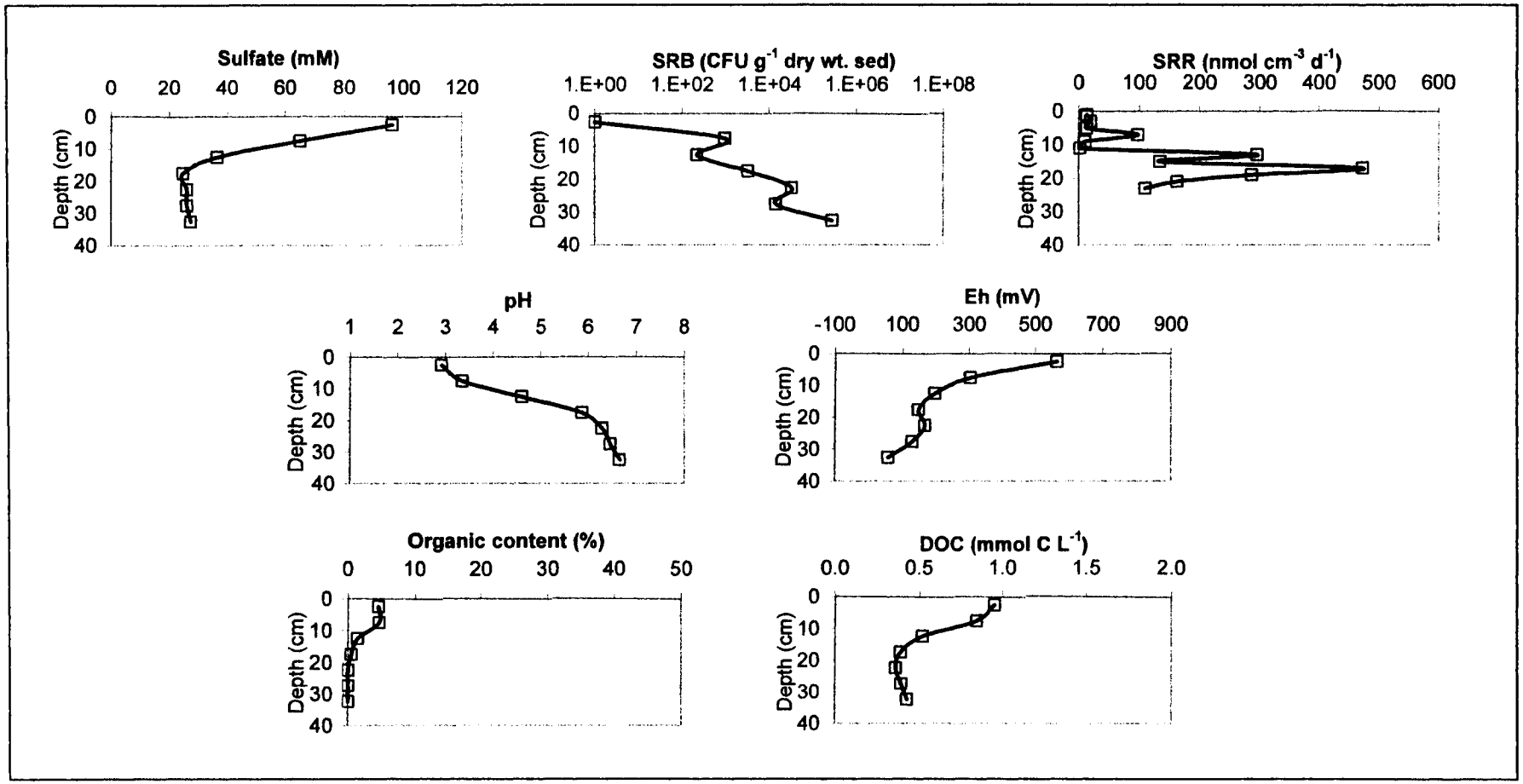

Figure 2-1. Sulfate concentration, sulfate-reducing bacteria (SRB) abundance, sulfate reduction rates, pH, Eh, organic carbon content and dissolved organic carbon (DOC) in tailings and porewaters from the Potter mine site in 2004.

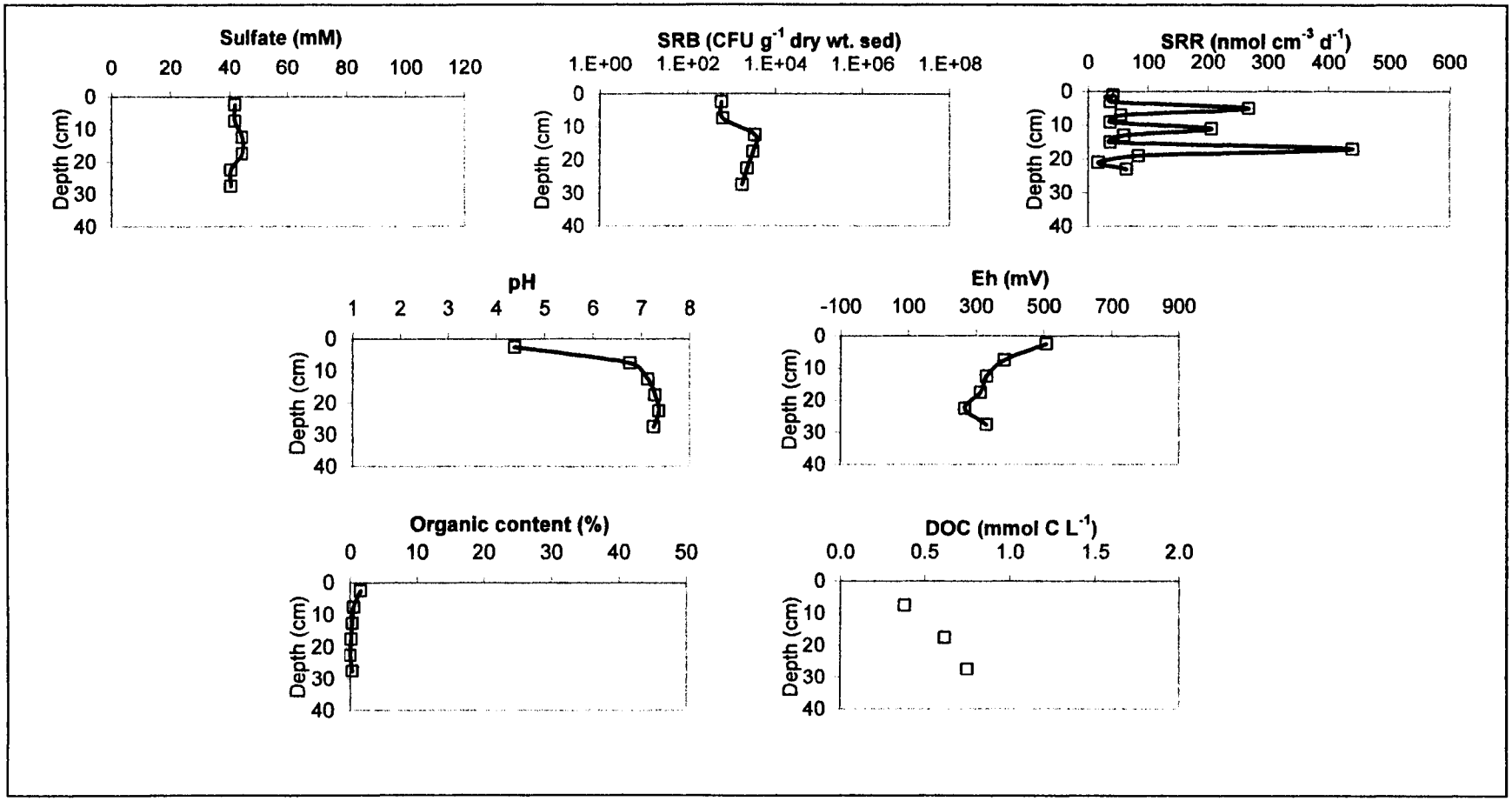

Figure 2-2. Sulfate concentration, sulfate reducing baceria (SRB) abundance, sulfate reduction rates, pH, Eh, organic carbon content and dissolved organic carbon (DOC) in tailings and porewaters from the Broulan mine site in 2004.

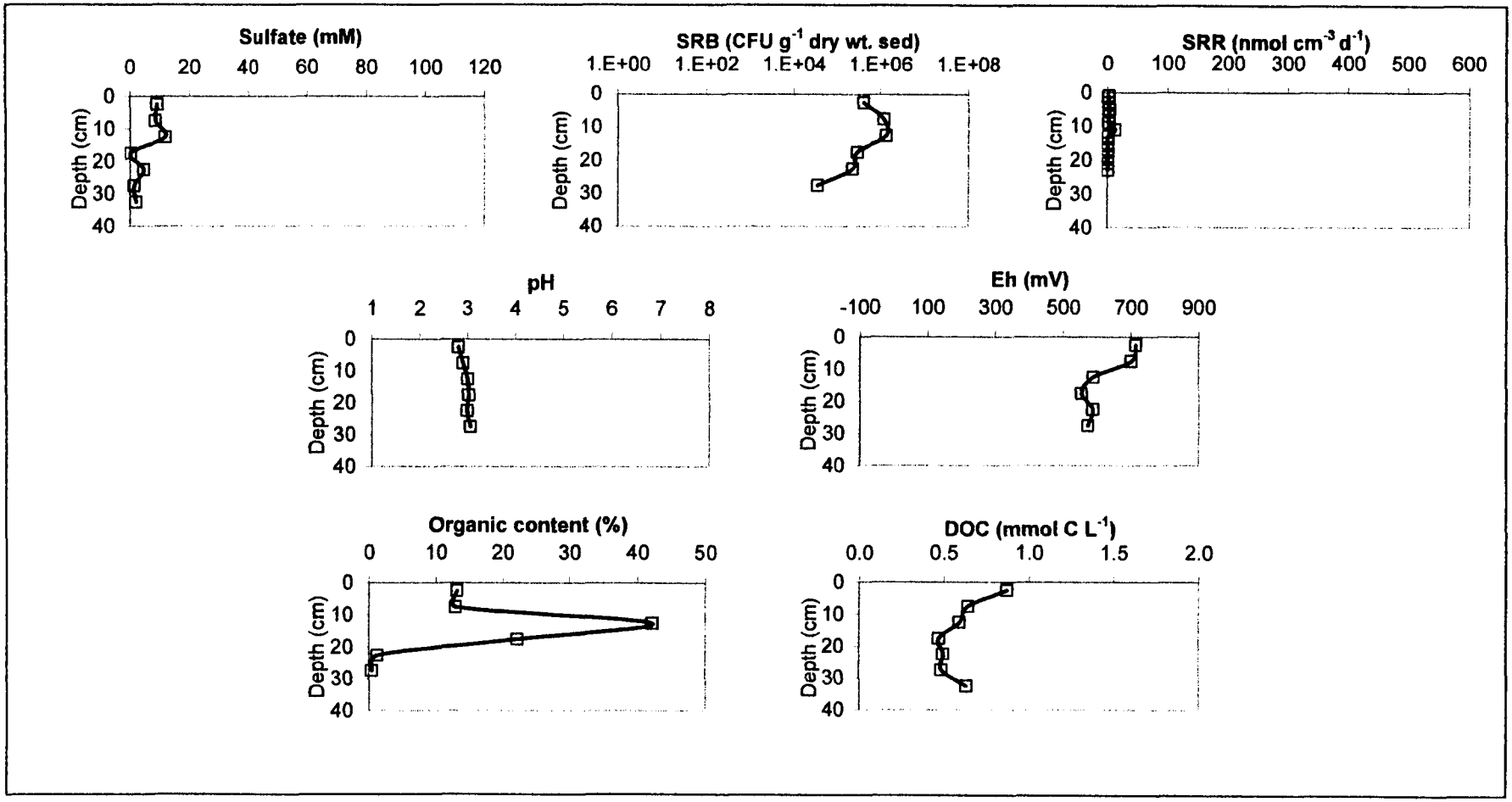

Figure 2-3. Sulfate concentration, sulfate-reducing bacteria (SRB) abundance, sulfate reduction rates, pH, Eh, organic carbon content and dissolved organic carbon (DOC) in tailings and porewaters from the Kam Kotia mine site in 2004.

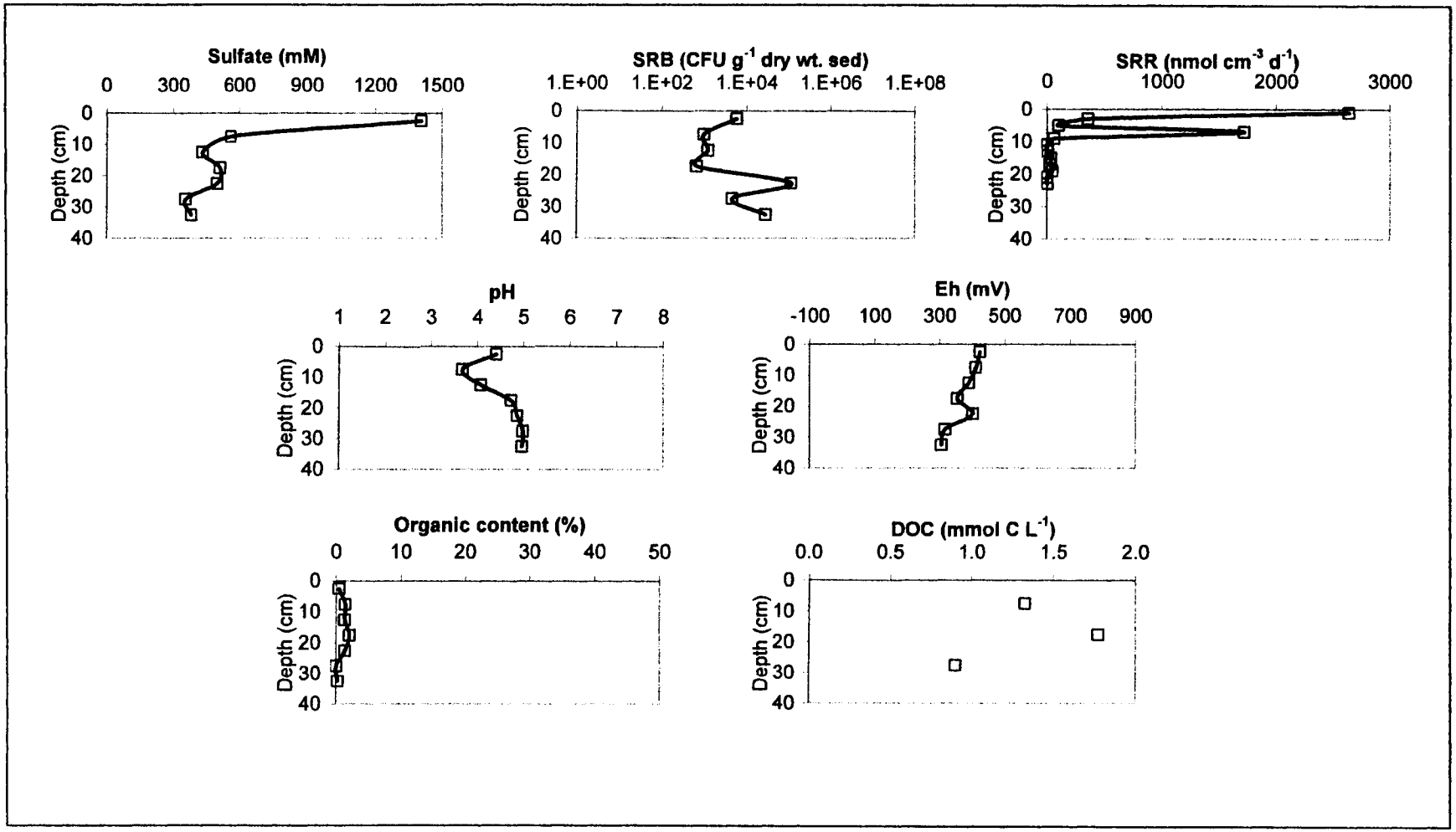

Figure 2-4. Sulfate concentration, sulfate-reducing bacteria (SRB) abundance, sulfate reduction rates, pH, Eh, organic carbon content and dissolved organic carbon (DOC) in tailings and porewaters from the Kidd metallurgical site.

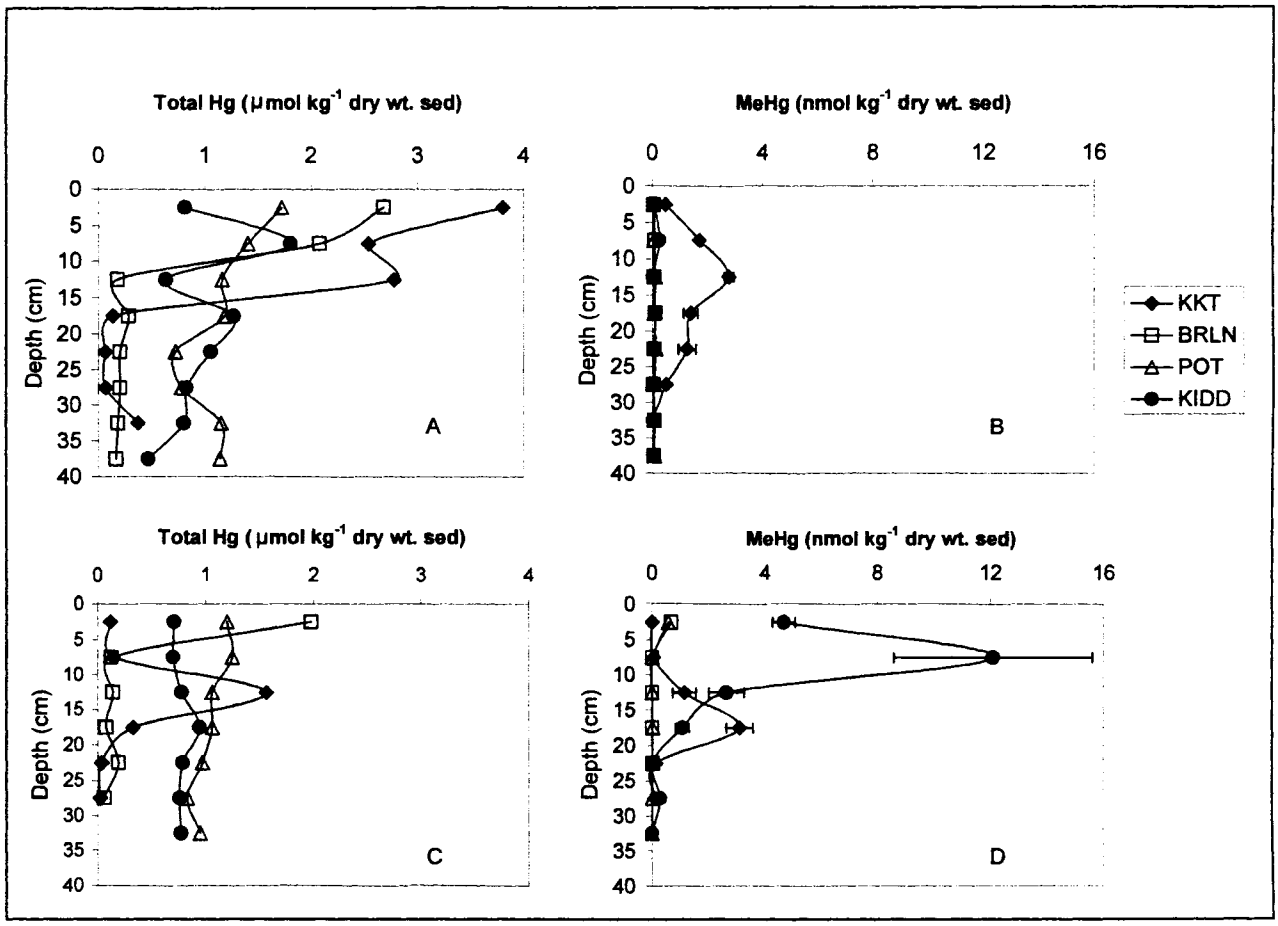

Figure 2-5. Total Hg (Hg_T) and methylmercury (MeHg) in bulk tailings at Kam Kotia (KKT), Broulan (BRLN), Potter (POT) and the Kidd Metsite (KIDD). A, B: 2003 values; C, D: 2004 values. Error bars (SD; n=3) are within 10% or smaller than symbols except where indicated.

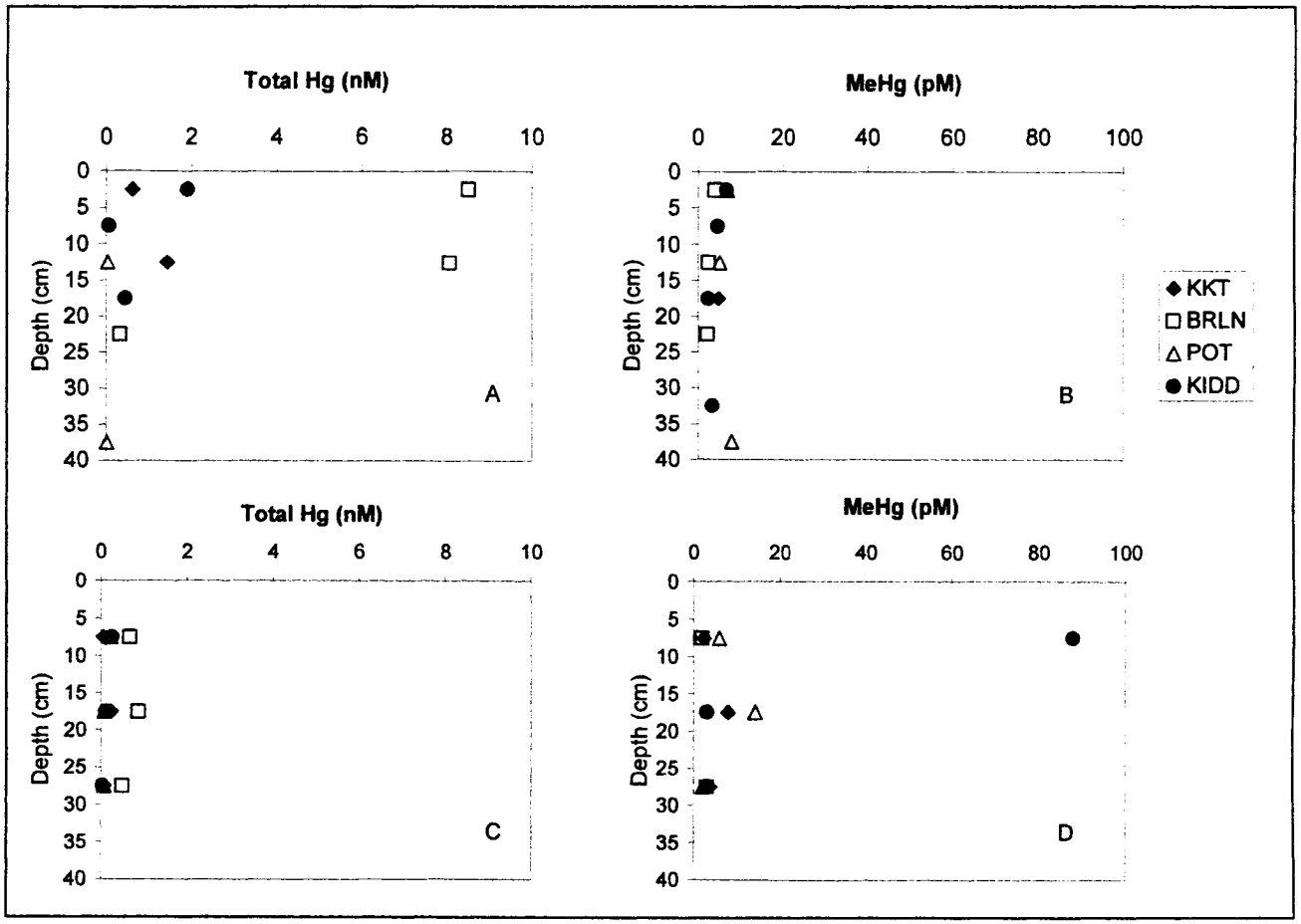

Figure 2-6. Total Hg (Hg_T) and methylmercury (MeHg) in tailings porewaters at Kam Kotia (KKT), Broulan (BRLN), Potter (POT) and the Kidd Metsite (KIDD). A, B: 2003 values; C, D: 2004 values. Relative standard error (SE/mean) was < 10% (n ≥2) for all values.

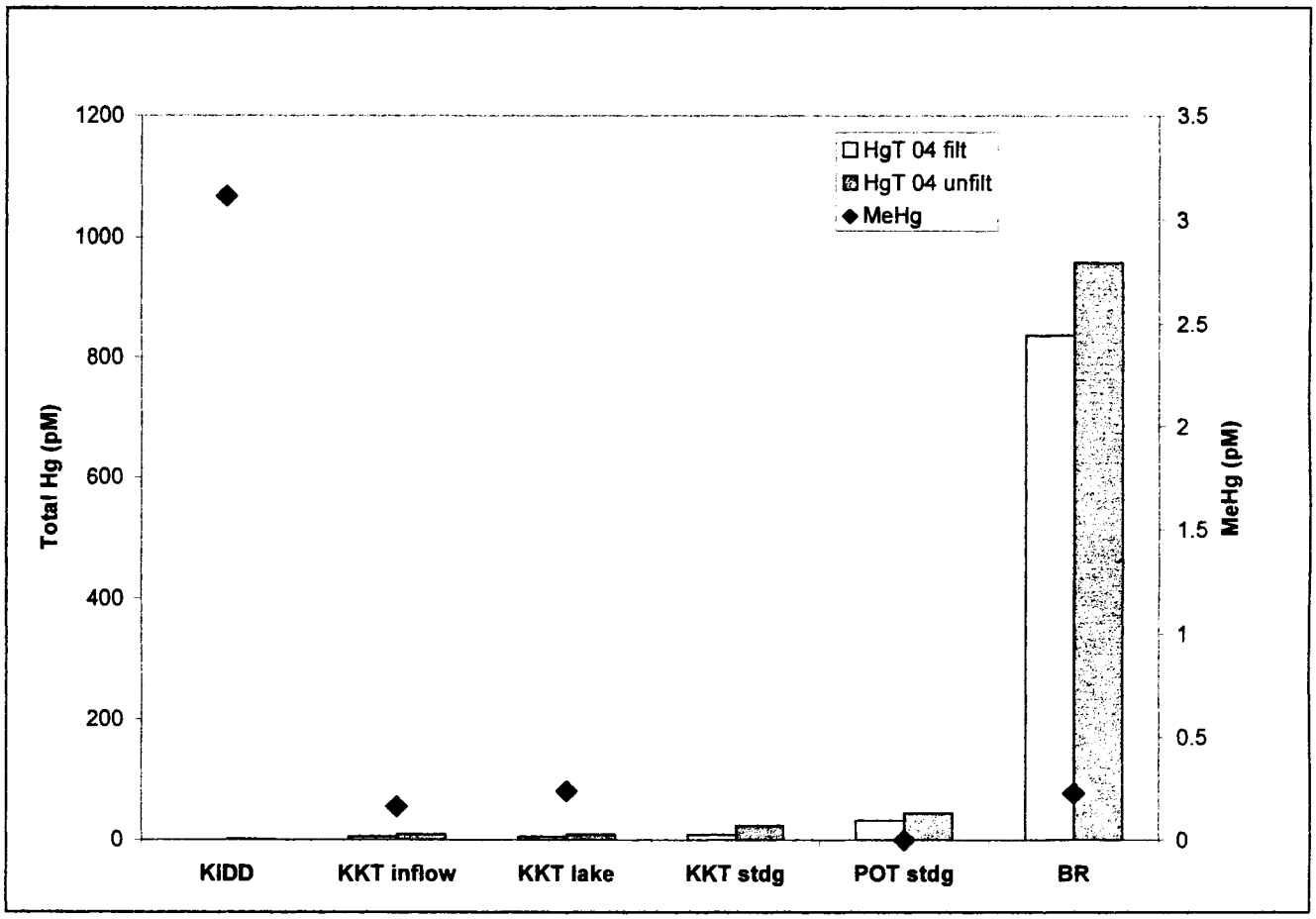

Figure 2-7. Total Hg (Hg_T) and MeHg values for surface water samples. Kidd = drainage from the Kidd Metsite; KKT inflow = inflow to Kam Kotia Lake from stream draining Kam Kotia mine tailings; KKT lake = Kam Kotia Lake site approx. 1 km from the inflow; KKT stdg = standing pond water near Kam Kotia sample pit; POT stdg = standing water from flooded area near Potter sample pit; BR = water collected from lake near Broulan sample pit. Filt = filtered; Unfilt = unfiltered. Relative standard error (SE/mean) < 10% ($n \geq 2$) for all values.

CHAPTER 3: FACTORS AFFECTING METHYLMERCURY LEVELS IN SURFICIAL TAILINGS FROM HISTORICAL NOVA SCOTIA GOLD MINES*

* Adapted from S. Winch, M. Parsons, D. Fortin, D. Lean, in prep.

3.1 INTRODUCTION

Large amounts of inorganic Hg were used in historic gold mining operations in Nova Scotia, Canada. Mining began in Nova Scotia around 1860, at which time Hg amalgamation was the primary method of gold extraction. Typical "lode"-type gold ores mined in 64 mining districts in Nova Scotia were predominantly associated with slates and greywackes of the Meguma group rocks of the southern mainland. Auriferous quartz veins lying within the slates and greywackes hosted gold-bearing arsenopyrite minerals. The ore was crushed to a fine particulate size in stamp mills or other milling installations. It was then passed over Hg-covered copper plates, where the gold would form an amalgam by dissolving into the Hg (Bates, 1987). The amalgam was later heated to volatilize the Hg leaving behind the gold. However, some portion of the Hg from the copper plates would be transported along with the "tails", or reject crushed rock, into the waste stream. Approximately 10-25% of the Hg used in the amalgamation process was lost to the tailings in the early 1900s (Wong et al., 1999). Cyanidation, the use of NaCN to recover gold, began to replace Hg amalgamation as early as 1880 (Bates, 1987), but Hg amalgamation continued to be used for several decades (Roach, 1937; Roach, 1940). This process produced tailings that were highly contaminated with Hg, and that were typically deposited near the mills in low-lying areas such as lakes or wetlands. Although Hg amalgamation was discontinued around 1940, the tailings from these early mining operations still contain large quantities of Hg, and cover large land areas in Nova Scotia. Total Hg concentrations in the Goldenville gold mining area of Nova Scotia have previously been reported in the range of 24-324 $\mu mol\ kg^{-1}$ (Wong et al., 1999) but very little information is available regarding the speciation of Hg in amalgamation tailings.

Methylmercury (MeHg) is the form of Hg that is of greatest concern in the environment, as it is highly toxic and bioconcentrates up the food web from algae to zooplankton, fish and eventually top predators and humans (Hammerschmidt et al., 2002; Pickardt et al. 2005). Sulfate-reducing bacteria (SRB) are known methylators of Hg in various sedimentary environments (Choi & Bartha, 1994; Compeau & Bartha, 1985; Compeau & Bartha, 1987; Devereux et al., 1996; King et al., 1999; King et al., 2000; King et al., 2001). Gold cyanidization tailings from ores in northern Ontario that are mineralogically similar to Nova Scotia gold ores have been shown to host active SRB populations (Fortin et al., 2000; Fortin et al. 2002). If SRB were present in Nova Scotia gold tailings, these tailings might be highly contaminated with MeHg. This is the first study to examine MeHg and SRB in Nova Scotia gold tailings.

As human populations grow and smaller centres expand, pressure mounts to develop once-remote areas. Abandoned mine sites are often used for recreational activities by local populations who may or may not be aware of the hazards present. In some locations, cottages, houses or whole communities are built on mine wastes, and governments across Canada are becoming increasingly concerned about liability issues associated with abandoned mines, as evidenced by the recent establishment of the National Orphaned/Abandoned Mines Initiative (NOAMI – NRCan, 2006). For these reasons, it is essential to investigate the potential hazards to humans and ecosystems represented by Hg and other forms of contamination in these environments.

Depth profiles of MeHg and Hg_T concentrations in Au tailings deposits were compiled and compared with organic content, *in situ* abundance and activity of SRB, pH, Eh, SO_4^{2-}, dissolved sulfide and other geochemical variables in tailings from two former mining areas in Nova Scotia. This study sought to characterize the relationships between MeHg and sulfate reducers in tailings from lode-type Au ores, and to determine whether MeHg and Hg_T were elevated in waters receiving drainage from tailings dumps.

It was hypothesized that SRB would be present but neither abundant nor highly active in these environments, since sulfate levels were likely to be low. Nonetheless, it was expected that even modest populations of SRB would lead to significant accumulation of MeHg in these tailings, given the high concentrations of Hg_T. Surface waters in the vicinity of mine sites were also analyzed for MeHg or Hg_T contamination.

3.2 MATERIALS AND METHODS

3.2.1 Environmental Setting

Tailings were sampled from abandoned tailings dump sites in September, 2003 and May, 2004. During these two sampling periods, six sites were sampled in two watersheds: Lake Catcha (Figure 3-1) and Upper/Lower Seal Harbour (Figure 3-2).

Lake Catcha mining operations were active from 1887-1961 (Bates, 1987). For a period of time, this was one of Nova Scotia's most productive mines, producing a total of 17,961 oz gold

(Bates, 1987). The Lower Seal Harbour (LSH) mine was in operation from 1894-1949, during which time it produced 34,188 oz gold (Bates, 1987). MacKenzie (1907) documents the installation of a stamp mill at LSH in 1905, specifying the use of Hg amalgamation, but gold was mined at this site since 1894 (Bates, 1987). The mine later closed and was re-opened and expanded in 1935. A cyanidation plant was added in 1936 (Roach, 1937) and from that time, presumably until the mine closed in 1949 (Bates, 1987), gold was extracted using Hg amalgamation in a rod mill followed by cyanidation (Roach, 1937; Roach, 1940). Tailings were initially dumped just south of the mill (Roach, 1937), and later pumped through 900 ft of pipe (Roach, 1940), i.e. in the vicinity of LSH-1 (Figure 3-2). Later, it is assumed the tailings were pumped further away from the mill, eventually reaching the areas where the LSH-2 (Figure 3-2) and FP samples were collected.

The Boston-Richardson mine is located on the shore of Gold Brook Lake in Upper Seal Harbour, Nova Scotia (Figure 3-2). 57,946 oz of gold were produced from this mine between 1893 and 1958 (Bates, 1987). This was the site of a bromo-cyanide plant that was used to treat arsenopyrite concentrates from mines in the area, including LSH. MacKenzie (1907) mentions sending concentrates from LSH to the Boston-Richardson mine to be cyanided. After 1910, the Boston-Richardson mine was in operation until 1958 (Bates, 1987). Tailings were discharged into what is now the floodplain of Gold Brook Lake, near the mill.

Tailings were initially sampled from two pits (ELC and LCL) at Lake Catcha and two at Lower Seal Harbour (LSH-1 and LSH-2) in September, 2003 (Figure 3-2). Lake Catcha sites could not be re-sampled in May 2004 due to hurricane debris, therefore LSH-1 and LSH-2 were re-

sampled and the Seal Harbour watershed study area was expanded to include Upper Seal Harbour (USH) and First Pond (FP). At the time of sampling in May 2004, the tailings had been thawed for < 3 weeks. After analysis of 2003 and 2004 field data, the Lake Catcha site was re-sampled in May and October 2005 to collect tailings for subsequent laboratory experiments, sulfate reduction rate measurements and analysis of microbial DNA (Chapter 4).

In 2003, water samples were collected from Lake Catcha, as well as the bog adjacent to the ELC pit, and several sites downstream. In 2003 and 2004, water samples were collected from a stream flowing from a bog near the LSH site over the tailings field and into East Brook, then downstream to the outflow of East Brook into the ocean, with additional samples taken in 2004 from the USH area and a background site upstream of the system (Figure 3-2).

3.2.2 Sampling

50 mL sterile polypropylene (Falcon) tubes (BD Biosciences) were used to collect tailings at 5 cm intervals up to ~ 40 cm depth, depending on the depth of the water table at each site. The tailings were scooped directly into the tubes, which were immediately sealed and placed on ice, then frozen within three hours and kept frozen until use. One set of samples was used in the field for pH and Eh measurements. Samples for enumeration of SRB were collected in previously sterilized 20 mL glass vials using a sterilized spatula. Vials were sealed and stored at 4°C in the dark until they were used for bacterial enumeration, within 24 hrs of returning to the laboratory.

In Sept. 2003, tailings samples for general geochemical analysis of porewaters (sulfide, ferrous iron, sulfate) were collected in previously sterilized 20 mL glass vials using a spatula sterilized with 70% ethanol. Vials were sealed and kept at 4°C until porewater was extracted (within 24 hrs) using a sterile syringe degassed with nitrogen, and then filtered through a 0.22 μm syringe filter into 7 mL glass vials for chemical analysis. Reagents for sulfate, sulfide and ferrous iron analysis were added immediately and samples were analyzed in the laboratory within 24 hr. In May, 2004, plexiglass dialysis chambers (called "peepers" - Carignan et al. 1985; Carignan & Lean, 1991) were installed 2-3 weeks prior to sampling tailings. Peepers were acid-washed and fitted with 0.22 um filter paper that was previously soaked for several days in deionized water. They were placed into sealed containers, submerged in deionized water and degassed for 5 d with nitrogen, then transported in the sealed containers. Peepers were inserted vertically into the tailings piles. On retrieval, peepers were immediately rinsed with deionized water. Porewater was removed in the field, using sterile syringes degassed with nitrogen, injected into previously sterilized, sealed, degassed 20-mL septum vials and refrigerated. Porewaters were analyzed within 24 hrs for HS^-, SO_4^{2-} and Fe(II).

Larger quantities of tailings were required to extract porewater for Hg_T and MeHg analysis. Acid-washed 250-mL HDPE (Nalgene) bottles were used for this purpose. Six bottles of tailings were collected at two to four depths at each sampling site, immediately placed on ice and frozen within three hours. Prior to analysis, samples were thawed and centrifuged at 5000 rpm for 20 min. Porewater was immediately drawn off with a syringe, transferred to a clean 250 mL bottle and re-centrifuged to remove residual solid matter. Water from all samples at a given site and depth was pooled to obtain sufficient quantities for Hg_T, MeHg and DOC

measurements. Once extracted and re-centrifuged, it was divided into acid-washed 50 mL polypropylene tubes. One tube was acidified with 0.5% trace metal grade HCl for MeHg analysis. A second tube was preserved with 1% BrCl for Hg_T analysis, and a third sub-sample was filtered through a 0.45 um Pall Gelman Acrodisk 25 mm syringe filter for DOC analysis. All samples were immediately stored in the dark at 4°C. In samples where only small amounts of porewater could be extracted, priority was given to Hg_T and MeHg analysis.

Due to restrictions on transport of radioactive materials, sulfate reduction rates (SRR) were not measured in 2003. Sampling for sulfate reduction rates in 2004 (and 2005 at the ELC site only) was carried out as described in Praharaj and Fortin (2004) based on the method of Jorgensen (1978). Briefly, two 12-cm long cores were collected across the redox boundary at each site, using plexiglass corers with injection ports drilled at 2-cm intervals and covered with silicon sealant. The cores were collected near the tailings sampling pits such that the first core contained tailings up to 12-cm depth and the second from 12-24 cm. Filled corers were immediately sealed and stored in air-tight plastic bags to prevent oxygen infiltration. In the laboratory, cores were immediately injected with $^{35}SO_4^{2-}$ and incubated at the *in situ* temperature (LSH-1, LSH-1, USH: top 12 cm = 18°C, 12-24 cm = 9°C; FP: 0-24 cm = 9°C) for 24 h (7 h for ELC 05 sediments). After incubation, the cores were immediately sectioned at 2 cm intervals and fixed with 20% Zn-Acetate to terminate microbial activity and fix sulfides as ZnS.

Surface water samples to be analyzed for MeHg were collected in duplicate in previously cleaned 1 L HDPE bottles, and rinsed five times with sample water. Samples were collected

using appropriate clean techniques with the bottle mouth pointed upstream, acidified immediately in the field with 0.5% concentrated, trace-metal grade HCl (OmniTrace), sealed inside two Ziplock bags and kept at 4°C in the dark until used. Additional surface water samples were collected in 50 mL acid-washed polypropylene tubes for total Hg and DOC measurements. Hg_T samples were preserved with 0.5 mL of bromine chloride (27 g of KBr in 2.5 L of HCl followed by 38 g of $KBrO_3$) as outlined in EPA Method 1631 (USEPA, 2002). pH and Eh measurements of tailings and porewaters were taken on site using a VWR portable meter with Corning pH and redox probes.

3.2.3 Laboratory Analysis

Total Hg and MeHg in tailings were measured as μmol kg^{-1} and nmol kg^{-1} wet weight, respectively, and concentrations were normalized to dry weight based on percent water content, in order to avoid losses of Hg in the drying process and to determine how much of the Hg_T or MeHg in tailings was in the solid and liquid phases.

Total Hg in wet tailings was measured by high temperature combustion using an NIC automatic Hg analyzer (SP-3D; MDL = 0.1 ng). All samples were analyzed at least in duplicate with mean relative standard error (standard error/mean; RSE) of 8.3%.

MeHg was extracted from wet tailings using the solvent extraction-GC/AFS method described elsewhere (Cai et al., 1997). Where MeHg concentrations were off-scale, samples were rerun at lower injection volume. Samples were analyzed at least in duplicate with mean RSE =

11.8%. Recoveries of MeHg were determined from duplicate samples from at least one depth at each sampling site spiked with 0.75 ng MeHg. Mean recoveries of 94% were obtained. Organic content of tailings was estimated by loss on ignition (LOI) after drying and heating to 400°C for 8 hr.

Sulfate reduction rates (SRR) provide a measure of SRB activity and have been shown to be related to mercury methylation rates in sediments (King et al., 1999). SRR were measured as outlined in Chapter 2. SRB were enumerated using the most-probable-number (MPN) method, as described in Praharaj and Fortin (2004) and outlined in Chapter 2. The enumeration was performed within 1 to 3 days after sampling. A modified Postgate medium containing four electron donors (lactate, acetate, formate and pyruvate) was used, along with a reducing agent. All solutions were adjusted to pH 7.5 with 2 M NaOH. Tubes were incubated at room temperature in the dark for two weeks and growth was assessed by the presence of black Fe-sulfides in the tubes. MPN values were calculated from statistical tables (Cochran 1950) and expressed in colony forming units per gram of dry weight of sediments (CFU g^{-1} dry wt).

MeHg analysis of porewater and surface water samples was carried out by capillary gas chromatography/atomic fluorescence spectrometry (GC/AFS) after solid-phase extraction onto sulfhydryl cotton columns and acidic potassium bromide elusion (Cai et al., 1996). The volume of surface water samples was 1 L for MeHg analysis and 40 mL for Hg_T. Porewater volumes for MeHg analysis ranged from 10 to 40 mL, depending on the amount that could be extracted from each sample. In order to assess recovery of MeHg from surface waters and porewaters, spiked blanks were prepared using 1 ng MeHg in 1 L (5 pM) of deionized water for surface

waters and 1 ng MeHg in 50 mL of deionized water for porewaters (100 pM). Recoveries were 77% for porewaters and 94% for surface waters. Concentrations of MeHg in blanks were below the detection limit of 0.1 pM. Field blanks contained no detectable MeHg.

Total Hg in porewater and surface water samples was measured using a Series 2600 Tekran Hg analysis system according to the dual stage gold pre-concentration method (modified USEPA method 1631, MDL = 1.0 pM). Porewater samples were diluted in DIW containing 1% BrCl at a ratio between 1:2 and 1:400, depending on Hg concentration and brought to a final volume of 40 mL. Immediately prior to analysis, 5 μL of hydroxylamine (previously bubbled with Ar) was added per mL of sample or standard. Peak areas from blanks of DIW containing 1% BrCl were subtracted from sample and standard peak areas prior to calculation of Hg_T concentration.

DOC in porewater and surface water samples was measured using an OI Analytical 1010 total organic carbon analyzer (persulfate oxidation method; DL = 15 μM), and potassium biphthalate (Acros Organics, New Jersey, USA) standards. DOC samples were filtered through 0.45 μm filters (Pall Gelman Acrodisk 25 mm syringe filters). Samples were diluted where necessary for sufficient volume, and placed into acid-washed glass vials. Peaks for DIW blanks were subtracted from sample peaks.

Porewaters were analysed for SO_4^{2-} by the $BaSO_4$ turbidimetric method (Rodier, 1975). HS^- content was measured by using Cline reagents and reading absorbance at 670 nm with a UV-

Visible spectrophotometer after calibration with standard reagents. Fe(II) was measured using the ferrozine method (Stookey, 1970).

3.3 RESULTS

3.3.1 Porewater chemistry and microbial characteristics

With few exceptions, the tailings sampled from all sites were pH-circumneutral (6-7), but slightly more acidic near the surface. Eh values decreased with depth at all sites. Sulfate values were low and appeared to vary seasonally, with a maximum value of 4 mM measured in Sept. 2003. Sulfide content was also low, with maximum values ~ 2 μM, and Fe(II) concentrations were < 60 μM. Most tailings contained <1% organic matter, with occasional organic layers (max. 58%). DOC varied widely between sites, from 0.1-19.1 mmol C L^{-1}. SRR ranged from detection limits to 64.5 nmol cm^{-3} d^{-1} at all sites, while SRB MPN counts ranged from below detection limits to 2.9 x 10^7 CFU g^{-1} dry wt.

The first sampling of Seal Harbour tailings took place in September, 2003, when samples were collected for depth profiles at LSH-1 and LSH-2. In May, 2004, these two sites were re-sampled, and the Upper Seal Harbour (USH) and First Pond (FP) sites were also included (Figure 3-2). Geochemical and microbiological results for LSH-1, LSH-2, USH and FP appear in Figures 3-3 through 3-6. Lake Catcha bog (ELC) and beach (LCL) tailings were sampled in September 2003, and additional tailings samples were collected from ELC in May and October,

2005. Geochemical and microbiological data for the Lake Catcha samples are shown in Figures 3-7 and 3-8.

In September 2003, LSH-1 sulfide concentrations increased and Eh values decreased steadily with depth below 25-30 cm, suggesting sulfate reduction activity in this zone (Figure 3-3). Both sulfate and sulfide were higher in Sept. 2003 than in May 2004. One measurement of porewater DOC was made in Sept. 2003 (1.3 mM 35-40 cm), and the range of DOC values measured in May 2004 was 0.2-0.4 mM. DOC, sulfate and sulfide values likely increased over the summer months due to microbial activity. SRB cell count estimates, however, were < 250,000 CFU g^{-1} dry wt at all depths in Sept. 2003 (Figure 3-3).

In May 2004 at LSH-1, Eh showed a sharp downward spike at 15-20 cm, and a peak in Fe(II) at 20-25 cm, possibly indicating the presence of a Fe(III) reduction zone within the tailings (Figure 3-3). SRB were estimated to be 2.6 x 10^6 CFU g^{-1} dry wt at 5-10 cm depth. This is somewhat surprising, since with this exception, SRB MPN values measured in the spring were < 250,000 CFU g^{-1} dry wt in all samples from all sites. The slightly elevated organic content (1.3%) at the surface in this depth profile may have promoted SRB growth. SRR, on the other hand, were only above detection limits at depths > 10 cm, increasing with depth, but only to 5 nmol cm^{-3} d^{-1} at the maximum sampling depth of 24 cm (Figure 3-3). These data indicate that although SRB were present in these tailings, they were not very active in May 2004.

In the Sept. 2003 samples, LSH-2 featured an organic-rich layer (6.4%) at 15-20 cm, just below a local minimum in Eh (Figure 3-4). SRB were most abundant in the bottom depth sampled (3.1

x 10^6 CFU g^{-1} dry wt at 25-30 cm), but a smaller maximum was also observed at 5-10 cm (8.1 x 10^5 CFU g^{-1} dry wt). Sulfate, sulfide and DOC were higher in Sept. 03 than in May 04, as they were at LSH-1. Although only one porewater DOC measurement was made in 2003 (19.1 mM at 10-15 cm), it was well beyond the range observed for the spring 2004 (1.0-1.8 mM). In 2004, a lesser peak (0.8%) in organic matter was present in LSH-2 samples at 10-15 cm. Although SRB cell count estimates and SRR are low, profiles of both of these parameters as well as Fe(II) may indicate some microbial activity at or just below the organic-rich layer.

At the FP site (sampled in May 2004 only), pH increased from 6.1 to 7.0 and Eh decreased very gradually with depth (299-222 mV) (Figure 3-5). FP organic content was <1%, and HS^- was undetectable except at 30-35 cm (0.15 μM). Fe(II) was low (≤ 20 μM) throughout and no discernable pattern was observed with depth, whereas sulfate was near detection limits throughout. SRB were recovered from all but the bottom sample, but estimated numbers reached a maximum of only ~ 6,800 CFU g^{-1} dry wt. Sulfate reduction rates peaked at ~ 8 cm depth, reaching 17 nmol cm^{-3} d^{-1}. Sulfide was negligible except at 30-35 cm (0.15 μM); DOC also increased to a maximum of 1.9 mM at this depth, suggesting increased microbial activity at greater depth than was sampled.

pH (4.6-5.3) was lower at USH than at the other Seal Harbour sites (Figure 3-6). Although sulfate was consistently < 0.2 mM, SRB counts, Fe(II), HS^-, DOC and organic content all increased below 20 cm, pointing to the likelihood that microbial activity in general, and particularly of SRB, increased with greater depth. SRR were neglible but were only measured

to a depth of 24 cm, therefore no direct measurement of sulfate reduction activity is available at the maximum sampling depth of 30 cm.

Eh was considerably higher in the May 2005 sampling at ELC than in Sept. 03 (Figure 3-7). Sulfate was slightly higher (max. 4 mM) in Sept. 03 than May 05 for ELC. ELC tailings featured an organic-rich layer (59%) at the surface (removed for May 05 and Oct 05 sampling), but organic content was $\leq 2\%$ below 10 cm depth. SRB populations were larger at ELC than at any other site sampled in 2003, with the greatest abundance observed at depths > 20 cm (max. 2.9×10^7 CFU g^{-1} dry wt, corresponding to a drop in Eh and an increase in pH. SRR at ELC reached a maximum of 43 nmol cm^{-3} d^{-1} at ~ 10 cm depth in May 2005, dropping to near detection limits by ~ 20 cm depth, then rising again to 7.4 nmol cm^{-3} d^{-1} at 30 cm. SRR were measured again in samples collected from ~ 10 cm and ~ 30 cm depth in Oct. 2005, by which time SRR had increased to 64.5 nmol cm^{-3} d^{-1} at 10 cm and to 44.4 nmol cm^{-3} d^{-1} at ~ 30 cm depth.

LCL samples contained very little organic matter ($\leq 1.2\%$ throughout; Figure 3-8). Oddly, sulfate, sulfide and SRB cell counts all peaked near the surface of the LCL tailings, while Eh was lower at 0-5 cm than at 5-10 cm. Subsequent to the Sept. 03 sampling, it became apparent that a group of gold prospectors had been operating a sluice at this beach only a few days prior to the sampling date. Photographs published in an online prospectors' newsletter (Nova Scotia Prospectors Association, 2003) depict a 4" dredge being used to pump tailings from the littoral lake sediments to a sluice that emptied onto the beach within 1-2 m of where the sampling pit

was subsequently located. The porewater chemistry and MPN counts, therefore, were likely influenced by the addition of lake sediments to the beach area tailings.

3.3.2 Methylmercury and total mercury in bulk tailings and porewaters

Contrary to expectations, not all of the gold mine tailings contained high levels of total Hg. At the Seal Harbour site (Figure 3-9 A & B), the maximum total Hg value decreased with increasing distance from the Lower Seal Harbour stamp mill, i.e. from LSH-1 ($\leq$10.6 μmol kg^{-1}) to LSH-2 ($\leq$3.8 μmol kg^{-1}) to FP ($\leq$0.4 μmol kg^{-1}). Similarly, the USH sample site (Figure 3-9 B) was ~ 150 m from the stamp mill at the Boston-Richardson mine and had 8.0-21 μmol kg^{-1} Hg_T. The Lake Catcha beach tailings (LCL – Figure 3-9 C), located near the GH Anderson 10-stamp mill, also contained high Hg_T (5.1-16.9 μmol kg^{-1}), especially in the uppermost, disturbed layer, which contained 73.5 μmol kg^{-1} Hg_T where lake sediments were poured on the beach. This high value may reflect the enrichment of Hg in lake sediments by sedimentation processes. Hg_T concentrations in the ELC tailings (Figure 3-9 C) were 4.4-32.6 μmol kg^{-1}. These samples were collected downslope of the Oxford Gold Company mill, a ten-stamp mill installed in 1902.

As with Hg_T, a wide range of MeHg concentrations was observed, but visual inspection of depth profiles showed that distribution of MeHg did not closely match patterns of Hg_T distribution with depth (Figure 3-9). Regression analysis was therefore carried out for the full data set to determine which parameters most closely correlated with MeHg in tailings and porewaters. Positive correlation was observed between MeHg in bulk tailings and Hg_T, but this

correlation was not strong. The strongest overall correlation was between porewater MeHg and porewater Hg_T (slope = 0.0024; r^2 = 0.67). Table 3-1 gives the slope, r^2 and sample size for all regressions of tailings MeHg and porewater MeHg vs. each parameter. Porewater data are listed in Table 3-2.

DOC and sulfate were both positively correlated with porewater MeHg. These correlations, although weak (r^2 = 0.21 for DOC, 0.22 for sulfate), were considerably stronger than correlations of porewater MeHg with pH, Eh, Fe(II), sulfide, % organics, SRR or SRB cell count estimates. DOC likely serves as a carbon substrate and electron donor for SRB activity. The correlation between porewater MeHg and sulfate may be constrained by the low sulfate concentrations in these tailings.

Although linear regression analysis of the whole data set identified a number of factors that correlated with MeHg in the bulk tailings, these relationships were weak. On the other hand, visual inspection of the data revealed stronger trends within individual depth profiles than in the overall data set. Analysis of MeHg vs. each variable measured in individual profiles reduced the sample size and power of each test, but it allowed some quantification of this very complex data set. Linear regression analysis on a site-by-site basis was used to compare MeHg against each parameter where at least three data points were obtained (Table 3-1). For tailings data, MeHg values were first divided by the standard deviation of MeHg values for their corresponding depth profile in order to improve comparability between sites containing different ranges of MeHg values. To improve model fit, four outliers were removed for the

purposes of the regression analysis: organic carbon content for ELC 03, 0-5 cm; tailings MeHg content for USH, 30-35 cm; and porewater Hg_T content for LCL, 15-20 cm and 25-30 cm. Regression analysis of individual sites showed that MeHg in bulk tailings was more often correlated with Hg_T, % organic content, pH and Eh than with sulfate, sulfide, DOC, SRB abundance or Fe(II) although many of these factors are intertwined (Table 3-1).

Nine out of ten data sets showed positive correlations between MeHg and Hg_T, although six of them had low coefficients of determination (r^2). Strong correlations (r^2 = 0.4-0.9) between MeHg and Hg_T in bulk tailings were observed in LSH-2 03, FP and ELC 03 samples, where MeHg ranged between 0.06 -0.1% of Hg_T (Table 3-1).

Six out of eight data sets showed positive correlation between MeHg and % organic content, with the highest correlation coefficients occurring at LSH-2 03 and FP (0.76 and 0.72, respectively) (Table 3-1). The strong correlation in the LSH-2 03 data is clearly influenced by a zone rich in organic content that trapped both MeHg and Hg_T. This finding is consistent with other studies (Karlsson and Skyllberg, 2003; Benoit et al. 1998; Bloom et al., 2003; Hammershmidt and Fitzgerald, 2004) and with findings outlined in Chapter 2. For the ELC 03 profile, the extremely organic-rich sample was not included in the regression analysis, as it would render the results meaningless. Nonetheless, visual inspection of the depth profile clearly shows accumulation of MeHg and Hg_T in the uppermost, organic layer.

Tailings MeHg was negatively correlated with Eh in all the depth profiles except ELC 03 and ELC 05, where the influence of the surficial organic layer reversed this trend (Table 3-1). The

positive correlation was weakest at LSH-2 03, also due to the presence of an organic-rich layer, and strongest at FP. MeHg was positively correlated with pH in six of nine profiles, the exceptions again being ELC 03 and ELC 05, due to the organic-rich layer, and LCL, probably due to the disturbance of surficial tailings. With these exceptions, it appears that MeHg concentrations tended to be highest in pH-neutral, reducing environments, which are also conducive to growth of SRB. Although MeHg did not correlate well with SRB, overall SRB abundance was greatest at ELC, where the MeHg:Hg_T ratio (0.08-0.15%) was also highest for the high-Hg_T tailings. Some evidence of MeHg correlation with Fe(II) was found at LSH-1 04 and LSH-2 04, indicating that Fe(III)-reducing bacteria may contribute to MeHg production at these sites (Fleming et al., 2006).

Given the low Hg and MeHg values at FP, it was used as a "control" site for comparison with the other locations. Enrichment factors for Hg_T and MeHg in all other tailings and porewaters were calculated by dividing each value by the highest value obtained in the corresponding depth profile for FP. For example, since the highest Hg_T value measured in FP tailings was 0.4 $\mu mol\ kg^{-1}$, each Hg_T value at all of the other sites was divided by 0.4 to obtain an enrichment factor for each sample. These values as well as mean values for each sampling pit are presented in Table 3-2. From this analysis it is apparent that USH, LCL and ELC are all highly contaminated with Hg and that ELC became highly contaminated with MeHg between spring and fall. Two other samples (LSH-1 03, 30-35 cm depth and USH, 30-35 cm depth) had MeHg concentrations > 5 $nmol\ kg^{-1}$. They were both collected from the bottom of the depth profiles, and featured relatively low Eh values. Samples collected from the upper, drier layers at the same sample sites and at LCL had low MeHg content, despite high Hg_T, suggesting that

hydrologic conditions are important to MeHg production and/or accumulation, even in tailings highly contaminated with Hg_T.

The ELC tailings represented a situation where many factors came together to create an environment highly conducive for MeHg production and accumulation. They contained high Hg_T and abundant, active SRB, and were re-vegetated and therefore featured an organic-rich layer at the surface. Overall, the highest MeHg value measured in bulk tailings across all sites was 56.4 nmol kg^{-1}, and was found at the bottom of the USH profile, where a similar combination of factors was present. Tailings were high in Hg_T at this site, and porewater Hg_T concentrations were also elevated at the bottom of the depth profile (20.6 nM), therefore high concentrations of inorganic Hg were available for methylation. An increase in organic content to 4.6% in the deepest layer sampled also contributed to MeHg accumulation.

The high MeHg concentrations found at the ELC 03 site were unique among the tailings samples containing > 5 μmol kg^{-1} Hg_T, except for USH 30-35 cm (described above) and LSH-1 03 30-35 cm. The common factor among all of these samples was their location in zones likely to be permanently saturated. This prompted an experiment to measure MeHg concentrations in ELC tailings as they were dried and re-wetted over time to determine whether demethylation might be important at the drier sites. Surprisingly, however, the tailings collected in May 2005 for the demethylation study contained much less MeHg than the Sept. 2003 sample, even though Hg_T levels remained roughly constant. MeHg had already been lost from these tailings, either demethylated or washed away during spring melt. In ELC tailings collected a third time in October 2005, MeHg concentrations had risen again, demonstrating a

seasonal cycle of MeHg loss and replenishment. Korthals and Winfrey (1987) observed similar seasonal cycles of Hg methylation in oligotrophic lake sediments, where net methylation was greatest during mid to late summer. Lower net methylation at other times of the year was attributed to aerobic conditions which inhibit sulfate reduction and promote demethylation.

The bulk tailings collected from the beach at LCL contained higher Hg_T (74 μmol kg^{-1}) than any other location, and > 1000 nM Hg_T in the porewaters. MeHg in bulk tailings was < 5 nmol kg^{-1}, but porewater MeHg was 35-52 pM, higher than at any other locations sampled in this study. Hg_T and MeHg measured at this location may be indicative of a serious contamination problem in the lake sediments. The Canadian interim sediment quality guideline (ISQG) for Hg_T is 0.85 μmol kg^{-1} (dry wt), and probable effect level (PEL) = 2.4 μmol kg^{-1} (CCME, 2002). The ISQG is the concentration deemed safe for all aquatic life throughout all aspects of their aquatic life cycles during an indefinite period of exposure. PEL is defined as the level above which adverse biological effects are usually or always observed (CCME, 2002). The LCL beach tailings contain highly toxic levels of Hg_T. No guidelines have yet been established for MeHg in sediments.

3.3.3 Methylmercury and total mercury in surface waters

Water sampled from Lake Catcha near the beach site where the sampling pit was located had the highest Hg_T concentration (419 pM) of all the surface water samples collected in this study, including the highly contaminated stamp mill foundations at USH (Table 3-3). This value exceeds the background concentration for this area by a factor of 7.8. The high Hg_T in the lake

reflected the extremely high Hg_T in LCL porewaters (1.1 mM), although the elevated porewater concentrations may have resulted from lake sediments being poured on the beach. Water draining from the east tailings area (near ELC) into Lake Catcha contained higher MeHg (4.6 pM) and lower Hg_T (12 pM) than the lake water itself, although surface water from the bog next to the ELC sampling pit contained 360 pM Hg_T.

Water samples were collected from the Seal Harbour watershed in Sept. 2003 at 100 m intervals starting at the bog upstream of the LSH mill site and moving downstream to West Brook (W1 to W11), and from West Brook above and below the confluence (W12 and W13, respectively) and two sites just upstream of West Brook's outflow to the ocean (W14 and W15) (Figure 3-2, Table 3-3). The bog site (W1) was intended as a background sample, but the water contained 57 pM Hg_T and 7.2 pM MeHg, indicating that this site was probably somewhat contaminated. Both Hg_T and MeHg values initially increased as the stream passed the mill site (local maxima of 107 pM and 8.7 pM, respectively), then dropped to below the background concentrations (minimum 33.4 pM and 3.0 pM, respectively). The sites that were furthest downstream (W14 and W15) contained the highest concentrations of Hg_T (124 and 120 pM, respectively), but MeHg continued to fall to 3.1 pM just before reaching the ocean.

The large increase in Hg_T observed with distance downstream in West Brook led us to speculate that a previously un-sampled tailings deposit at First Pond might be contributing Hg_T to the system. The FP site was therefore sampled in May 2004. FP tailings and porewaters were the lowest in Hg_T and MeHg of all sites, and the surface water MeHg and Hg_T concentrations were similar to porewater values.

Surface water samples (W17-W21) were also collected upstream and downstream of the Boston-Richardson Mill/USH pit location in 2004, in addition to sites W1-W16. W17 was a background site sampled well upstream of any mining activity in May 2004, which had 24.6 pM Hg_T and 2.0 pM MeHg. Two sites sampled in the vicinity of the Boston-Richardson mine featured higher Hg_T and MeHg levels: W18, which was standing water from within the old foundations of the stamp mill building and draining into Gold Brook Lake contained 297 pM Hg_T and 12.1 pM MeHg; W21, a small stream receiving drainage from some highly oxidized tailings from the East Gold Brook mine located across Gold Brook Lake from the Boston-Richardson mill, contained 122 pM Hg_T and 8.6 pM MeHg. In the water samples (W19 and W20) collected from the stream draining the tailings pond, however, MeHg dropped slightly below background to 1.4 pM, and Hg_T increased gradually to 42.2 pM.

In samples W1-W15 (and W16, added in May 04), the same trends were observed in 2004 as in Sept. 2003, but both MeHg and Hg_T concentrations were lower. Hg_T reached a maximum of 72 pM at W3 and a minimum of 43 pM at W9, then increased to 69 pM at West Brook (W12) and dropped off slightly to 62 pM before reaching the ocean (W15). MeHg declined steadily from 6.3 pM at W1 to 1.9 pM at W15, which was equivalent to the background concentration measured at W17.

Linear regression analysis of MeHg in surface waters compared with Hg_T, DOC, pH and Eh showed no correlation with $r^2 > 0.1$ (data not shown).

3.4 DISCUSSION

Although the Au mine tailings examined in this study were quite uniform in geochemical profiles (near-neutral pH, low sulfate and sulfide, low organic matter, oxidizing to slightly reducing redox conditions), and they were all produced from the same type of ore, a wide range of Hg_T and MeHg values in the sediments and porewaters was observed, even among tailings produced from the same mill. This study demonstrates that although Hg_T levels reflect the original composition of tailings at deposition, MeHg concentrations result from dynamic processes *in situ*, and identifies a complex set of parameters that influence MeHg levels in Nova Scotia gold mine tailings.

Much of the variation in the Hg_T in tailings can be attributed to proximity to the mill site, with higher-Hg_T sediments being located closer to the mill sites and lower-Hg_T tails further away. This is a reflection of both the extraction processes used and the grade of ores being processed. Mines were originally established near deposits of high-grade Au ores, some of which contained visible Au. Over time, high-grade ores became depleted and miners extracted Au from progressively lower-grade ores by dissolution of arsenopyrite minerals by cyanidation (Bates, 1987). Simultaneously, tailings accumulated to great depths around the mill sites, making it necessary to pipe them further away. From 1935 until the mine closure in 1949 arsenopyrite concentrates were extracted by Hg amalgamation followed by cyanidation, and the remaining low-grade ores were cyanided to recover small quantities of Au (Roach, 1937; Roach, 1940; Bates, 1987). The resultant tailings from these operations contained mostly cyanided greywacke, slate and quartz minerals, but periodically tails from the amalgamation

process were deposited along with lower-Hg residues (Roach, 1940), which would account for the heterogeneity of Hg_T content at LSH-1 between the 2003 and 2004 sampling pits. Wong et al. (1999) found similar heterogeneity with depth in the tailings at Goldenville. The trend toward decreasing Hg concentrations with distance from the LSH mill is seen in samples from LSH-1, LSH-2 and FP.

Since the Hg in tailings was added as Hg^0 in the extraction process, it is reasonable to assume that MeHg levels in fresh tailings would have been negligible, and any MeHg now present in tailings was produced *in situ*. It was hypothesized that MeHg would be correlated with SRB MPN counts and/or SRR. Although SRB abundance distribution with depth did not match depth profiles of MeHg concentration in the tailings, the highest SRB cell count estimates were observed at ELC, the site most highly contaminated with MeHg. Further, although SRR were quite low at all of the sites sampled, they were higher at ELC than any other sample site. Although FeRB have been shown to methylate Hg (Fleming et al., 2006) they are not likely contributors to Hg methylation at this site, as ferrous iron concentrations were negligible (Figure 3-7). Abiotic methylation under natural conditions has been shown to be less than 1/10 that of microbial methylation (Berman and Bartha, 1986). SRB, therefore, remain the most likely agents of Hg methylation in the ELC tailings.

MeHg concentration in a given tailings environment is, however, also influenced by other factors, including the amount of dissolved Hg_T in porewater that is available for methylation, the presence of organic material to trap MeHg, the presence of sufficient DOC and sulfate to sustain SRB growth, and hydrological conditions that in turn influence oxygen levels.

Accumulation of MeHg was clearly evident in the organic-rich layers at LSH-2 (2003). This observation is consistent with results presented in Chapter 2. Bloom et al. (2003) observed that in gold mine tailings subjected to a sequential extraction procedure, most MeHg was found in the organo-chelated fraction, as estimated by their "F3" extraction (85% KOH). When Hg(II) from gold tailings was added to organic-rich sediments, it was rapidly distributed to the organo-complexed fraction and resulted in high MeHg.

Seasonal fluctuations of MeHg concentration were observed at ELC, where multiple overlapping factors created an environment conducive to MeHg production. Increased MeHg was accompanied by a rise in SRR and a decrease in Eh between spring and fall. Higher Eh in spring was attributed to the influx of oxygenated water after snowmelt. Although methylation and demethylation processes are known to proceed simultaneously in high-Hg_T environments, redox conditions have been shown to influence the demethylation pathway. Under aerobic conditions, reductive demethylation (RD) dominates, whereby Hg^{2+} released during degradation of MeHg is reduced to Hg^0 and lost through volatilization to the atmosphere, whereas oxidative demethylation (OD) proceeds more readily under anaerobic conditions, and likely leads to the remethylation recycling of Hg^{2+} after demethylation (Schaefer et al., 2002). As a result, for a given concentration of Hg_T in contaminated sediments, MeHg tends to be lower in aerobic environments and higher in anerobic situations. The balance between methylation and demethylation in the ELC tailings appears to have oscillated seasonally with changes in redox conditions, resulting in net demethylation in spring but shifting to net methylation by the end of the summer.

This finding raises questions about the USH site, which was only sampled in the spring, and at the edge of a large, partially-submerged floodplain full of Hg-contaminated tailings. The extremely high MeHg measurement from the bottom of this profile indicates that Hg methylation, particularly in the surrounding floodplain may be a serious concern later in the season. Since tailings were very often deposited in low-lying, wet locations, these findings indicate that future research on MeHg production in tailings should take into account seasonal variability.

Wong et al. (2002) measured Hg-flux rates that were 20 to > 100 times greater from Nova Scotia gold mine tailings than from natural Nova Scotia soils, and Beauchamp et al. (2002) found that Hg flux rates from tailings at two abandoned gold mine sites in Nova Scotia were two orders of magnitude higher than those observed over undisturbed native soils of similar parent material. Since RD is the dominant demethylation process in aerated sediments, microbial activity is likely responsible for the large fluxes of Hg^0 to the atmosphere from Nova Scotia gold mine wastes.

In addition to being demethylated in spring, MeHg may also have been flushed out of ELC tailings during spring snowmelt. Balogh et al. (2004) found that Hg and MeHg were flushed from watersheds during hydrological events. They also hypothesized that warm weather prior to flushing events might promote Hg methylation, increasing MeHg concentrations in surface waters after summer rainfall. Further study is therefore warranted in submerged or saturated

locations such as ELC and USH to determine whether MeHg is flushed from tailings during summer flushing events.

The tailings piped to First Pond (FP), approximately 1.5 km from the Lower Seal Harbour stamp mill, were extracted by cyanidation only, and from relatively low-grade ores containing little arsenopyrite. FP tailings therefore contained negligible levels of Hg_T and MeHg, and served as an excellent control site for comparison with other tailings. This site could be used as a reference site for other studies investigating Hg in gold mine tailings in the same area.

Total Hg and MeHg in bulk tailings and porewaters sampled at LCL were likely influenced by sluicing activities immediately prior to sampling, therefore observations at this site are likely not representative of undisturbed gold mine tailings. The elevated Hg_T in the LCL surficial tailings and porewater, and high porewater MeHg may, however, indicate a serious Hg contamination problem in the lake sediments. Further research would be required to ascertain Hg and MeHg levels in the sediments, but this property is now private "waterfront" property and the site of a recently constructed cottage.

Surface water samples collected from both the Lake Catcha and Seal Harbour watersheds showed that MeHg is not greatly elevated over background concentrations in surface waters. Hg_T, however, is more of a concern. The highest Hg_T in surface water was in Lake Catcha. Background levels of Hg_T range from ~25-54 pM, and Canadian water quality guidelines specify a maximum of 130 pM Hg_T to protect freshwater aquatic life. Lake Catcha water exceeded background Hg_T by a factor of 7.8, and exceeded the Canadian water quality

guidelines by a factor of 3.2. It also surpassed levels detected in the foundations of the Boston-Richardson stamp mill. Although Hg_T in the drainage from the ELC bog area that flows into Lake Catcha was not elevated, surface water collected in the bog contained 360 pM Hg_T. During high rainfall events, therefore, Hg may be transported from the ELC bog area into Lake Catcha.

Analysis of Seal Harbour area surface waters showed that Hg_T concentrations increased as the water coursed over the tailings-contaminated landscape to the ocean at Seal Harbour, but overall, Hg_T contamination was low and MeHg concentrations had diminished to background levels at the point of discharge into Seal Harbour.

Although Hg and MeHg concentrations were the focus of this study, it is important to bear in mind that Nova Scotia gold mine tailings are also very high in arsenic. Wong et al. (1999, 2002) found average arsenic values of ~ 400 mmol kg^{-1} in the Goldenville and Caribou mining districts, and arsenic concentrations in the beach tailings at Lake Catcha reached a maximum of 1,260 mmol kg^{-1}. Arsenic contamination is of serious concern in these environments. A comparison of arsenic, cadmium and Hg toxicity in an aquatic ecosystem, however, found that Hg was the most toxic of these metals, and arsenic was the least toxic (Abdelghani et al., 1995). Further, the same researchers found evidence of a synergistic effect of arsenic and Hg. No information was available concerning possible additive or synergistic effects of arsenic and MeHg. Evaluations of ecosystem effects of Nova Scotia gold mine tailings, therefore, should examine the combined impact of arsenic and Hg, or arsenic and MeHg contamination.

3.5 CONCLUSIONS

Waste materials from two historical gold mining areas in Nova Scotia were analyzed for methylmercury content and related parameters. Sulfate-reducing bacteria are known to produce MeHg, and have been recovered from gold mine tailings in other locations. It was hypothesized that SRB would be present in Nova Scotia gold mine tailings, and that depth profiles of MeHg concentration would be positively correlated with SRB cell count estimates. SRB were recovered at all sites, and the location containing the highest MeHg concentration hosted the most abundant SRB population. MeHg depth profiles, however, did not correlate with SRB abundance patterns or with sulfate reduction rates, but MeHg levels depended on a complex set of factors, including total Hg concentrations in the bulk tailings and porewaters, organic content, hydrological conditions, presence of SRB, DOC and sulfate, and demethylation processes. Overall, the strongest correlation was between porewater MeHg and porewater Hg_T, indicating that bioavailability is an important factor in methylation. MeHg was shown to undergo seasonal fluctuations at one Hg contaminated site, that appeared to be governed by microbial production in the summer and demethylation and/or advection resulting from an influx of oxygenated water during spring snowmelt. Surface water concentrations of MeHg were not greatly increased downstream of gold mine tailings in either of the watersheds investigated. Total Hg concentrations increased in both watersheds, and although Hg_T contamination was low in the Upper/Lower Seal Harbour watershed, concentrations were particularly elevated in Lake Catcha.

In light of modern pressures to develop old mine sites for human use, whether for roadways, habitat or recreational activities, it is increasingly important to understand Hg contamination issues related to mine wastes. This is the first interdisciplinary study to examine highly toxic methylmercury and sulfate reduction in Nova Scotia gold mine tailings. Future research should investigate transport of Hg_T and MeHg from tailings dumps to surface waters during spring snowmelt and high rainfall events, particularly where tailings are saturated or submerged and contain > 5 μmol kg^{-1} Hg_T and/or high organic content. Seasonal variation in MeHg concentrations should be taken into account during evaluation of Hg contaminated gold mine tailings in northern regions, as springtime observations of low MeHg content may be misleading. Any future toxicological studies on Nova Scotia gold mine tailings should consider possible combined effects of Hg and/or MeHg and arsenic contamination.

Table 3-1. Results of linear regression of MeHg vs. geochemical and microbiological variables. LSH-1 03: Lower Seal Harbour site #1, 2003; LSH-1 04: Lower Seal Harbour site #1, 2004; LSH-2 03: Lower Seal Harbour site #2, 2003; LSH-2 04: Lower Seal Harbour site #2, 2004; USH: Upper Seal Harbour (2004); FP: First Pond (2004); ELC 03: East Lake Catcha, 2003; ELC 05: East Lake Catcha, 2005; LCL: Lake Catcha beach tailings (2003).

MeHg in TAILINGS

variable:	Tailings Hg_T			Porewater Hg_T			% org			pH			Eh			SRR			DOC			SRB			SO_4^{2-}			HS^-			Fe(II)		
	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^2	n	slope	r^3	n
Site																																	
LSH-1 03	0.63	0.40	8				3.30	0.03	8	0.34	0.03	8	-0.009	0.97	8							0.00	0.09	8	-0.19	0.02	3	1.60	0.77	3			
LSH-1 04	-0.32	0.10	7	0.00	0.98	3	-1.60	0.30	7	1.50	0.40	7	-0.004	0.37	7	0.53	0.76	5	0.03	0.00	6	0.00	0.08	7	-60.60	0.72	6	-36.40	0.36	6	1.32	0.70	6
LSH-2 03	0.95	0.91	6				0.40	0.80	6	0.95	0.20	6	-0.001	0.01	6							0.00	0.37	6	1.10	0.99	3	0.38	0.16	3			
LSH-2 04	0.05	0.00	7	-0.02	0.73	3	-0.30	0.01	7	1.70	0.70	7	-0.007	0.78	7	0.40	0.05	5	0.17	0.31	7	0.00	0.01	7	-7.60	0.17	7	3.50	0.01	7	1.38	0.46	7
USH*	0.58	0.40	*6	0.00	0.16	3	0.58	0.40	6	1.30	0.20	6	-0.004	0.30	6	0.01	0.00	5	1.10	0.57	6	0.00	0.04	6	34.00	0.80	6	-6.20	0.13	6	-0.27	0.01	7
FP	0.85	0.72	6	0.14	1.00	3	0.85	0.72	6	2.30	0.70	6	-0.025	0.67	6	-0.27	0.46	5	0.17	0.19	6	0.00	0.01	6	-12.40	0.38	6	n/a			-0.01	0.00	6
ELC 03	0.67	0.45	6				0.39	0.42	*4	-1.30	0.60	6	0.006	0.72	6							0.00	0.17	6							-9.90	0.09	5
ELC 05	-1.70	0.00	6				-0.15	0.02	8	-6.10	0.65	8	0.004	0.06	8	-0.12	0.41	6							-2.20	0.25	3						
LCL	0.44	0.19	6			**	0.44	0.19	6	-3.00	0.40	6	-0.007	0.36	6							0.00	0.15	6	-0.69	1.00	3	0.48	0.16	6			
all sites	0.31	0.20	*52	1.60	0.10	**16	0.16	0.03	58	-0.09	0.00	60	-0.003	0.15	60	-0.11	0.14	26	0.03	0.09	28	0.00	0.03	52	0.16	0.02	39	0.24	0.02	40	-0.63	0.17	37

MeHg in POREWATER

all sites	0.00	0.13	21	0.00	0.67	**16	0.47	0.01	19	0.61	0.02	21	0.003	0.02	21	-0.11	0.02	8	0.06	0.21	15	0.00	0.00	21	1.20	0.22	17	1.40	0.07	18	-0.60	0.10	18

*removed outlier for regression

** removed LCL extreme values for Hg_T in porewater

Table 3-2. Porewater total Hg and methylmercury concentrations for tailings samples collected at East Lake Catcha (ELC), Lake Catcha beach site (LCL), Lower Seal Harbour sites 1 and 2 (LSH-1 and LSH-2), First Pond (FP) and Upper Seal Harbour (USH). SE indicated in parenthesis where RSE > 10%. RSE < 10% for all Hg_T values. nd: not done.

Sample site	Year	Depth (cm)	Hg_T (nM)	MeHg (SE) (pM)
LSH-1	2003	22.5	nd	43.3
	2004	7.5	1.0	3.6
		17.5	1.8	3.5
		27.5	3.7	5.2
LSH-2	2003	12.5	0.7	3.2
		27.5	0.2	3.3
	2004	7.5	1.0	4.5
		17.5	0.6	1.2
		27.5	0.6	2.7
USH	2004	7.5	2.5	3.5
		17.5	5.1	9.0
		27.5	20.6	14.4
FP	2004	7.5	0.6	0.0
		17.5	0.7	2.1
		27.5	0.7	2.1
ELC	2003	2.5	4.2	15.0
		17.5	nd	19.4
		27.5	nd	11.4 (2.4)
LCL	2003	17.5	1125	51.9
		27.5	1108	34.7 (10.0)

Table 3-3. Water chemistry variables for surface water samples collected from the Lake Catcha and Upper/Lower Seal Harbour mining districts. SE indicated in parenthesis where RSE > 10%. nd: not done.

Location	Sample name	mean MeHg(SE) (pM)	mean Hg_T(SE) (pM)	DOC (mM)	pH	Eh (mV)	Temp (°C)
Seal Harbour Run (2003)	W1	7.2	57.1	3.28	5.3	415	23.9
	W2	7.8	73.3	3.31	4.8	472	19.3
	W3	8.7(1.4)	96.7	4.19	4.7	441	16.1
	W4	8.7	106.6	5.54	6.0	354	17.2
	W5	8.3(2.1)	90.7	7.72	6.6	254	20.9
	W6	4.7(0.7)	90.8	7.13	6.7	302	16.6
	W7	3.8(0.6)	48.3	7.10	6.4	286	17.5
	W8	4.8	44.5	6.76	6.5	328	19.2
	W9	3.5	44.3	7.09	6.5	328	18.5
	W10	4.6(1.6)	33.4	6.87	6.6	315	16.7
	W11	4.4	68.9	6.76	6.7	350	16.4
	W12	3.3	70.2	3.19	4.5	433	18.1
	W13	3.0	88.6	2.97	5.1	395	18.5
	W14	3.9	123.7	3.03	4.9	400	18.3
	W15	3.1	119.6	2.93	5.9	394	18.9
Seal Harbour Run (2004)	W1	6.3	48.0(7.9)	0.86	5.7	303	18.0
	W2	5.2	57.4	0.80	5.1	316	14.0
	W3	3.6	71.7	0.78	5.0	322	10.0
	W4	3.4	64.2	0.84	5.8	287	12.2
	W6	2.6	62.3	0.87	6.2	282	17.2
	W7	2.5	51.0	0.86	6.6	278	18.6
	W8	2.5	46.5	0.79	6.5	280	17.6
	W9	2.4	42.9	0.78	6.5	280	17.7
	W10	2.7	44.8	0.75	6.5	301	15.7
	W11	2.2	46.5	0.75	6.5	296	15.2
	W12	2.0	68.8	0.84	4.7	320	nd
	W13	2.1	65.8	0.81	5.2	284	13.2
	W14	2.2	63.5	0.80	5.4	303	14.6
	W15	1.9(0.4)	62.3	0.81	5.4	309	14.6
	W16	2.3	64.7	0.80	5.4	315	14.4
	W17	2.0	24.6	0.78	4.7	385	8.8
	W18	12.1	297.3	0.11	5.8	341	8.9
	W19	1.4	32.5	0.77	4.6	388	10.6
	W20	1.6	42.2	0.79	4.6	408	10.1
	W21	8.6	122.4	0.84	4.9	382	9.5
Lake Catcha (2003)	LC 16	3.1(0.6)	53.6	2.95	6.2	340	14.4
	LC Lake	2.4(0.2)	419.0	1.24	6.6	366	18.3
	LC Inlet	4.6(1.4)	11.8	4.88	6.1	275	16.7
	LC East	3.1	360.5	2.95	6.2	166	17.0

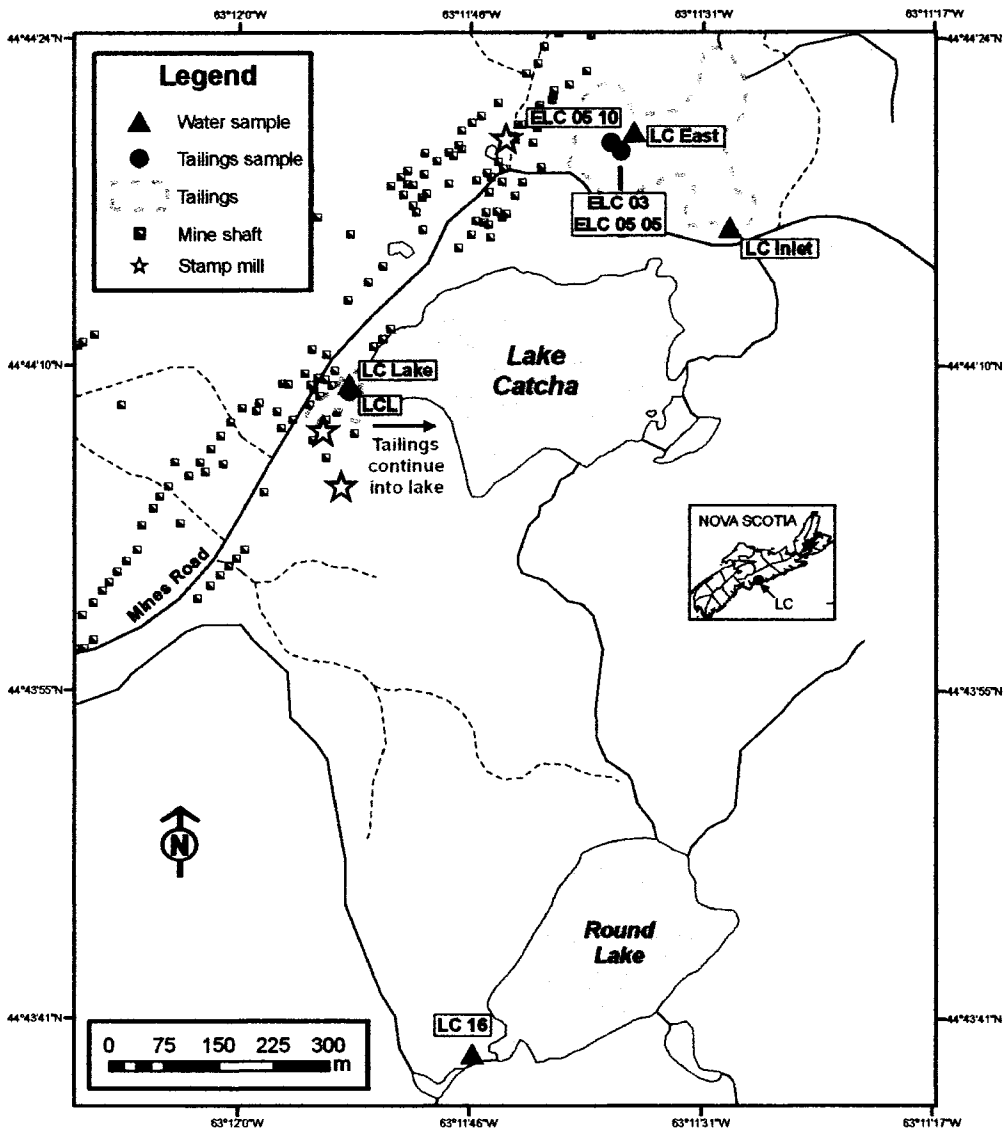

Figure 3-1. Map showing sample locations in Lake Catcha mining district, Nova Scotia.

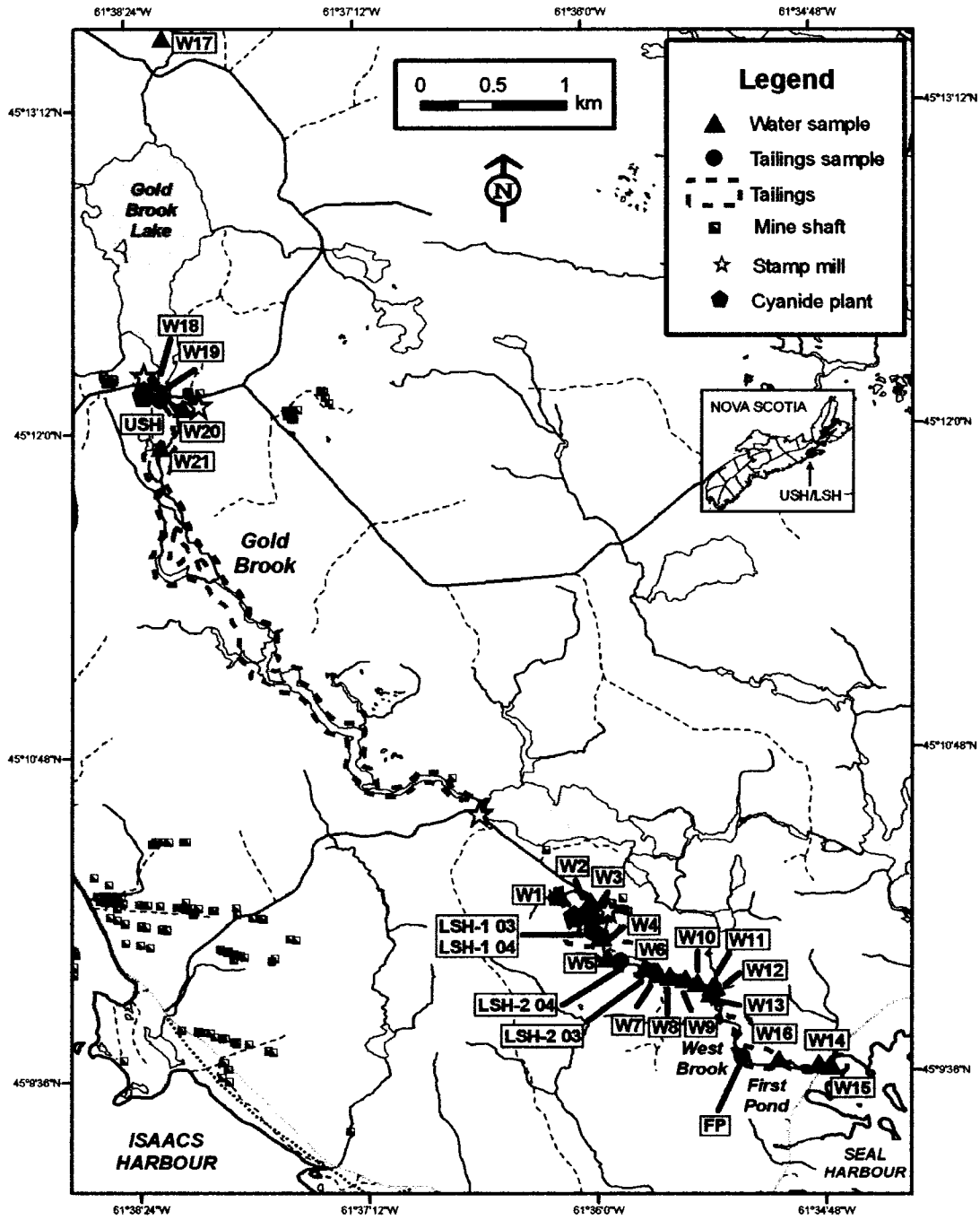

Figure 3-2.Map showing sample locations in the Upper Seal Harbour and Lower Seal Harbour mining districts, Nova Scotia.

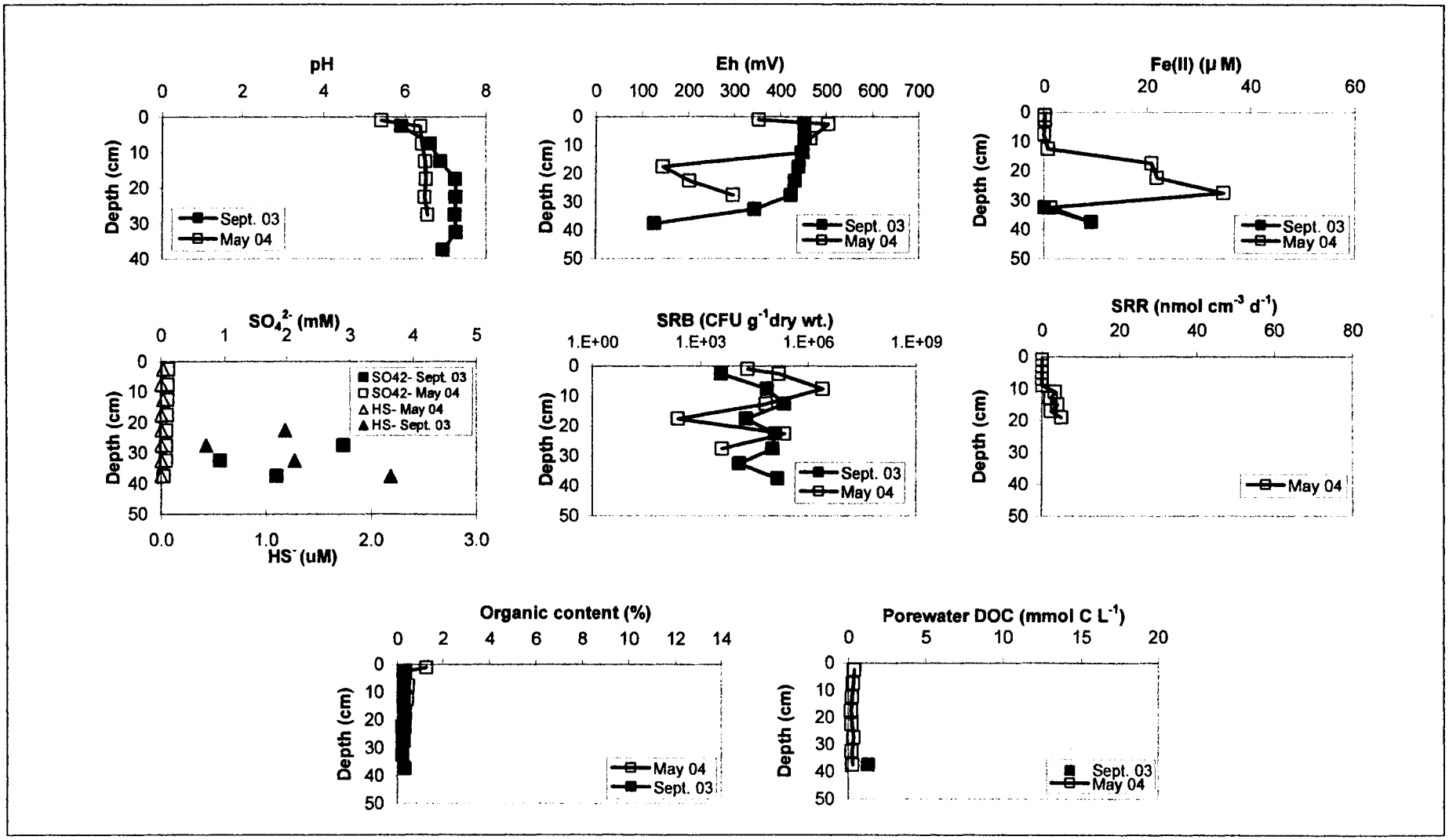

Figure 3-3. Depth profiles of geochemical parameters, SRB abundance and SRR measurements for LSH-1.

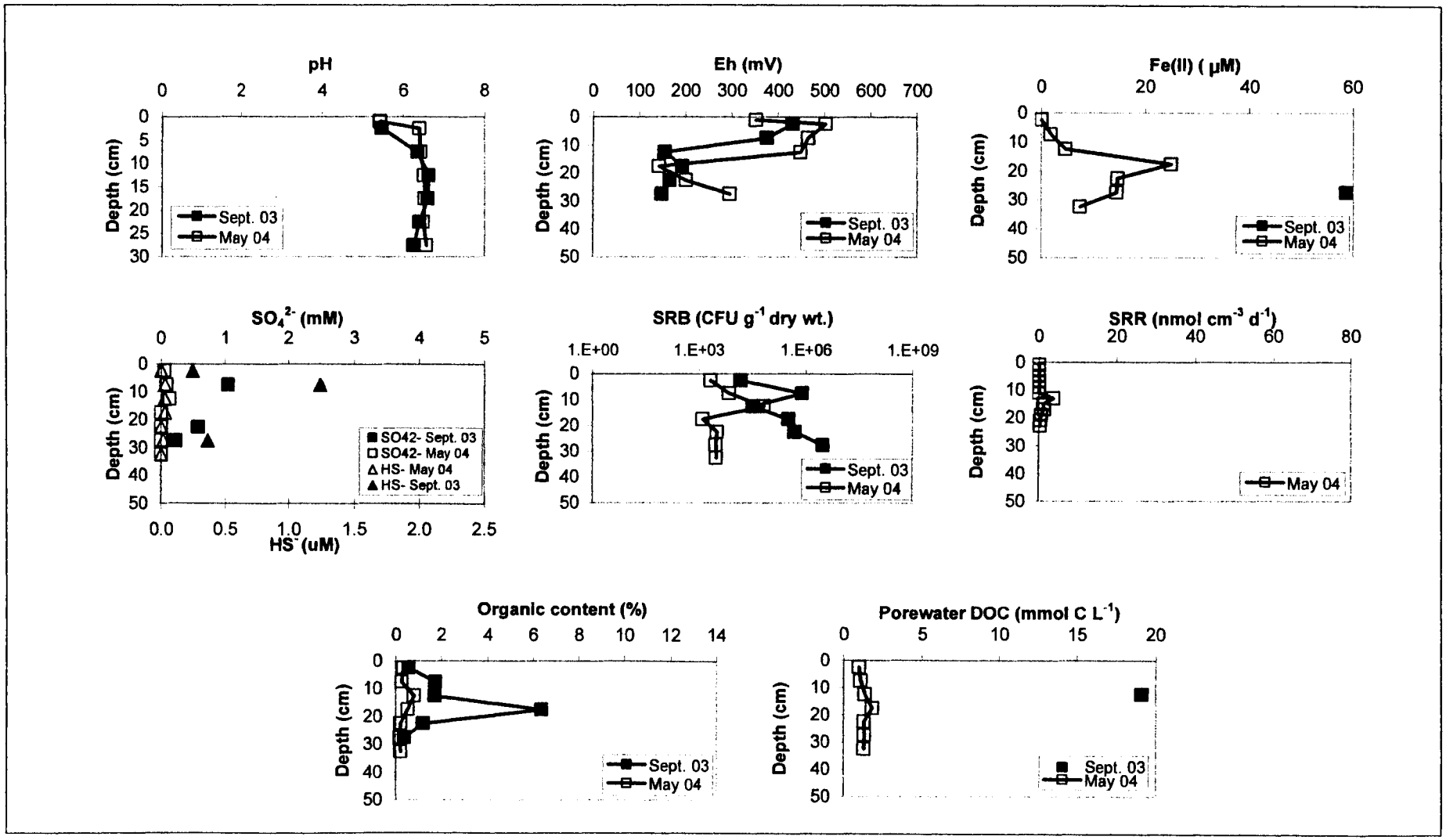

Figure 3-4. Depth profiles of geochemical parameters, SRB abundance and SRR measurements for LSH-2.

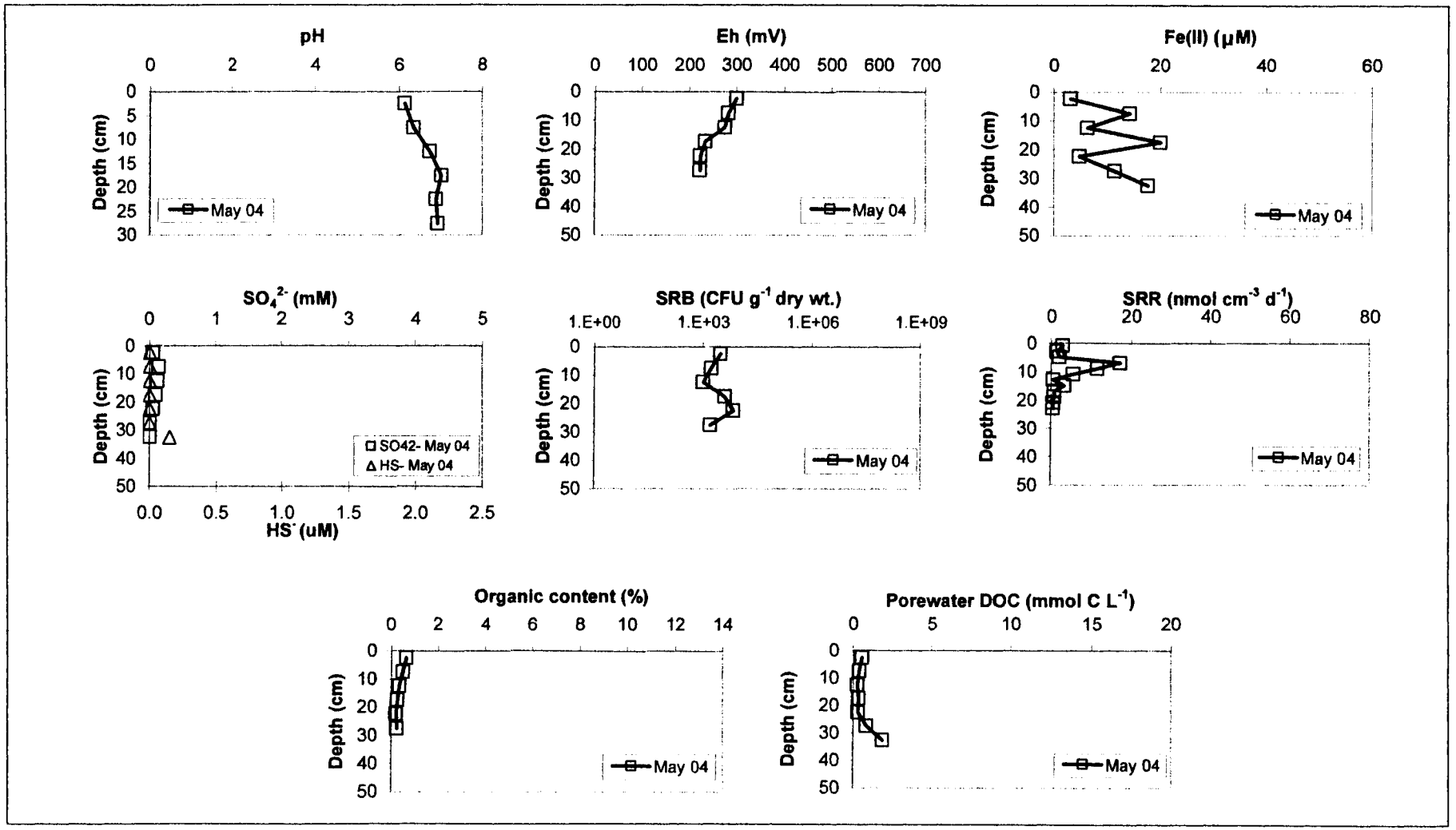

Figure 3-5. Depth profiles of geochemical parameters, SRB abundance and SRR measurements for FP.

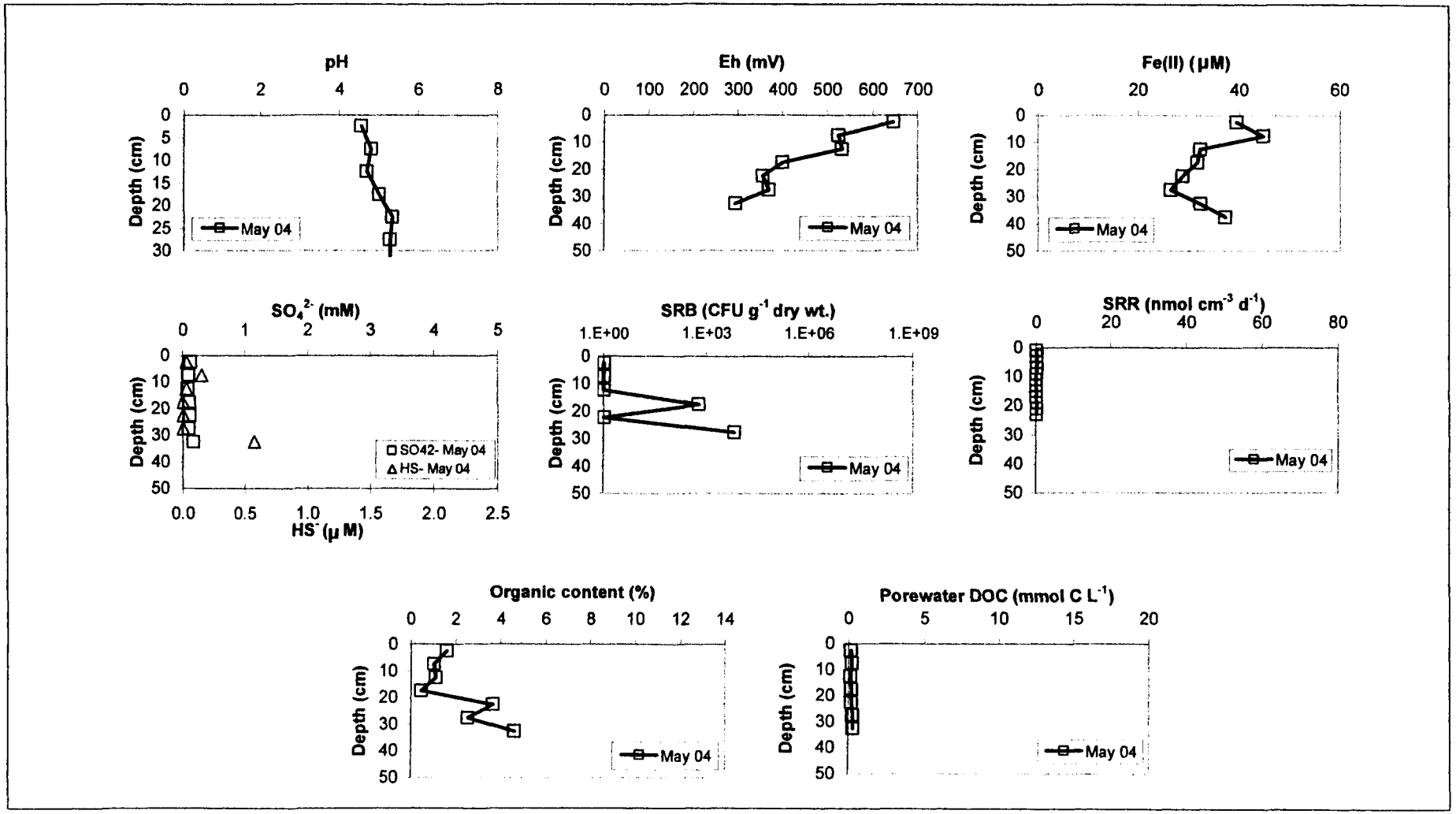

Figure 3-6. Depth profiles of geochemical variables, SRB abundance and SRR measurements for USH.

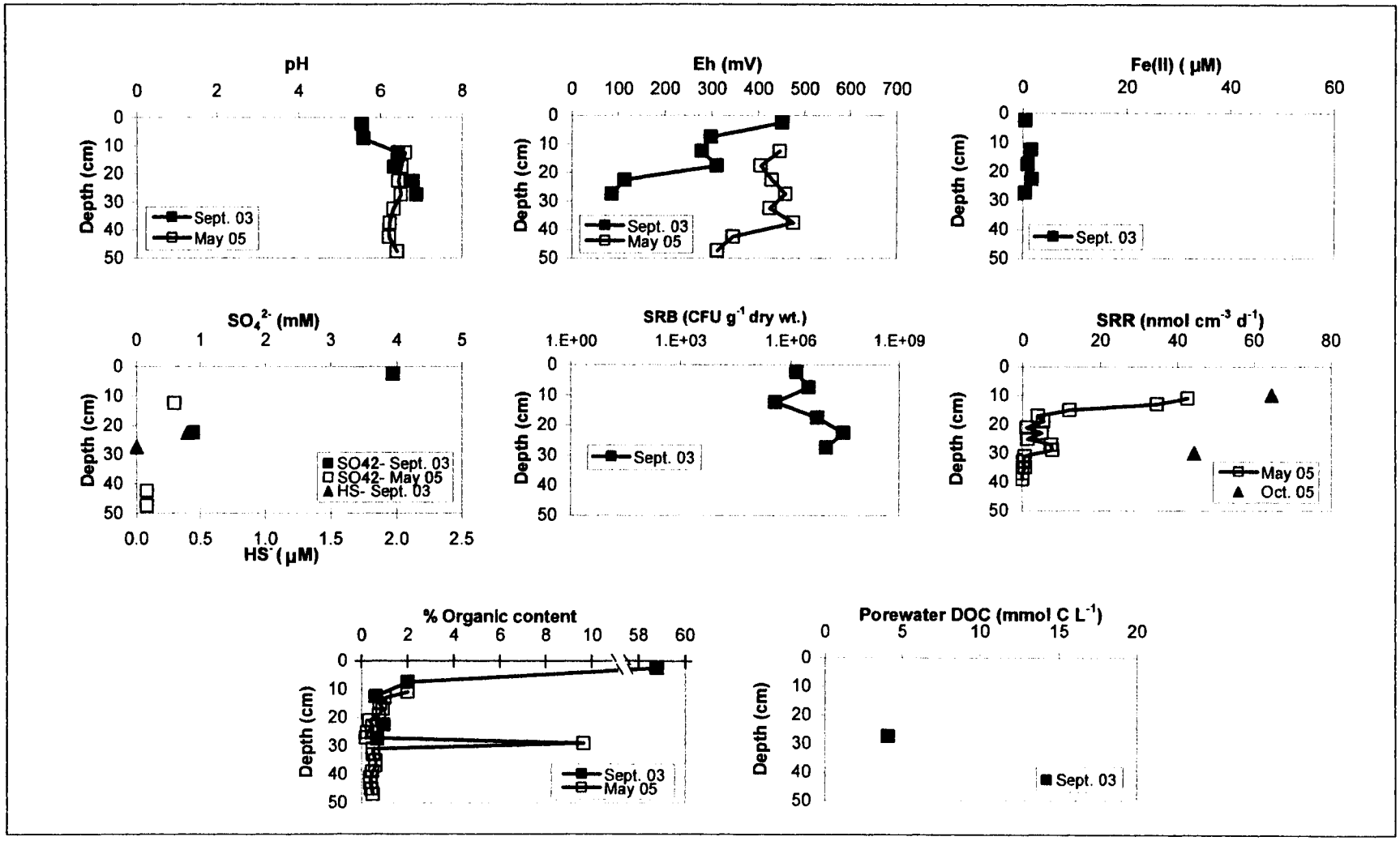

Figure 3-7. Depth profiles of geochemical parameters, SRB abundance and SRR measurements for ELC.

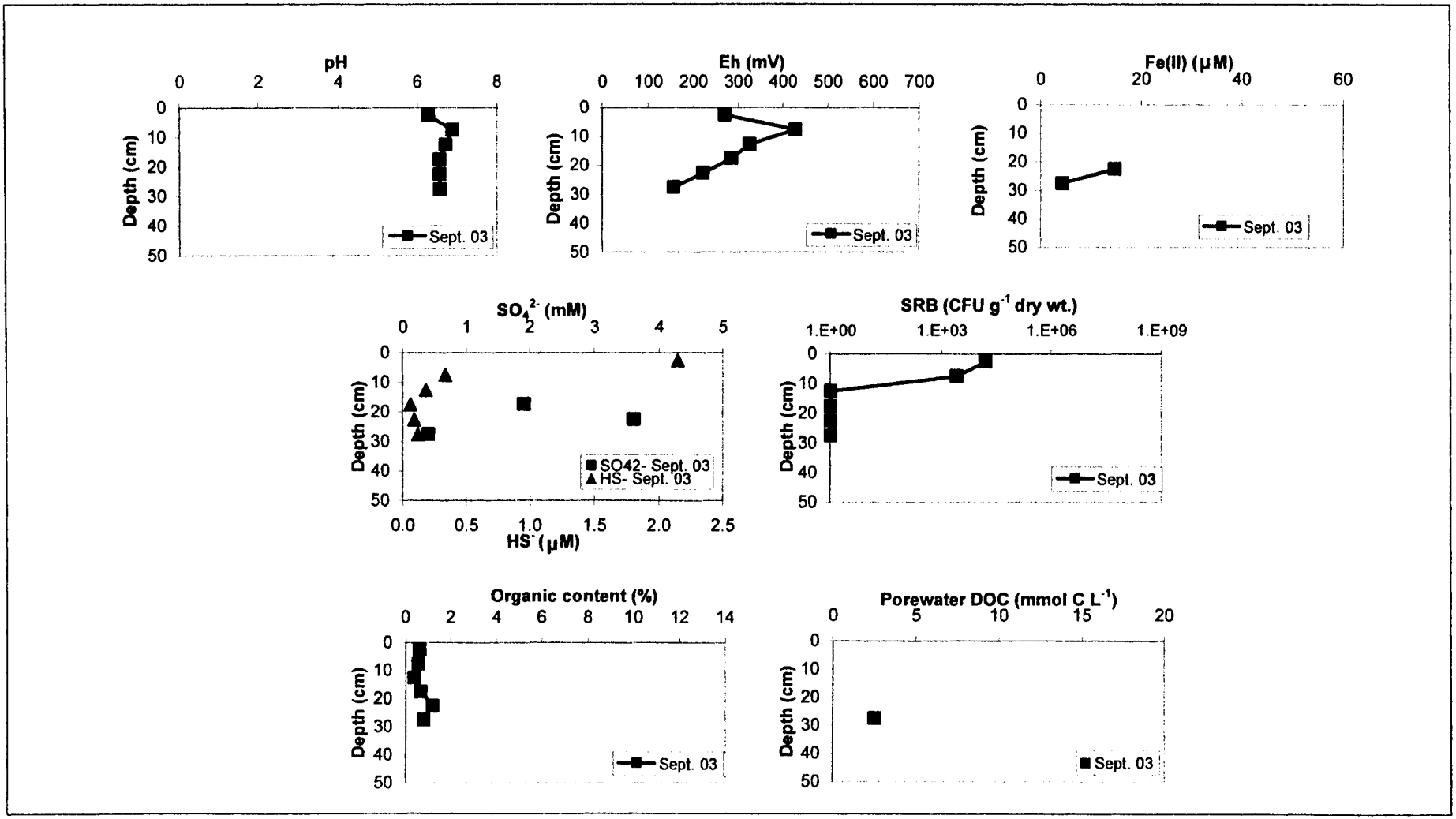

Figure 3-8. Depth profiles of geochemical parameters, SRB abundance and SRR measurements for LCL.

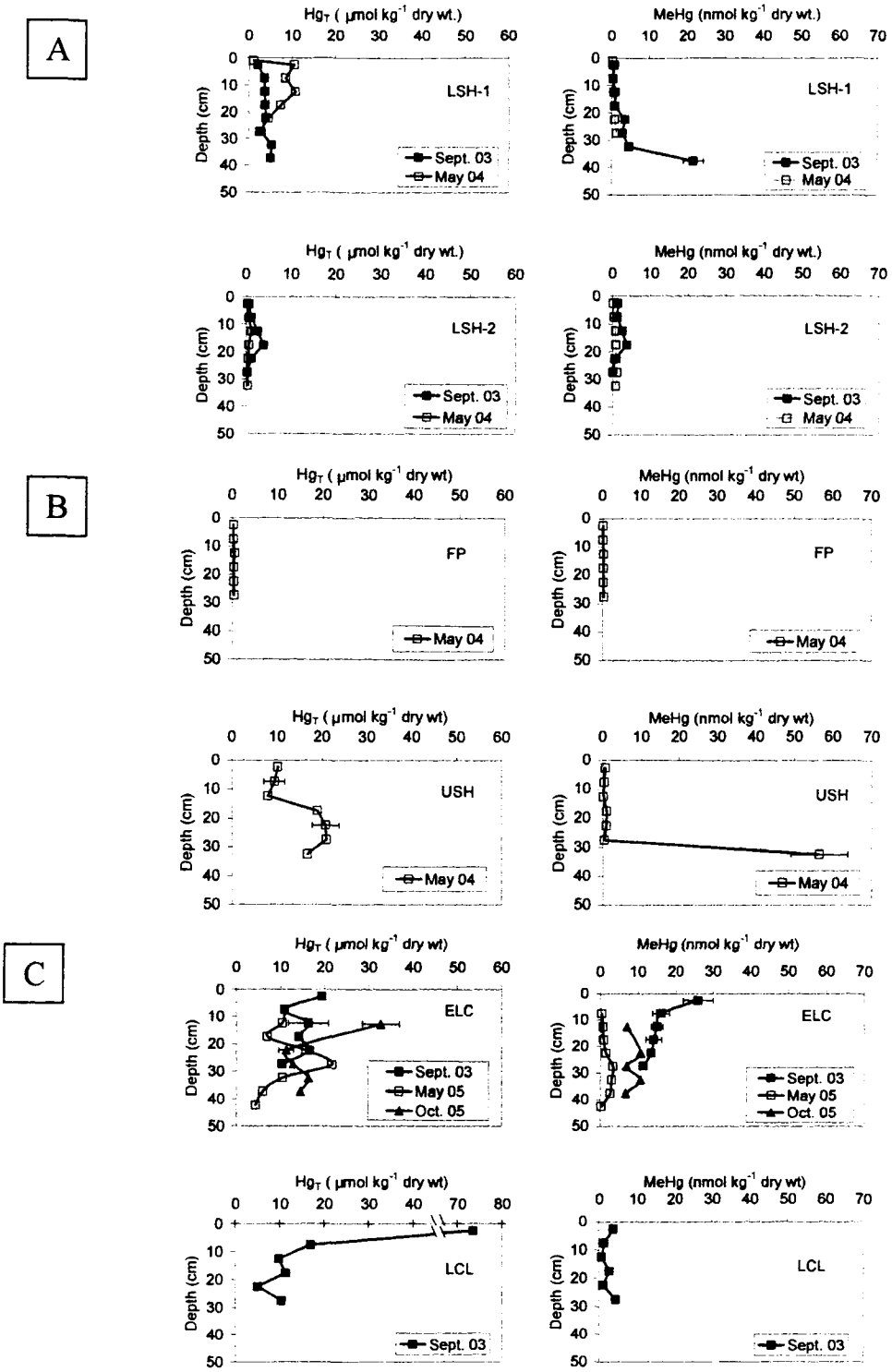

Figure 3-9. Depth profiles of total Hg (Hg_T) and methylmercury (MeHg) concentrations in bulk tailings. Error bars = SE (smaller than symbols except where shown; LCL 0-5 cm SE = 10.8).

CHAPTER 4: IDENTIFICATION OF SULFATE-REDUCING BACTERIA IN METHYLMERCURY CONTAMINATED MINE TAILINGS BY ANALYSIS OF SSU RIBOSOMAL RNA GENES*

* Adapted from Winch S., Mills H., Kostka J.E., Fortin D., Lean D. (in prep.)

4.1 INTRODUCTION

In the previous studies described in this thesis (Chapters 2 and 3), methylmercury (MeHg) contamination was evident in two geochemically distinct mine tailings dumps. Mercury methylation has repeatedly been linked to microbial activity in sediments, and specifically to the activity of sulfate-reducing bacteria (SRB) (Choi and Bartha, 1994; Compeau and Bartha, 1985; Compeau and Bartha, 1987; Devereux et al., 1996; King et al., 1999; King et al., 2000; King et al., 2001). Previous studies suggested conditions in acidic, oxidizing layers of mine tailings were not conducive to the growth of SRB, therefore Hg methylation in these environments was not considered. Indirect, culture-based cell count estimates and sulfate reduction rates (SRR) used in several studies (e.g. Fortin et al., 1995; Fortin et al., 2000; Fortin et al., 2002; Fortin et al., 1996; Praharaj and Fortin, 2004) later revealed that SRB are present and active in both pH-neutral gold mine tailings and in acidic base-metal tailings from the region surrounding Timmins, Ontario, raising the possibility that methylmercury (MeHg) might be produced at significant rates in mine tailings. Until now, however, no studies have examined bacterial nucleic acids extracted from gold and base-metal tailings to determine which SRB are present.

Chapters 2 and 3 of this thesis showed that SRB were present and active in both pH-neutral tailings from historical gold mining in Nova Scotia and acidic base-metal tailings from mining of Cu-Pb-Zn from massive sulfide ores in the vicinity of Timmins, northern Ontario. Tailings from one site in each of these regions featured markedly higher MeHg to total Hg (Hg_T) ratios than others from each corresponding region. MeHg content in the tailings varied

according to *in situ* conditions, e.g. total Hg concentration, organic content, sulfate concentration, hydrologic conditions and demethylation processes, the latter being in turn influenced by redox conditions. The study presented in this chapter sought to determine whether the MeHg contamination observed at these two sites might also be partially attributable to the presence of one or more SRB species common to both sites. Due to the low pH, low organic content (Chapter 2) and elevated metals (Al et al., 1994; Blowes et al., 1995) at the Kidd Metsite, and high levels of Hg (Chapter 3), arsenic and other metals in Nova Scotia gold mine tailings (Chapter 3, Wong et al., 1999, Wong et al., 2002), low overall biodiversity and few SRB species were expected at both sites.

Unlike other acidic sites studied (Chapter 2), which featured low SRR, low to negligible abundance of SRB and MeHg concentrations in the oxidized, acidic layers near the surface of the tailings, the Kidd Metallurgical site (Kidd) tailings featured a large peak in MeHg in both the bulk tailings and the porewaters, coinciding with a zone of high SRR. Although SRB were detected in these layers by indirect, culture-based cell counts (MPN), their abundance was not elevated. It was therefore hypothesized that these tailings might host a population of acidophilic sulfate-reducing bacteria that was highly active and produced MeHg, but that did not grow well in the circumneutral pH modified Postgate's medium used in the MPN cultures.

Due to the low pH in the Kidd tailings, dominant SRB were expected to be spore-forming, Gram-positive SRB, such as *Desulfotomaculum* spp.. On the other hand, MeHg was extremely elevated at this site, and since all known Hg methylators to date are Gram-

negative bacteria of the *Deltaproteobacteria* phylum, it was expected that one or more *Deltaproteobacteria* species would also be present.

In the pH-neutral gold mine tailings, *Deltaproteobacteria* were expected to dominate the SRB community. Further, since evidence for demethylation was found in Chapter 3, it was hypothesized that demethylating bacteria would be present.

In order to identify SRB in these two contrasting mine tailings environments, and to characterize their respective microbial community structures, two overlapping primer sets were used to extract, clone and sequence small subunit rRNA gene (DNA) sequences from mine tailings sediments. Clones were also derived from SRB enrichment cultures inoculated with mine tailings. After digest by restriction endonucleases, restriction fragment length polymorphism (RFLP) patterns were used to group together identical clones.

Results of sequencing, RFLP and phylogenetic analysis revealed that although most of the sequences derived from the two tailings environments could be grouped into common phyla, at the sub-phylum level the microbial community structures featured many novel species and reflected the unique properties of each environment. Biodiversity was not limited in the gold mine tailings, but was relatively much lower in the acidic tailings. SRB communities also differed between the two environments. The strong presence of two closely related novel acidophilic *Deltaproteobacteria* spp. was detected in the acidic tailings, while *Deltaproteobacteria* clones derived from gold mine tailings were found in small numbers

and were more closely related to several cultured lineages. Evidence of demethylating and Hg-resistant bacteria was also found in the gold mine tailings.

4.2 MATERIALS AND METHODS

4.2.1 Sample sites

Of the tailings deposits previously investigated (Chapters 2, 3), the Kidd Metsite (acidic base-metal site) and East Lake Catcha (pH-neutral gold tailings site) were chosen for more in-depth microbiological and molecular analysis. These samples were chosen based on the geochemical and initial microbiological analysis of tailings from four acidic tailings dumps in Northern Ontario (Kidd Metsite, Kam Kotia, Broulan and Potter mines), and six gold mine tailings dumps in Nova Scotia (East Lake Catcha, Lake Catcha Beach, Lower Seal Harbour #1, Lower Seal Harbour #2, Upper Seal Harbour and First Pond). Specifically, the two sites were selected based on their MeHg content and SRR.

Tailings from the Kidd Metsite (one of the four acidic, base-metal tailings dumps described in Chapter 2) featured high SRR corresponding with high MeHg in tailings (~ 2.5 ug kg^{-1}) and porewater (18 ng L^{-1}) in the acidic, oxic layers, in spite of low Hg_T (< 200 ppb) in the tailings. Indirect SRB cell count estimates by the most-probable-number method for the same sample were relatively low (< 6 x 10^4 CFU g^{-1} dry wt), but this might be attributable to the use of a pH-neutral, modified Postgate's medium for culturing cells which did not mimic

the acidic conditions *in situ*; if acidophilic sulfate reducers were present, they might not grow well in a pH-neutral environment.

Of the six Nova Scotia gold tailings dumps described in Chapter 3, the East Lake Catcha (ELC) site featured high Hg_T levels (10-30 μmol kg^{-1}) and a MeHg : Hg_T ratio of ~ 1:1000, therefore MeHg concentrations were > 5 nmol kg^{-1} in the tailings. The MeHg : Hg_T ratio was unusually high compared with other high-Hg_T gold tailings, probably due to MeHg demethylation by Hg-resistant bacteria in more aerobic, high-Hg_T tailings (Schaefer et al., 2002). This site also had the highest sulfate reduction rates (≤ 64.5 nmol cm^{-3} of sediment d^{-1}) and SRB MPN cell count estimates ($3.8 \times 10^5 - 2.9 \times 10^7$ colony forming units g^{-1} dry wt of sediment) of all the gold mine tailings dumps studied.

Frozen field samples from the Kidd Metsite obtained in August, 2004 and cultures grown from a fresh sample of oxidized tailings obtained in June 2005 from the same location were used for DNA analysis. A fresh core of gold tailings from ELC was obtained in October 2005 and immediately used to inoculate SRB enrichment culture medium. Remaining samples were frozen for DNA extraction and Hg_T and MeHg analysis.

4.2.2 Culturing

Enrichment cultures inoculated with gold mine tailings (nSRB) were grown in pH-neutral freshwater medium (Widdel and Bak, 1992) containing 28 mM sulfate, 2.5 mM $FeCl_2$ and a final concentration of 10 mM lactate or acetate. Medium was inoculated under anaerobic

conditions with 1 mL tailings, then serially diluted in tenfold steps. Cultures were incubated at 30°C and monitored for signs of cell growth. SRB growth was indicated by the development of a black precipitate, formed when sulfide produced through sulfate reduction reacted with reduced iron in the medium. Cultures from the highest dilutions with observable growth were transferred to fresh medium under anaerobic conditions and incubated at 30°C.

Base-metal tailings from the oxidized, acidic upper layer of the Kidd Metsite tailings were used to inoculate acidic medium to enrich for acidophilic (or acid-tolerant) sulfate reducers (aSRB). Enrichment cultures were inoculated under anaerobic conditions and grown in a basal salts/yeast extract medium based on Sen and Johnson (1999), with glucose as electron donor, and incubated at 30°C. Once growth was observed, cultures were transferred into fresh medium with glucose, ethanol or glycerol as electron donor and incubated until growth was observed.

4.2.3 DNA isolation from tailings sediments

The Kidd Metsite sample was taken from a depth of 5-10 cm to correspond to the zone of greatest MeHg concentration in the tailings and porewaters. For the ELC site, samples from 10-15 cm and 30-35 cm depth were selected for DNA analysis.

Prior to DNA extraction, glassware and plastics were either autoclaved or baked at 250°C for 24 h. Genomic DNA was extracted from tailings sediments using the Hurt et al. (2001)

method from 2 g (wet weight) of sediment. In brief, sediment samples stored at -20°C were repeatedly thawed during physical grinding in the presence of a denaturing solution (4 M guanidine isothiocyanate, 10 mM Tris-HCl [pH7.0], 1 mM EDTA, 0.5% 2-mercaptoethanol) and refrozen by immersion in liquid N_2. The sediment samples were incubated for 30 min at 65°C in pH 7.0 extraction buffer (100 mM sodium phosphate [pH 7.0], 100 mM Tris-HCl [pH 7.0], 100 mM EDTA [pH 8.0], 1.5 M NaCl, 1% hexadecyltrimethylammonium bromide [CTAB], and 2% sodium dodecyl sulfate [SDS] and centrifuged (1,800 x g for 10 min). The supernatant was extracted with 24:1 (vol/vol) chloroform-isoamyl alcohol, and centrifuged (1,800 x g for 20 min). The nucleic acids were precipitated at room temperature with isopropanol (30 min), pelleted by centrifugation (16,000 x g for 20 min), resuspended in water, and subsequently purified into DNA-only aliquots (Hurt et al. 2001) using the QIAGEN DNA/RNA kit (Midi).

4.2.4 DNA isolation from cultures

DNA was extracted from liquid cultures for use as template in a standard PCR. Cells were pooled from 2-6 replicates of each culture type (aSRB with ethanol, aSRB with glucose, nSRB with lactate and nSRB with acetate, as described above). DNA was isolated using an UltraClean Soil DNA Isolation Kit (MoBio).

4.2.5 PCR amplification with SSU rRNA gene-specific primers

The DNA extracted from sediments and cultures was used as the template for a standard PCR using *Bacteria* domain-specific forward and reverse primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Johnson, 1994) and 1392R (5'-ACGGGCGGTGTGTRC-3') (Wilson et al., 1990), respectively. Each PCR amplification mixture contained 0.3 μL Taq polymerase (5 U μL^{-1}) (TaKaRa Shuzo), 5 μL of each primer (5 μM), 5 μL dNTP mixture (supplied) (10X), 5 μL of 10X LA PCR buffer (supplied) and 1 μL of DNA template. The mixture was adjusted to a final volume of 50 μL with distilled water. 16S rRNA gene amplification reactions were cycled in an Eppendorf thermocycler.

PCR product was visualized using gel electrophoresis (0.7% SeaKem LE agarose gel in 0.5X TBE buffer stained with ethidium bromide and illuminated with UV) and compared with molecular size standards to determine the presence of the desired size fragment. For cloning and sequencing with *Bacteria* domain-specific SSU rRNA gene primers, 20 μL of PCR product was used in gel electrophoresis, and the relevant bands were excised from the gel and purified using Qiaex II gel extraction kit, according to the manufacturer's recommendations.

To increase detection of *Deltaproteobacteria*, primers specific to this class were also used to amplify DNA from tailings and cultures. The forward and reverse primers 385F (5'-CTG ACG CAG CRA CGC CG-3') and 1492R (5'-TAC GGY TAC CTT GTT AYG ACT T-3') were extensively tested by Klepac-Ceraj et al. (2004) for their specificity to a broad range of

Deltaproteobacteria. 47% of clones detected with these primers were related to *Deltaproteobacteria*, but other groups were also detected due to the broad specificity of the 385F primer, which was designed to cover all known SRB groups within the *Deltaproteobacteria* (Klepac-Ceraj et al., 2004). This primer set, however, remains the most specific for detection of *Deltaproteobacteria*-related sequences. *Deltaproteobacteria* gene amplification reactions were cycled in an Eppendorf thermocycler. PCR product was visualized using gel electrophoresis and eluted from the gel as described above in preparation for cloning.

In order to detect Gram-positive SRB, we attempted to amplify DNA derived from tailings and cultures using primers specific for *Desulfotomaculum* spp. (forward and reverse primers DFM 140-158, 5'-TAG MCY GGG ATA ACR SYK G-3', and DFM 842, 5'-ATA CCC SCW WCW CCT AGC AC-3', respectively). No significant amplification was detected from any samples using these primers.

For cloning and sequencing using *Deltaproteobacteria* primers, only 5 μL of PCR product was subjected to gel electrophoresis for visualization, to confirm the presence of bacterial DNA. PCR amplification of DNA (from the clean-up stage) was then done using 385F/1492R primers. DFM140/842 (Daly et al., 2000) were used for Gram-positives, but in all samples, the quantity of the corresponding band that was produced was insufficient to proceed with cloning.

4.2.6 Clone library construction

Purified pooled *Bacteria* domain specific or *Deltaproteobacteria* amplicons were cloned into the TOPO TA cloning vector pCR2.1 according to the manufacturer's instructions (Invitrogen). Inserts were subsequently PCR amplified from lysed colonies with primers specific for the vector, M13F (5'-GTAAAACGACGGCCAG-3') and M13R (5'-CAGGAAACAGCTATGAC-3'), and then were digested (2 h, 37°C) with the restriction enzymes *Hae*III and *Msp*I.

Restriction digest profiles were analyzed using 2% agarose gel electrophoresis (SeaKem LE agarose). Clones from each library were grouped according to their restriction fragment length polymorphism (RFLP) patterns into phylotypes. Prior to sequencing, DNA from selected clones was purified using the QIAquick PCR purification kit and then sequenced in both forward and reverse directions to provide 2x coverage. In highly diverse samples, a representative from each phylotype representing multiple identical RFLP patterns was sequenced to assure phylotype integrity. A random selection of those phylotypes represented by single RFLP patterns were also sequenced. Prior to comparative sequence analysis, vector sequences flanking the inserted amplicon were manually removed.

4.2.7 Sequence analysis

Contiguous sequences were assembled with the program BioEdit v7.0.0 (Hall, 1999). Previously identified sequences with high sequence similarity to the clones obtained in this

study were determined using the BLAST algorithm against the GenBank database available from National Center for Biotechnology Information (NCBI) (Altschul et al., 1990). Clone sequences were checked for chimeras using the program Chimera Check from the Ribosomal Database Project II (Cole et al., 2003). All clone sequences and reference sequences were aligned in the ARB software package using the Fast Aligner algorithm, incorporating ribosomal secondary structure data (Strunk and Ludwig, 1997). Neighbor-joining trees incorporating a Jukes–Cantor distance correction were created from the alignments using the ARB software package (Strunk and Ludwig, 1997). Bootstrap data represented 1000 samplings. An application to GenBank to obtain nucleotide sequence accession numbers is in progress.

4.2.8 Statistical analysis

Statistical analyses were used to determine the sampling efficiencies and diversity differences within clone libraries based on RFLP analysis. Rarefaction curves were calculated using Analytic Rarefaction 1.3 (Heck et al., 1975; Holland, 2003). EstimateS (Colwell et al., 2004) was used to estimate species richness nonparametrically with Chao1 and to calculate the Shannon–Wiener and the reciprocal of Simpson's (1/D) indices. The percent coverage (C) of the clone libraries was calculated according to the equation $C = [1 - (n_1/N) \times 100]$, where n_1 is the number of unique clones as determined by RFLP analysis and N is the total number of clones in the library (Mills et al., 2004; Good, 1953). Clone library sequence data were used to compare phylogenetic diversity between samples. Clone

sequence diversity indices for gene and nucleotide diversity (Nei, 1987), and $\theta(\pi)$ (Tajima, 1983) were calculated using Arlequin (Schneider et al., 2000).

4.2.9 X-ray diffraction

The precipitates formed by SRB cultures inoculated from acidic mine tailings and grown in acidic cultures under anaerobic conditions (aSRB) were analyzed using X-ray diffraction (XRD). Culture tube stoppers were first pierced with two 22-gauge syringe needles. The tubes were immediately placed into vacuum bottles, sealed and attached to the freeze-drier, which removed all the air from the bottles and tubes. The precipitates were allowed to dry for 3 days, after which the vacuum was released while flushing the freeze-drier intake with N_2 gas to avoid contact with oxygen. Tubes containing dried precipitates were also flushed with N_2 gas before removal of the syringe needles from the stoppers. The tubes were also sealed with parafilm to prevent gas exchange after removal of the needles. XRD analysis was performed using a Philips X'Pert diffractometer, with a Cu Kα source operating at 45 kV and 40 mA, and a Kevex Si (Li) solid-state detector. The sample holder for the XRD was loaded with precipitates in an anaerobic chamber, and then placed into a 2 L wide-mouth bottle and sealed before removal from the anaerobic chamber for transport to the goniometer. The goniometer was outfitted with a gas line and attachment so the sample holder could be continuously flooded with N_2 gas during the operation of the instrument. All samples were run in step-scan mode at 0.02°/60s from 5° to 75° (2θ).

4.3 RESULTS

4.3.1 RFLP and statistical analysis of clone libraries

Nucleic acids were successfully extracted from mine tailings sediment samples and sulfate-reducing bacteria (SRB) enrichment cultures inoculated with mine tailings. Clone libraries were constructed using amplified DNA from SSU rRNA gene targets (Tables 4-1, 4-2).

In total, 502 clones were generated - 266 from gold mine tailings sediments and enrichment cultures, and 236 from acidic base-metal mine tailings sediments and cultures. RFLP analysis of the DNA derived clones indicated 120 distinct phylotypes were detected using *Deltaproteobacteria*-specific primers (94 from gold sediments and cultures; 26 from acidic sediments and cultures), and 110 phylotypes with *Bacteria* primers (84 from gold sediments and cultures; 26 from acidic sediments and cultures).

Both acidic tailings and gold mine tailings were highly biodiverse, as indicated by species richness and biodiversity indices (Table 4-1). The gold mine tailings, however, were far more biodiverse than the acidic tailings. Additional sampling of gold tailings clones at the domain level would be necessary to fully describe the overall diversity, as rarefaction curves (Figure 4-1) did not indicate saturation (i.e. the slope was greater than zero - Heck et al., 1975), and percent coverage was $\leq$ 15%. Full description of the diversity in the gold mine tailings, however, was beyond the scope of this study, particularly since the intent was to identify sulfate-reducing bacteria, which appeared to constitute a relatively minor portion of

the overall microbial community structure. Sufficient sampling was carried out to establish the novelty and diversity of this environment, as shown by the indices of diversity and species richness (Table 4-1), and by the number of species that were <95% similar to cultured lineages (Table 4-2).

4.3.2 Results from the two primer sets

Comparative analysis of libraries from *Bacteria* primers and from *Deltaproteobacteria* primers indicated that a total of 28.6% of phylotype sequences obtained from *Bacteria* primer derived clone libraries had sequence similarity ≥ 96% to *Deltaproteobacteria* primer-derived phylotype sequences. Further, 11.4% of phylotype sequences obtained from sediments had 93-96% sequence similarity to sequences obtained from cultures. One RFLP pattern derived from gold mine tailings sediments matched one pattern from the acidic sediments, and one RFLP pattern from a gold-tailings inoculated culture matched one pattern from a culture inoculated with acidic tailings.

4.3.3 Phylogenetic analysis of the clone libraries

Both environments contained numerous species that were < 95% similar to known, cultured organisms. 33% of all sequences obtained from both primer sets were <90% similar to cultured lineages. An additional 39% were 90-94% similar to cultured lineages and only 28% were >95% similar to sequences derived from cultured organisms registered in the NCBI database (Table 4-2). Three *Deltaproteobacteria* sequences could only be classified at

the class level by ARB (Figure 4-2). An additional four sequences were 94-99% similar to clones from candidate divisions.

Primers specific for *Bacteria* detected phylotypes related to the *Proteobacteria* (*Delta*, *Beta* and *Gamma* classes), *Nitrospirae*, *Acidobacteria*, *Chloroflexi*, *Firmicutes* and *Actinobacteria*. *Deltaproteobacteria*-specific primers detected sequences most closely related to the *Deltaproteobacteria*, as well as *Nitrospirae*, *Acidobacteria*, *Firmicutes*, *Chloroflexi*, *Planctomycetes*, *Actinobacteria*, Candidate Divisions OP-8, WS-1 and OP-10.

Sediments and cultures from both environments contained sequences most closely related to the *Delta-* and *Gammaproteobacteria*, *Nitrospirae*, *Acidobacteria*, *Firmicutes* and *Actinobacteria*. In addition, sequences related to Candidate divisions OP-8, WS-1 and OP-10, *Chloroflexi*, *Betaproteobacteria* and *Planctomycetes* were detected only in gold mine sediments and corresponding cultures (Table 4-2, Figures 4-2, 4-3, 4-4).

Among the gold mine sediment sequences, no clear dominant phylotype could be discerned. Equal proportions (29% of all gold mine sediment derived clones) of sequences obtained using *Bacteria*-specific primers corresponded to the Gram-positive phyla *Actinobacteria* and *Firmicutes*, with smaller contributions from the other phylotypes. The largest proportion (22%) of sequences obtained using *Deltaproteobacteria*-specific primers were, not surprisingly, identified as *Deltaproteobacteria* sequences, followed by *Nitrospirae* and *Firmicutes* (21% each). (Calculations were based on the total number of clones associated with phylotypes of sequenced representatives.)

The dominant phylum detected in the acidic, base-metal sediment-derived clone libraries using *Bacteria*-specific primers was *Firmicutes* (55%), followed by *Actinobacteria* (24%), *Acidobacteria* and *Proteobacteria* (10% each). As in the gold mine sediment derived sequences, *Deltaproteobacteria* comprised the dominant group (55%) among sequences detected in acidic sediments using *Deltaproteobacteria*-specific primers. An additional 33% were most closely related to the *Firmicutes* phylum, 6% branched with the *Nitrospirae* and 4% with *Acidobacteria*.

Although many of the same phyla were detected in both types of tailings sediments and associated cultures, closer examination reveals that the genus- and species-level associations of the sequences detected in this study reflect the characteristics of two quite different environments. Specifically, sequences derived from pH-neutral gold mine sediments and cultures are characteristic of either uncontaminated soils, sediments and groundwaters or environments contaminated with heavy metals and arsenic. All sequences derived from the acidic, base-metal tailings, on the other hand, were found to be closely related to cultured lineages typically found in acid mine drainage situations. The following is a phylum-by-phylum comparison of the sequences extracted from the two types of tailings and cultures, which demonstrates the distinctiveness of each environment and the overall novelty of organisms detected in both of these under characterized environments.

4.3.3.1 *Proteobacteria*

With the exception of one clone that could not be classified beyond the class level, *Deltaproteobacteria* sequences derived from gold mine sediments were related to known lineages (*Syntrophobacter sulfatireducens* – 93% similar; *Syntrophus gentianae* – 93% similar; *Desulfobacca acetoxidans* – 90% similar), all of the order *Syntrophobacterales* (Figure 2). The nearest relatives (≥95% similarity) of the gold mine sediment derived clones, however, were unclassified clones detected in freshwater sediments (Nercessian et al., 2005; unpublished study), in Pb-, Cr- and organic-contaminated soils (Joynt et al., 2006) and in uranium mine wastes (unpublished study, NCBI database). The one clone that was not closely related to any cultured lineage was 97% similar to a reed bed reactor biofilm clone (unpublished study) and 96% similar to a *Deltaproteobacteria* clone isolated from an aquifer contaminated with hydrocarbon and chlorinated solvents (Dojka et al., 1998).

One *Deltaproteobacteria*-related phylotype detected by *Bacteria*-specific primers represented 86% of clones derived from acetate-amended cultures enriched for sulfate-reducing bacteria (SRB) and inoculated with gold mine tailings. It was 99% related to *Desulfovibrio ferrireducens* (str. CY2) (Table 4-2), a newly cultured species (Vandieken et al., 2006). *D. ferrireducens* is a motile, Gram-negative organism that can use lactate, formate, hydrogen, ethanol, propanol, fumarate and succinate as electron donors and sulfate, thiosulfate and sulfite as electron acceptors. It is also able to reduce Fe(III) in the form of Fe(III) oxide and Fe(III) citrate, without growth, and to disproportionate malate, pyruvate and fumarate. Its pH range is 6.3-7.5 and temperature growth range is between -2 and 30°C.

Strain CY2 was isolated from Lake Coeur d'Alene, an environment historically impacted by lead and silver mining, and has the capacity to tolerate high concentrations of heavy metals (Ramamoorthy, 2005). Other *Desulfovibrios* are able to methylate Hg: two of the six strains of *D. desulfuricans* tested to date produced detectible MeHg, and one other *Desulfovibrio* species (*D. africanus*) was also demonstrated to methylate Hg (Gilmour et al., 2006). No methylation studies have yet been done with *D. ferrireducens*.

In contrast with the gold mine sediments, the two *Deltaproteobacteria* phylotypes (K5_62 and K5_114) that jointly comprised 55% of clones derived from acidic mine sediments with *Deltaproteobacteria*-specific primers were not closely related to any cultured lineages. K5_62, however, was 99% similar to a clone detected in acidic sediment at an extreme acid mine drainage site in China (unpublished study in NCBI database), 99% similar to three clones isolated from an acidic (pH 2.5-3) reject coal pile (Brofft et al., 2002) and 98% similar to a clone extracted from Iron Mountain, California, a well-known extreme acid mine drainage environment (Bond et al., 2000). K5_114 was 99% similar to clones detected in two separate studies on Iron Mountain (Druschel et al., 2004; Bond et al., 2000). Clones K5_65 and K5_114 were 95% similar to each other.

Gammaproteobacteria-related sequences were extracted from both types of mine tailings sediments, and from the ethanol-amended cultures inoculated with acidic mine tailings (Figure 4-4, Table 4-2). All *Gammaproteobacteria* phylotypes were detected by the *Bacteria*-specific primer set. Not surprisingly, the two phylotypes detected in the acidic tailings and cultures were both 99% similar to *Acidithiobacillus ferrooxidans*, a well-known

Fe-oxidizer frequently implicated in the generation of acid mine drainage. The *Gammaproteobacteria*-related phylotype extracted from gold mine tailings sediments was 99% similar to *Acinetobacter johnsonii*, a neutrophilic organism tolerant of high concentrations of divalent metals (Boswell et al., 1999). Mercury-resistant strains of *Acinetobacter* have been isolated from numerous mercury mines in Russia (Kholodii et al., 2004). *Acinetobacter* spp. isolated from Hg-enriched soils have been shown to carry the *mer* operon (Khesin and Karasyova, 1984; Osborn et al., 1993; Osborn et al., 1997), as does *Acidithiobacillus ferrooxidans* (Olson et al., 1982; Shiratori et al., 1989).

4.3.3.2 *Firmicutes*

The *Firmicutes*-related sequences derived from gold mine sediments were most closely related (94-99% similar) to *Clostridium* spp. (*C. papyrosolvens* and *C. aurantibutyricum*, both cellulose degrading anaerobic sediment bacteria; Leschine 1995; Tamburini et al., 2003), to *Bacillus* spp. (common soil bacteria; Tzeneva et al. 2004), to clones detected in uranium-contaminated soil (Brodie et al., 2006) and to an organism derived from anoxic rice paddy soil (Hengstmann et al., 1999) (Figure 4-3). Several *Bacillus* spp. carry the *merB* gene and are capable of demethylating MeHg (Rochelle et al., 1991).

All of the *Firmicutes*-related phylotypes detected by both primer sets in acetate-amended SRB enrichment cultures inoculated with gold mine tailings were ≥ 96% similar to *Desulfosporosinus* spp., which are endospore-forming sulfate reducers. 96% of these clones were most closely related to *Desulfosporosinus* sp. Y5, a motile rod that is Gram-negative,

although phylogenetically it falls within the *Desulfosporosinus* genus (Liu et al., 2004), which primarily includes Gram-positive *Firmicutes*. It has previously been demonstrated that sp. Y5 is capable of dissimilatory arsenate reduction (Liu et al., 2004; Perez-Jimenez et al., 2005). Arsenic concentrations in the East Lake Catcha tailings are unknown, but geochemically similar gold mine tailings from Nova Scotia have been shown by other researchers to contain up to 3% wt/wt As (Wong et al., 1999; Wong et al., 2002) and nearby Lake Catcha beach tailings contained 0.09-0.94% As (unpublished data). These concentrations would likely select for *Desulfosporosinus* sp. Y5.

The largest proportion of *Firmicutes*-related phylotypes detected in acidic tailings sediments with both primer sets was 92% similar to *Alicyclobacillus pomorum* (Figure 4-3, Table 4-2), a thermophilic (optimum 45-50°C), heterotrophic, endospore-forming, strictly aerobic, Gram-positive, acidophile (optimum pH = 4.0-4.5) (Karavaiko et al., 2005). These phylotypes had as their closest relatives (92-93% similarity) two clones from separate studies, one of which was a Gram-positive, iron-oxidizing acidophile classified as *Alicyclobacillaceae*, and the other was implicated in the bio-oxidation of refractory gold ore (unpublished study, NCBI database). Clones derived from the oxidized tailings of an abandoned copper mine in Portugal contained sequences 89% related to *A. pomorum* (Bryan et al., 2006). Other *Alicyclobacillus*-related species have been shown to reduce Fe(III) and/or oxidize Fe^{2+}, S^0 or sulfide minerals (Karavaiko et al., 2005). Some differences are clearly evident between *A. pomorum* and the *A. pomorum*-type phylotypes isolated in this study, as the latter were cultured under anaerobic and mesophilic (30°C) conditions. None

have previously been grown under anaerobic conditions. This phylotype may therefore constitute a new species associated with acid mine drainage generation.

An additional *Firmicutes*-related phylotype detected in the acidic tailings sediments was 93% similar to *Desulfosporosinus orientis* (Figure 4-3, Table 4-2), a Gram-positive, endospore-forming sulfate reducer. A phylotype 96% related to *Desulfosporosinus orientis* was found in acidic lakes created by coal-mining activity in Germany (Kusel et al., 2001), and was found to have a pH range of 4.9-6.1 with optimum pH 5.5.

Like the acidic tailings sediments, cultures amended with glucose and inoculated with acidic mine tailings were dominated by phylotypes related to *Alicyclobacillus* spp. (Figure 4-3, Table 4-2). In these cultures, 100% of sequences detected using *Bacteria*-specific primers and 71% of sequences detected using *Deltaproteobacteria*-specific primers were 93% similar to *A. cycloheptanicus*. An additional 25% of clones detected by *Deltaproteobacteria* primers were 94% similar to *A. pomorum*, and one clone was 97% related to *Desulfosporosinus* sp. 5apy.

Sequences extracted from ethanol-amended acid mine tailings cultures included a much smaller proportion of *Alicyclobacillus*-related spp.: 13% of clones derived using *Deltaproteobacteria* primers were 93% similar to *Alicyclobacillus cycloheptanicus*, while the remaining 87% were 94% similar to *Desulfitobacterium hafniense* sp. Y51. Sequences detected with *Bacteria*-specific primers were similarly dominated (61% of clones) by

Desulfitobacterium-related species, with an additional 33% of clones being 93% similar to *Desulfosporosinus orientis* (Figure 4-3, Table 4-2).

Desulfitobacterium spp. are versatile anaerobic organisms, often found in contaminated environments or in bioprocesses treating sites contaminated with high concentrations of toxic metals (Villemur et al., 2006). *Desulfitobacteria* terminal electron acceptors include a wide range of metals including Mn(IV), Fe(III), U(VI) as well as Se(VI), As(V), hydrogen, sulfite, thiosulfate and sometimes sulfur. Temperature and pH for growth of *Desulfitobacterium* spp. reportedly range from 25 to 38°C and from pH 6.5 to 7.8, respectively (Finneran et al., 2002; Bouchard et al., 1996; Christiansen and Ahring, 1996; Sanford et al., 1996, Villemur et al., 2006). Most of the clones derived from the acidic tailings in this study were 94% similar to *Desulfitobacteria* sp. Y51. Although *Desulfitobacteria* generally stain Gram-positive, strain Y51 stains Gram-negative, and is the only *Desulfitobacterium* that has been shown to reduce both sulfate and sulfite (Suyama et al., 2001), therefore *Desulfitobacterium*-related species may contribute to sulfate reduction in the acidic mine tailings. The pH range previously determined for this class is considerably higher than the range observed in the acidic mine tailings (pH 3.7-4.4; Chapter 2), indicating that the species detected in this study may be unrelated to previously identified species.

All SRB enrichment cultures inoculated with acidic mine tailings developed a silvery precipitate that adhered to the inside of the culture tubes (Figure 4-5). XRD analysis of these precipitates identified a mixture of pyrite, marcasite, mackinawite and griegite, all Fe-sulfide minerals (Figure 4-6).

One *Firmicutes*-related phylotype (93% of total clones) derived from the lactate-amended cultures inoculated with gold mine tailings using *Deltaproteobacteria*-specific primers was 96% similar to *Sedimentibacter*, a Gram-positive, anaerobic, endospore-forming, fermenting organism often found in freshwater sediments and frequently implicated in dechlorination processes (Breitenstein et al., 2002) (Figure 4-3, Table 4-2). One species of this lineage, *Sedimentibacter saalensis* is known to form sulfide (Breitenstein et al., 2002), which may account for the black precipitate observed in lactate cultures in the apparent absence of sulfate-reducing bacteria.

4.3.3.3 *Nitrospirae*

Nitrospirae-related clones detected in acidic mine sediments also differed from those derived from gold mine sediments (Figure 4-4, Table 4-2). All *Nitrospirae* clones derived from acidic tailings (6% of total clones) were 99% similar to *Leptospirillum ferrooxidans*, which is one of the most-studied Fe-oxidizing bacteria implicated in acid mine drainage generation. Of the three *Nitrospirae* phylotypes derived from gold mine tailings, one was 89% similar to *Thermodesulfovibrio islandicus*, and the other two were not closely related to any cultured lineages. These three phylotypes were, however, 99% similar to other unclassified environmental clones derived from peat soil (unpublished study, NCBI database), river sediments (Crump and Hobbie, 2005), and a forested wetland (Brofft et al., 2002).

4.3.3.4 *Acidobacteria*

One *Acidobacteria* phylotype was extracted by each primer set from acidic mine tailings sediments (4% of clones derived from *Deltaproteobacteria* primers and 10% of clones detected by *Bacteria*-specific primers) (Figure 4-4, Table 4-2). Both phylotypes were ≥ 97% similar to an unclassified clone found in Fe-oxidizing nodules in reducing sediments (unpublished study, NCBI database). Two *Acidobacteria* phylotypes (one clone each) were also detected in gold mine sediments with *Deltaproteobacteria*-specific primers. One of these clones was 99% similar to a clone extracted from reed bed reactor soil (unpublished study, NCBI database), and the other was 92% related to an *Acidobacteria* clone extracted from the metal-laden waters of Mid-Atlantic Ridge deep sea hydrothermal systems (Garcia-Lopez et al., 2003).

4.3.3.5 *Actinobacteria*

One phylotype representing 9% of all clones derived from acidic mine tailings sediments (using *Bacteria*-specific primers) was closely related to the phylum *Actinobacteria*. Specifically, it was 99% similar to *Ferrimicrobium acidiphilum*, a heterotrophic, iron-oxidizing acidophile found in acid mine waters (Johnson and Roberto, 1997). One clone derived from the gold mine tailings with the same primer set was also 97% similar to this organism (Figure 4-4, Table 4-2). This was the only sequence detected in both acidic base-metal tailings and in gold mine tailings. Two additional *Actinobacteria*-related phylotypes were found in gold mine tailings sediments. One of these phylotypes, which represented

21% of clones detected with *Bacteria*-specific primers, was 97% similar to an unclassified clone derived from flooded, anoxic rice paddy soil (Ludemann and Conrad, 2000). The other, representing 17% of clones detected using the *Deltaproteobacteria*-specific primers, was derived from anoxic lake water (unpublished study, NCBI database).

One *Actinobacteria*-related clone was detected by *Deltaproteobacteria*-specific primers in lactate-amended cultures inoculated with gold mine tailings (Figure 4-4, Table 4-2). This organism was most closely related (95% similarity) to an unclassified clone extracted from subsurface water of the Kalahari shield in South Africa (unpublished study, NCBI database).

4.3.3.6 Other phyla

The remaining phyla (*Chloroflexi*, *Planctomycetes*, OP-8, WS-1 and OP-10, and the beta class of the *Proteobacteria*), were found only in the gold mine sediments and related cultures (Figure 4-4, Table 4-2).

Two *Chloroflexi*-related phylotypes were detected in the gold mine sediments. One of these (4% of total clones; *Deltaproteobacteria*-specific primers) was 99% similar to an unclassified clone from a hydrocarbon and chlorinated solvent contaminated aquifer (Dojka et al., 1998), and the other (14% of total clones; *Bacteria*-related primers) was 93% related to an unclassified clone from an anaerobic bioreactor that dechlorinates 1,2-dichloropropane (Schloetelburg et al., 2002).

The single *Planctomycete* clone found in the gold mine sediments (*Deltaproteobacteria* primer set) was 96% related to an unclassified clone derived from sediments heavily contaminated with nitrate, heavy metals, radionuclides, and halogenated organics (Abulencia et al., 2006).

5% of clones derived from the gold mine sediments with *Deltaproteobacteria* primers were 94-99% similar to other uncultured clones classified under Candidate divisions OP-8, WS-1, and OP-10. Of these, two were derived from groundwater contaminated with benzene or hydrocarbons and chlorinated solvents, and the other two were from mixed cultures used in dechlorination studies (Dojka et al, 1998; von Wintzingerode et al., 1999; unpublished studies, NCBI database). None were closely related to any characterized, cultured lineages.

All of the clones detected by *Bacteria*-specific primers in lactate-amended cultures inoculated with gold mine sediments were 99% similar to the *Microvirgula aerodenitrificans*, an aerobic, denitrifying Gram-negative freshwater *Betaproteobacterium.*

4.4 DISCUSSION

Mine tailings dumps occupy vast land areas in Canada and many other countries around the world and pose risks to human health and the environment, including the presence of toxic metals such as Hg. The risk of Hg toxicity is exacerbated and attenuated by the activity of Hg-methylating and MeHg-demethylating bacteria, respectively, in sedimentary environments. This study is the first to employ culture-independent methods to describe the

overall community structure of MeHg contaminated mine tailings, with particular emphasis on SRB as potential Hg methylators, and to compare two geochemically different mine tailings environments. Cloning and sequencing of bacterial DNA extracted from base metal and gold mine tailings using two overlapping primer sets detected *Deltaproteobacteria* that are potential Hg methylators in both environments, and that would not have been detected with a single primer set. Differences in the species and number of species of *Deltaproteobacteria* between the two environments indicated that MeHg was produced by different organisms. Cultures amplified the abundance of some organisms that were not detected in sediment samples, augmenting the spectrum of SRB that we were able to detect from the tailings. Further, genera were identified that may possess the capability to demethylate MeHg in both environments.

At the outset of this study, it was hypothesized that both types of tailings would host low microbial biodiversity, due to acidic conditions and/or contamination with toxic metals. On the contrary, biodiversity was observed in both environments, and by all indicators the gold mine tailings were characterized by much higher biodiversity than the acidic tailings. Hg contamination, and metal contamination in general, therefore, appeared to be less important in limiting biodiversity than was pH. Both communities featured species that previous studies have suggested would be adapted to the type of contamination present. Due to the nature of this study and the novelty of most of the bacterial sequences detected in both environments, it was necessary to infer the functionality of the corresponding microbes based on comparisons with known lineages and from the source environments of closely related unclassified clones from other studies.

The microbial community structure in gold mine tailings is under-characterized. Previous studies are few and narrowly focused. Anderson and Cook (2004) examined arsenic-contaminated, 100-yr-old gold mine tailings in New Zealand, from which they isolated one species of *Acinetobacter* and two *Bacillus* spp.. Mercury concentrations in these tailings were not described, but given the age of the tailings, Hg amalgamation was the most likely extraction method, therefore the tailings may have been highly contaminated with Hg. Blowes et al. (1998) found that of three *Thiobacillus* spp. enumerated in pH-neutral gold tailings from Joutel, Quebec, the dominant species was *Thiobacillus thioparus*, which was not found in the present study. Londry and Sherriff (2005) found that anaerobic enrichment cultures derived from Nopiming gold mine tailings in Manitoba were capable of methanogenesis, iron reduction and sulfate reduction. Evidence of the presence of iron reducers and/or sulfate reducers, as well as actinomycetes and two unknown microbial FAME structures was also found; *Desulfovibrio* spp. were absent. The present study is the first to show that a wide range of bacteria inhabit historic gold mine tailings, and that this community is well adapted to the presence of toxic metals and other contaminants.

In Chapter 3, seasonal fluctuations of MeHg concentrations in ELC tailings, and low ratios of MeHg:Hg_T at other high-Hg_T sites, led to the conclusion that MeHg levels were largely kept in check by demethylation processes in the Hg-contaminated gold tailings, except under prolonged low-redox, saturated conditions. Demethylation is carried out by Hg-resistant bacteria, which carry the *mer* operon. The *merA* gene, an essential gene in the *mer* operon, codes for mercuric reductase, which imparts Hg resistance to a wide range of bacteria by

reducing Hg^{2+} to volatile Hg^0. Some *mer* operons also include the *merB* gene, which codes for organomercurial lyase. This enzyme cleaves the bond between the methyl group and Hg^{2+}. Sequences related to the genera *Bacillus* and *Acinetobacter*, both of which include members that carry the *mer* operon, were detected in the gold mine tailings. Many *Bacillus* spp. have been shown to carry the *mer*B gene, therefore *Bacillus* spp. are potential MeHg demethylators in the ELC tailings. *Acinetobacter johnsonii* may also be Hg resistant, but the *mer* operon of *Acinetobacter calcoaceticus* does not include the *merB* gene (Kholodii et al., 2004), therefore it is unlikely that *A. johnsonii* demethylates Hg in the gold mine tailings.

Beauchamp et al. (2002) measured Hg flux to the atmosphere from Nova Scotia gold mine tailings that was two orders of magnitude higher than observed over undisturbed native soils of similar parent material. *Bacillus* and *Acinetobacter* spp. may both be contributors to these high Hg flux rates, as some members of these genera carry the *mer* operon (and, therefore, the *mer*A gene). Since these genera have also been found in historic gold tailings from New Zealand (Anderson and Cook, 2004), they may be important generators of Hg flux to the atmosphere in similar situations around the world.

In spite of low SRR and sulfate and high concentrations of toxic metals in the East Lake Catcha gold mine tailings, evidence was found for the presence of a diverse community of *Deltaproteobacteria*, living alongside Hg-resistant and demethylating bacteria.
The dominant sulfate reducers in circumneutral-pH gold tailings were expected to be Gram-negative *Deltaproteobacteria*. The most likely Hg methylators in the gold mine tailings would be *Desulfovibrio ferrireducens*, detected in the acetate cultures, and the four novel

species of *Deltaproteobacteria* derived from the gold mine sediments. *D. ferrireducens* is a newly cultured organism, for which Hg methylation studies should be conducted. The other organisms have yet to be isolated. Additional *Deltaproteobacteria*-related organisms may have been present in the gold mine tailings, however, the PCR-RFLP method used did not isolate organisms with relatively low population percentages. It is therefore probable that MeHg is produced by a more diverse suite of *Deltaproteobacteria* spp. in these tailings than in the acidic tailings.

Sequences closely related to Fe oxidizers conventionally associated with AMD production (e.g. *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Ferrimicrobium acidiphilum*) were extracted from the Kidd tailings, but the majority of clones detected in this environment using *Bacteria*-specific primers were novel species related to *Alicyclobacillus*. In addition, *Acidobacteria* clones closely related to unclassified clones derived from Fe-oxidizing nodules were detected by both primer sets, as were Gram-positive sulfate reducers, and Gram-negative sulfate reducers were detected by the *Deltaproteobacteria* primers. Acid mine drainage communities therefore appear to be more complex than previously documented by studies primarily focused on AMD production.

Findings from this study support the hypotheses derived from the geochemical and microbiological assessment of the Kidd Metsite tailings described in Chapter 2, with respect to the presence of both Gram-positive and Gram-negative sulfate-reducing bacteria. Of particular significance was the observation of two phylotypes of uncultured *Deltaproteobacteria* (K5_62 and K5_114) in the acidic mine tailings that had previously

been detected only in other acidic environments. Although this organism did not grow in the ethanol- or glucose-amended cultures, and has "defied cultivation" by other researchers (Baker et al., 2003), some aspects of its physiology can be inferred from circumstantial evidence. For example, closely related clones have now been detected in several different acidic environments, and none have been detected in pH-neutral environments, suggesting that these organisms are more likely genuine acidophiles than acid-tolerant neutrophiles. They may also be tolerant of extremely high sulfate levels and of heavy metals. Further, the sample was collected from a location and depth that featured a large spike in MeHg concentration in both bulk tailings and porewaters, despite low levels of Hg_T. The MeHg peak coincided with a zone of unusually high sulfate reduction rates that were particularly remarkable given the oxidizing and acidic nature of the tailings (Chapter 2). It appears, therefore, that these novel *Deltaproteobacteria* may be capable of producing MeHg at a substantial rate under oxidizing, acidic conditions such as exist in AMD environments, even where Hg_T is not elevated.

Since SRR and MeHg were elevated but SRB cell count estimates were only moderate in the Kidd sample (Chapter 2), we had hypothesized that the Kidd tailings might host an acidophilic SRB that did not grow well in the pH-neutral medium used for MPN cultures in the initial survey of this site. Observations from this study support this hypothesis, although the novel *Deltaproteobacteria* did not grow in acidic culture medium either, therefore the low cell count estimates from the MPN cultures described in Chapter 2 were not solely due to the neutral pH of the medium. In light of the lack of success in culturing the novel *Deltaproteobacteria* spp., and the large number of clones detected from these two

phylotypes in the Kidd tailings, it is likely that only the Gram-positive SRB (*Desulfosporosinus* spp. and/or *Desulfitobacterium* spp.) grew in the MPN cultures described in Chapter 2, and that SRR and MeHg observed in the sediment sample were reflective of the activity of the novel *Deltaproteobacteria* spp..

Sulfate reduction in acidic tailings may be dominated by one or both of the novel *Deltaproteobacteria* spp. detected in this study with further contributions from *Desulfosporosinus* spp., and perhaps *Desulfitobacterium* spp. *Deltaproteobacteria*-specific primers did not detect *Desulfovibrio* spp, even when they were clearly present, as in the acetate cultures inoculated with gold mine tailings, therefore *Desulfovibrio* spp. cannot be ruled out as possible sulfate reducers in the acidic mine tailings. Klepac-Ceraj et al. (2004) detected only 10 *Desulfovibrio* spp. clones in a ~ 2000-member clone library from salt marsh sediments using the 385F/1492R primer set, suggesting that these primers rarely detect *Desulfovibrio* spp..

The high concentration of MeHg in the Kidd tailings sample indicates that demethylation processes were not in evidence, in spite of the presence of *Acidithiobacillus ferrooxidans*, many strains of which are known to carry the *mer* operon (Takeuchi et al., 2005). Although *merB* has not been shown to be present in *A. ferrooxidans*, several strains have exhibited organomercurial lyase-like activity (Takeuchi et al., 2005). *A. ferrooxidans* str. QXS-1, found in this sample, may not possess this capability. On the other hand, induction of *mer* operons is highly dependent on Hg_T concentration (Schaefer et al., 2004; Takeuchi et al., 2005), therefore it is possible that *A. ferrooxidans* or any other potential demethylating

bacteria found in acid mine drainage situations may not function as demethylators in low-Hg_T environments. Therefore, if an organism capable of methylating Hg at a significant rate is present in low-Hg_T, acidic sediments, it is likely that net MeHg production would go unchecked by the demethylation processes that limit MeHg accumulation in higher-Hg_T environments. Future research efforts are needed to characterize the *mer* genes within these sites.

The appearance of iron-sulfide precipitates in the acidic cultures indicates that *Desulfosporosinus orientis* may contribute to the immobilization of metals under anoxic and low pH conditions. This phenomenon is viewed as a potentially important means of remediating acid mine drainage, although accumulated metal sulfides have also been shown to inhibit SRB activity by creating a barrier between bacterial cells and the nutrients they require (Utgikar et al., 2002).

XRD analysis first indicated the presence of sulfate salts and quartz (and likely other silicates), originating from the growth medium and the tailings particles (i.e., inoculum), respectively. X-ray data however showed some d-spacing values belonging to iron sulfide precipitates, such as pyrite, marcasite, mackinawite and griegite (Figure 6). The pyrite may be a carryover from the inoculum (Praharaj et al., 2004), but marcasite, mackinawite and griegite are quickly oxidized to Fe-hydroxide minerals when exposed to oxygen. These minerals, therefore, formed as a result of sulfate reduction and production of hydrogen sulfide in the cultures. It is unlikely that they would form in the oxidized surficial layers of the acidic tailings, but they may be important to natural bioremediation processes at depth.

Since these precipitates formed in both the ethanol- and glucose-amended acidic cultures, their formation is likely associated with *Desulfosporosinus orientis*, the Gram-positive sulfate reducer detected in both of these cultures. This finding is consistent with the observations of other researchers: Saunders et al. (2005) found that nanocrystalline metal sulfide minerals were formed in an aerobic, metal-contaminated, acidic (pH 3.1) groundwater aquifer injected with sucrose and diammonium phosphate to stimulate sulfate-reducing bacteria. The principal sulfate reducers involved in the bioremediation process were determined to be 96% similar to *Desulfosporosinus orientis*. Interestingly, *Alicyclobacillus*-related sequences were detected in the same location, suggesting that these two types of organisms may frequently colonize acidic, metal-contamined, oxidizing and/or aerobic environments.

4.5 CONCLUSIONS

DNA sequences revealed that methylmercury-contaminated mine tailings from historic gold mine tailings and acidic base-metal tailings are from common phyla, but at the sub-phylum level, microbial communities reflected the distinct properties of these two environments. Two novel species of *Deltaproteobacteria* detected in acidic, base-metal tailings hold the potential to be Hg methylators of some concern, particularly since they were 98-99% related to several clones derived from acidic or acid mine drainage environments worldwide. In gold mine tailings, methylation is likely carried out by a broader spectrum of *Deltaproteobacteria*, and kept in check by demethylation processes.

More research is required to cultivate and isolate novel species associated with mine tailings in order to determine their ecological functions in general, and specifically; to test the Hg methylation rates of novel *Deltaproteobacteria*.

Table 4-1. Statistical analysis of SSU rRNA gene clone libraries derived from mine tailings sediments using general *Bacteria* primers (bac) and primers specific for *Deltaproteobacteria* (del).

Samples	No. of clones	No. of phylotypes	Species richness	Shannon	1/D	Percent coverage	θ(π)	Nucleotide diversity	Gene diversity
Kidd del	94	20	58 (29, 170)*	2.01	5.18	85	51.9 ± 25.1	0.14 ± 0.07	0.74 ± 0.02
Kidd bac	48	24	194 (94, 435)	2.56	8.19	58	242 ± 119	0.17 ± 0.08	0.67 ± 0.07
gold del	90	83	1123 (423, 3266)	4.38	435	11	78.2 ± 39.0	0.20 ± 0.10	0.96 ± 0.03
gold bac	86	79	596 (281, 1403)	4.32	446	15	314 ± 160	0.21 ± 0.11	0.91 ± 0.04

*The numbers in parentheses are 95% confidence limits.

Table 4-2a. Frequency of characterized *Deltaproteobacteria*- and *Firmicutes*-related clone types in libraries derived from tailings and cultures.

Clone	Source	Primer set	# related clones	Phylogenetic group	Nearest relatives	Nearest environment	% similarity	Accession #	Nearest cultured relative	% similarity	Accession #
K5_62	acidic tailings	385F/1492R	27	δ-Proteobacteria	clone H74	acidic mine sediment, China	99	DQ328626	none		
					RCP1-24	forested wetland impacted with reject coal	99	AF523882			
					RCP1-85	forested wetland impacted with reject coal	99	AF523883			
					RCP2-16	forested wetland impacted with reject coal	99	AF523884			
					BA71	Iron Mountain, CA	98	AF225447			
K5_114	acidic tailings	385F/1492R	17	δ-Proteobacteria	clone AS6	Iron Mountain, CA	99	AF543496	none		
					BA18	Iron Mountain, CA	99	AF225446			
ELC_10_13	gold tailings	385F/1492R	1	δ-Proteobacteria	clone RB344	reed bed reactor biofilm	97	AB240344	none		
					delta clone WCHB1-27	hydrocarbon and chlorinated solvent contaminated aquifer	96	AF050538			
ELC_10_36	gold tailings	385F/1492R	2	δ-Proteobacteria	FW clone pLW-45	freshwater lake sediments	99	DQ067029	Desulfobacca acetoxidans	90	AF002671
					delta clone Sh765B-TzT-29	U mine wastes	95	AJ519630			
ELC_30_18	gold tailings	385F/1492R	1	δ-Proteobacteria	soil clone HS9-74	Pb-, Cr- & organic-contaminated soils	95	AY221614	Syntrophus gentianae	93	X85132
ELC_30_41	gold tailings	385F/1492R	1	δ-Proteobacteria	clone c5LKS15	Lake Kinneret sediments	98	AM086112	Syntrophobacter sulfatireducens str. TB8106	93	AY651787
A37	gold cult/acetate	16S	18	δ-Proteobacteria	Desulfovibrio ferrireducens str. CY2	freshwater lake sediments	99	AJ582758	same		
K23	acidic tailings	16S	15	Firmicutes	Alicyclobacillaceae clone SLC66	mineral leaching cultures	92	AY040739	Alicyclobacillus pomoram	92	AB089840
K5_128	acidic tailings	385F/1492R	26	Firmicutes	clone G26	gold ore biooxidation	93	DQ364429	Alicyclobacillus pomoram	92	AB089840
G9	acidic cult/glucose	385F/1492R	6	Firmicutes	Alicyclobacillus pomorum		94	AB089840	same		
G13	acidic cult/glucose	16S	24	Firmicutes	Alicyclobacillus cycloheptanicus		93	AB042059	same		
G23	acidic cult/glucose	385F/1492R	19	Firmicutes	Alicyclobacillus cycloheptanicus		93	AB042059	same		
E17	acidic cult/EtOH	385F/1492R	2	Firmicutes	Alicyclobacillus cycloheptanicus		93	AB042059	same		
E43	acidic cult/EtOH	385F/1492R	1	Firmicutes	Alicyclobacillus cycloheptanicus		93	AB042059	same		
K22	acidic tailings	16S	1	Firmicutes	Desulfosporosinus orientis		93	AJ493052	same		
E25	acidic cult/EtOH	16S	6	Firmicutes	Desulfosporosinus orientis		93	AJ493052	same		
G15	acidic cult/glucose	385F/1492R	1	Firmicutes	Desulfosporosinus sp. 5apy		97	AF159120	same		
A51	gold cult/acetate	385F/1492R	1	Firmicutes	Desulfosporosinus sp. 5apy		98	AF159121	same		
A27	gold cult/acetate	385F/1492R	19	Firmicutes	Desulfosporosinus sp. Y5		97	AY233860	same		
A11	gold cult/acetate	16S	3	Firmicutes	Desulfosporosinus sp. Y5		96	AY233860	same		
E11	acidic cult/EtOH	385F/1492R	20	Firmicutes	Desulfitobacterium hafniense Y51		94	AP008230	same		
E15	acidic cult/EtOH	16S	7	Firmicutes	Desulfitobacterium hafniense clone 3174		94	AF403181	Desulfitobacterium sp. Viet-1	93	AF357919
E27	acidic cult/EtOH	16S	2	Firmicutes	Desulfitobacterium sp. Viet-1	river sediment, Vietnam	94	AF357919	same		
E41	acidic cult/EtOH	16S	2	Firmicutes	Desulfitobacterium sp. RPf35Ei	reactor treating AMD	94	AY548779	same		
ELC_10-6	gold tailings	385F/1492R	1	Firmicutes	clone AKAU3868	uranium contaminated soil	94	DQ125755	Clostridium papyrosolvens	94	X71852
ELC_30_37	gold tailings	385F/1492R	2	Firmicutes	Bacillus sp. IDA4917		98	AY289504	same		
ELC_30_21	gold tailings	16S	2	Firmicutes	anoxic bulk soil clone BSV82	anoxic soil of a flooded rice microcosm	99	AJ229226	Clostridium sp. FA2/18	98	AY188849
ELC_30_34	gold tailings	16S	2	Firmicutes	clone AKAU4085	uranium contaminated soil	98	DQ125854	Bacillus sp. IDA4921	98	AY289500
L45	gold cult/lactate	385F/1492R	14	Firmicutes	Sedimentibacter sp. C7		96	AY766466	same		

Table 4-2b. Frequency of characterized clone types related to *beta-* and *gamma-Proteobacteria*, *Nitrospirae*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *Actinobacteria*, Candidate Divisions WS-1, OP8 and OP10 in libraries derived from tailings and cultures.

Clone	Source	Primer set	# related clones	Phylogenetic group	Nearest relatives	Nearest environment	% similarity	Accession #	Nearest cultured relative	% similarity	Accession #
L13	gold cult/lactate	16S	24	β-Proteobacteria	Microvirgula aerodenitrificans str.LMG 4329		99	AJ487013	same		
K41	acidic tailings	16S	3	γ-Proteobacteria	clone fg11	Tinto River, Spain (extreme acidic environment)	99	DQ303276	Acidithiobacillus ferrooxidans str. QXS-1	99	DQ168465
E29	acidic cult/EtOH	16S	1	γ-Proteobacteria	AF clone KF/GS-JG36-22	U mine waste pile sediment	99	AJ295655	Acidithiobacillus ferrooxidans str. QXS-1	99	DQ168465
ELC_30_27	gold tailings	16S	2	γ-Proteobacteria	Acinetobacter johnsonii str. S35		99	AB099655	same		
K5_130	acidic tailings	385F/1492R	5	Nitrospirae	clone fe12	mine waste rock	99	DQ303277	Leptospirillum ferrooxidans str. P3a	99	AF356837
ELC_10_8	gold tailings	16S	2	Nitrospirae	clone HSM-SS-003	peat soil, Japan	99	AB238766	Thermodesulfovibrio islandicus	89	X96726
ELC_10_10	gold tailings	385F/1492R	2	Nitrospirae	FW clone IRD18B03	river sediments, USA	99	AY947906	none		
ELC_10_47	gold tailings	385F/1492R	1	Nitrospirae	clone FW19	forested wetland (Broft; x acidic)	99	AF524005	none		
ELC_10_21	gold tailings	385F/1492R	1	Cand. Div. OP8	clone WFeA1-59	hydrocarbon and chlorinated solvent contaminated aquifer	95	AF050555	none		
ELC_30-43	gold tailings	385F/1492R	1	Cand. Div. OP8	clone SHA-124	mixed culture with 1,2-Dichlorpropan (bioremed study)	97	AJ306781	none		
K30	acidic tailings	16S	3	Acidobacteria	clone: TakashiAB-B21	Fe-oxidizing nodules in reducing sediments	97	AB254782	none		
K5_76	acidic tailings	385F/1492R	3	Acidobacteria	clone: TakashiAB-B21	Fe-oxidizing nodules in reducing sediments	99	AB254782	none		
ELC_10_49	gold tailings	385F/1492R	1	Acidobacteria	clone BS073	reed bed reactor soil	99	AB240251	none		
ELC_30_8	gold tailings	385F/1492R	1	Acidobacteria	clone AT-s3-28	Mid-Atlantic Ridge hydrothermal sediment	92	AY225646	none		
ELC_30_13	gold tailings	385F/1492R	1	Cand. Div. WS-1	clone SJA-43	anaerobic, trichlorobenzene-transforming microbial consortium	94	AJ009463	none		
ELC_30_46	gold tailings	385F/1492R	1	Cand. Div. OP10	clone ZZ12AC2	benzene-contaminated groundwater	99	AY214187	none		
ELC_30_3	gold tailings	385F/1492R	1	Planctomycetes	clone 655077	nitrate and heavy metal contaminated soils	96	DQ404737	none		
ELC_10_12	gold tailings	385F/1492R	1	Chloroflexi	clone WCHB1-44	hydrocarbon and chlorinated solvent contaminated aquifer	99	AF050565	none		
ELC_30_7	gold tailings	16S	2	Chloroflexi	clone SHD-71	anaerobic bioreactor that dechlorinates 1,2-dichloropropane	93	AJ278167	Dehalococcoides sp. BHI80-15	90	AJ431246
K15	acidic tailings	16S	7	Actinobacteria	clone D3-7	acid mine drainage, China	99	DQ464144	Ferrimicrobium acidiphilum	99	AF251436
ELC_10_21	gold tailings	16S	1	Actinobacteria	clone D3-7	acid mine drainage, China	99	DQ464144	Ferrimicrobium acidiphilum	99	AF251436
ELC_10_31	gold tailings	16S	3	Actinobacteria	actinobacterium clone ARFS-2	flooded anoxic rice paddy soil	97	AJ277687	Acidimicrobium ferrooxidans	87	U75647
ELC_30_45	gold tailings	385F/1492R	4	Actinobacteria	clone 122	anoxic lake water	92	AJ536836	none		
L17	gold cult/lactate	385F/1492R	1	Actinobacteria	Uncultured actinobacterium clone DR546BH1103001SAD23	subsurface water of Kalahari shield, S. Africa	95	DQ234844	Eggerthella sinensis str. HKU14	91	AY321958
K5_72	acidic tailings	385F/1492R	2	unclassified	clone DUNssu007 (+1B)	acidic forest soil, Germany	94	AY724094	none		

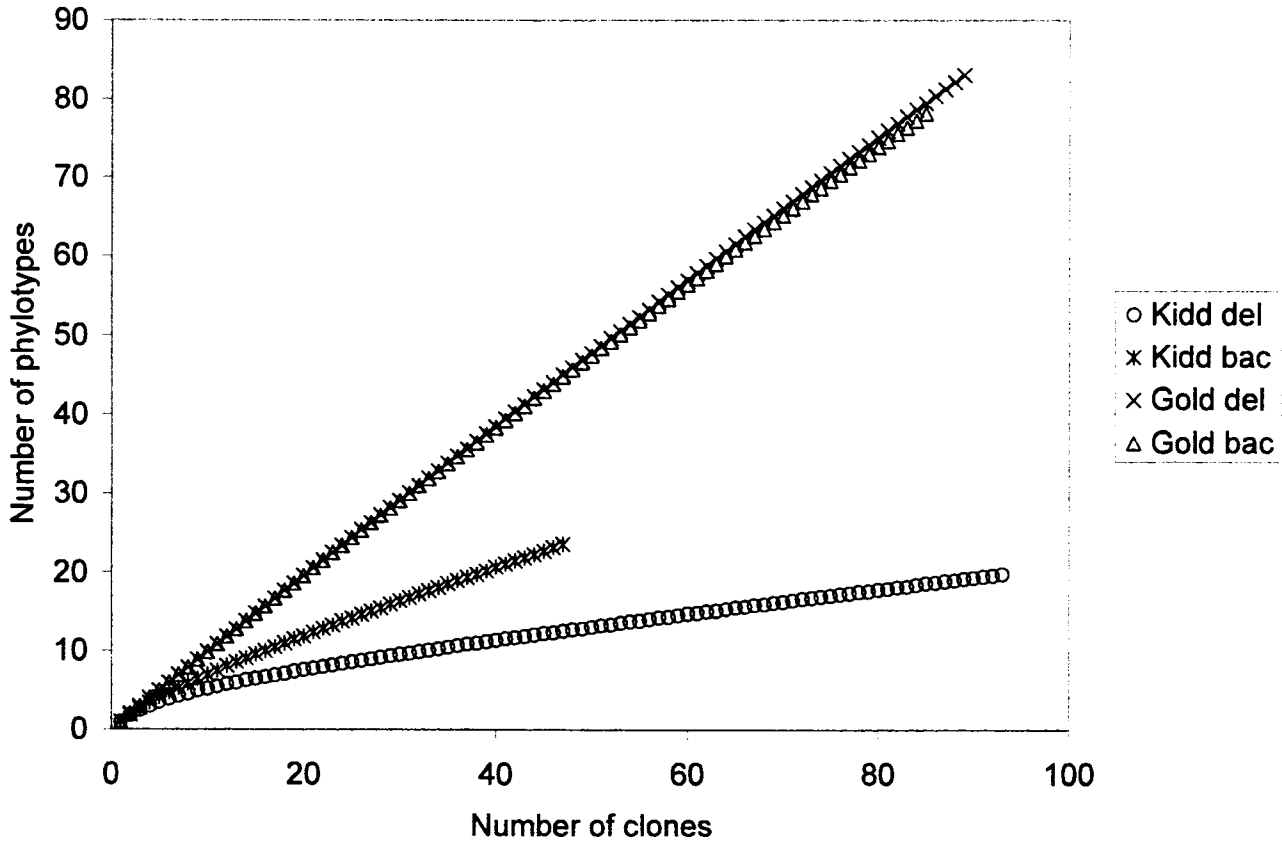

Figure 4-1. Rarefaction curves determined for the various phylotypes of SSU rRNA gene clones derived from Kidd Metsite and ELC sediment samples using *Bacteria*-specific (bac) and *Deltaproteobacteria*-specific (del) primers. Phylotypes were defined as distinct RFLP patterns resulting from digestion of clone sequences with restriction endonucleases HaeIII and MspI. Rarefaction analysis was performed using equations reported by Heck et al. (1975).

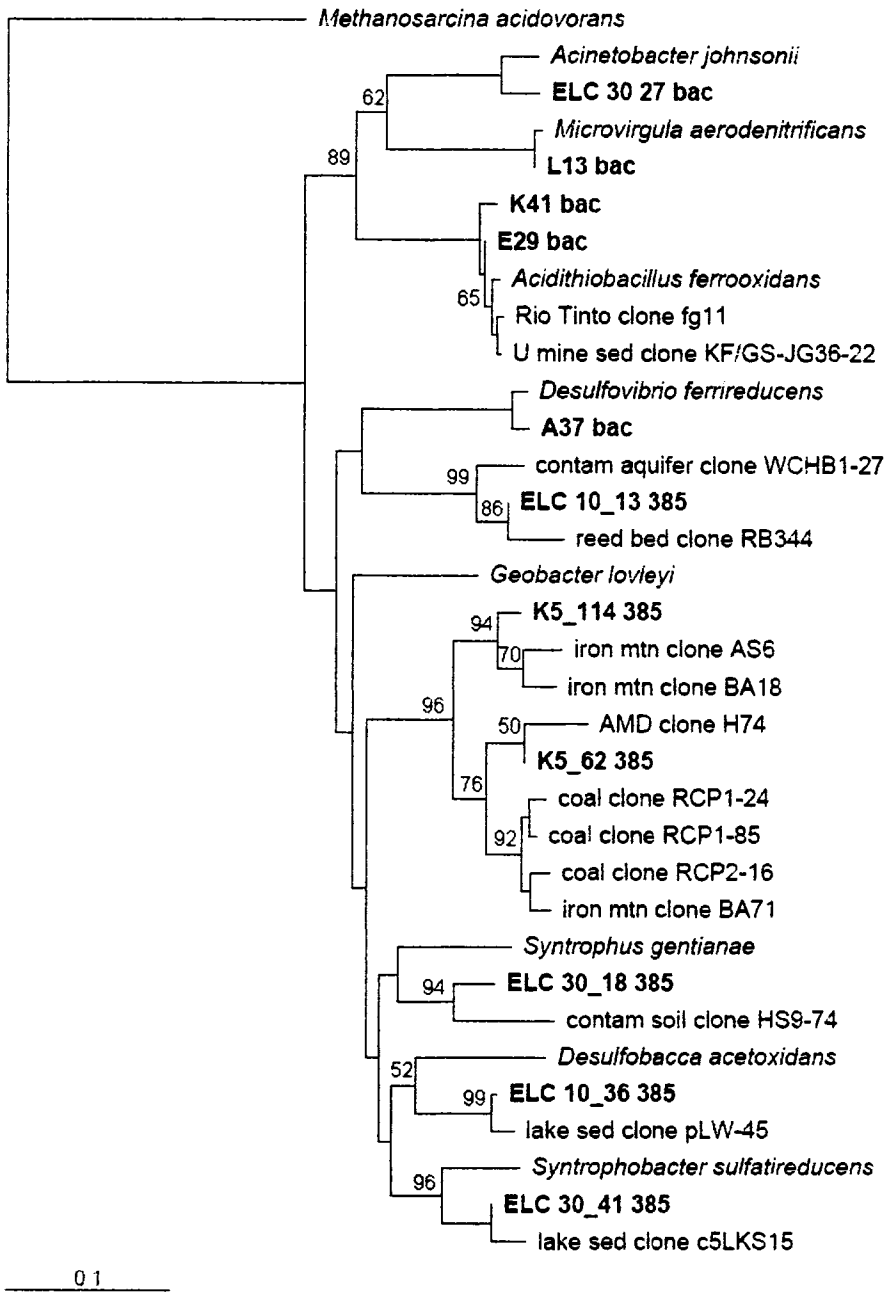

Figure 4-2. Neighbor-joining phylogenetic tree of *Proteobacteria*-related sequences, incorporating a Jukes-Cantor distance correction, based on SSU rRNA genes from tailings and cultures from the Kidd Metsite (denoted K5), East Lake Catcha (ELC) and ELC cultures amended with acetate (A). Sequences from this study and close relatives were aligned using the Fast Aligner algorithm, verified by hand, and compared to the *E. coli* SSU rRNA secondary structure using the ARB software package. Bootstrap analyses were conducted on 1,000 samples. *Methanosarcina acidovorans* was used as the outgroup. Bar, 0.10 change per nucleotide position.

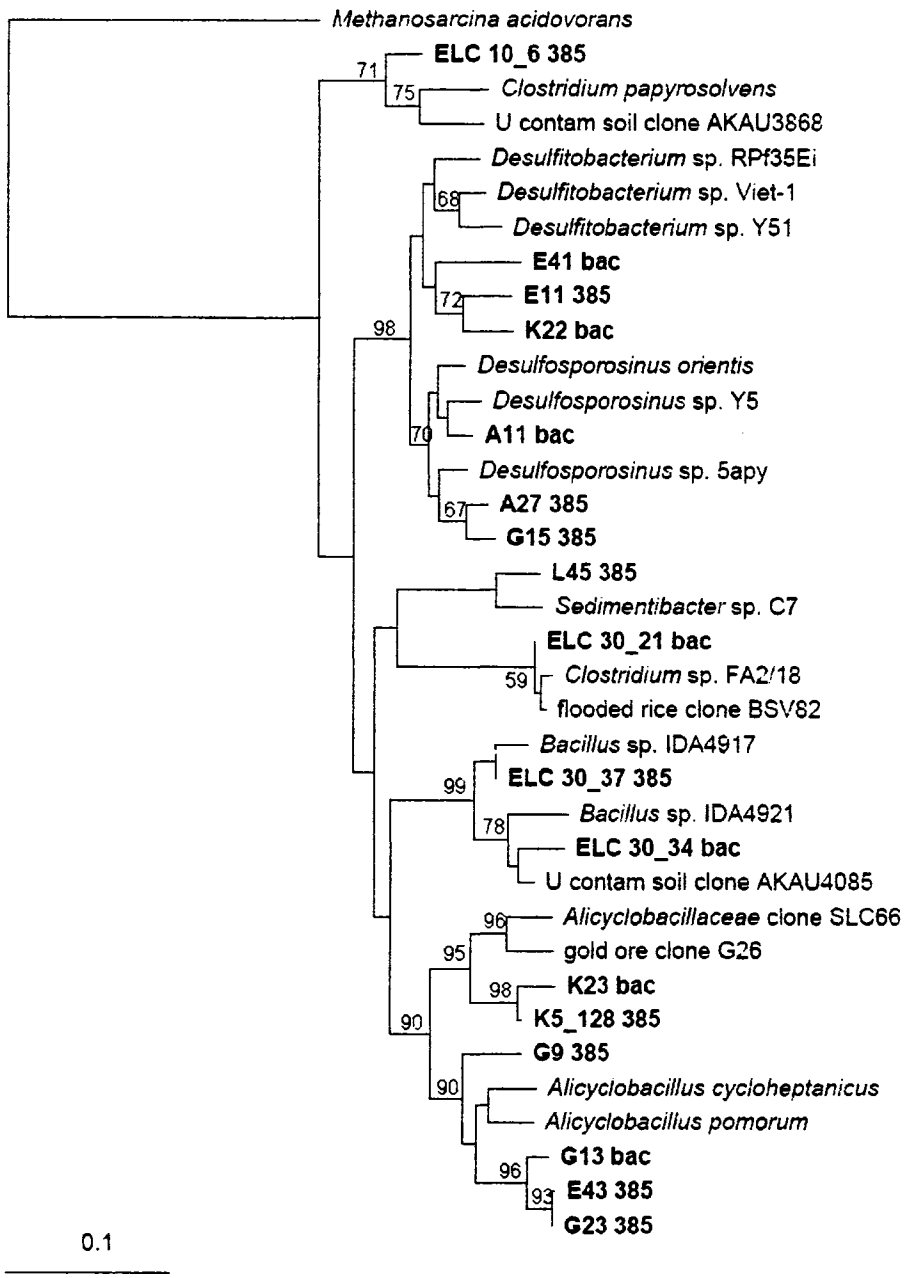

Figure 4-3. Neighbor-joining phylogenetic tree of *Firmicutes*-related sequences, incorporating a Jukes-Cantor distance correction, based on SSU rRNA genes from tailings and cultures from the Kidd Metsite (denoted K5, K), East Lake Catcha (ELC), ELC cultures amended with acetate (A) or lactate (L), and Kidd Metsite cultures amended with ethanol (E) or glucose (G). Sequences from this study and close relatives were aligned using the Fast Aligner algorithm, verified by hand, and compared to the *E. coli* SSU rRNA secondary structure using the ARB software package. Bootstrap analyses were conducted on 1,000 samples. *Methanosarcina acidovorans* was used as the outgroup. Bar, 0.10 change per nucleotide position.

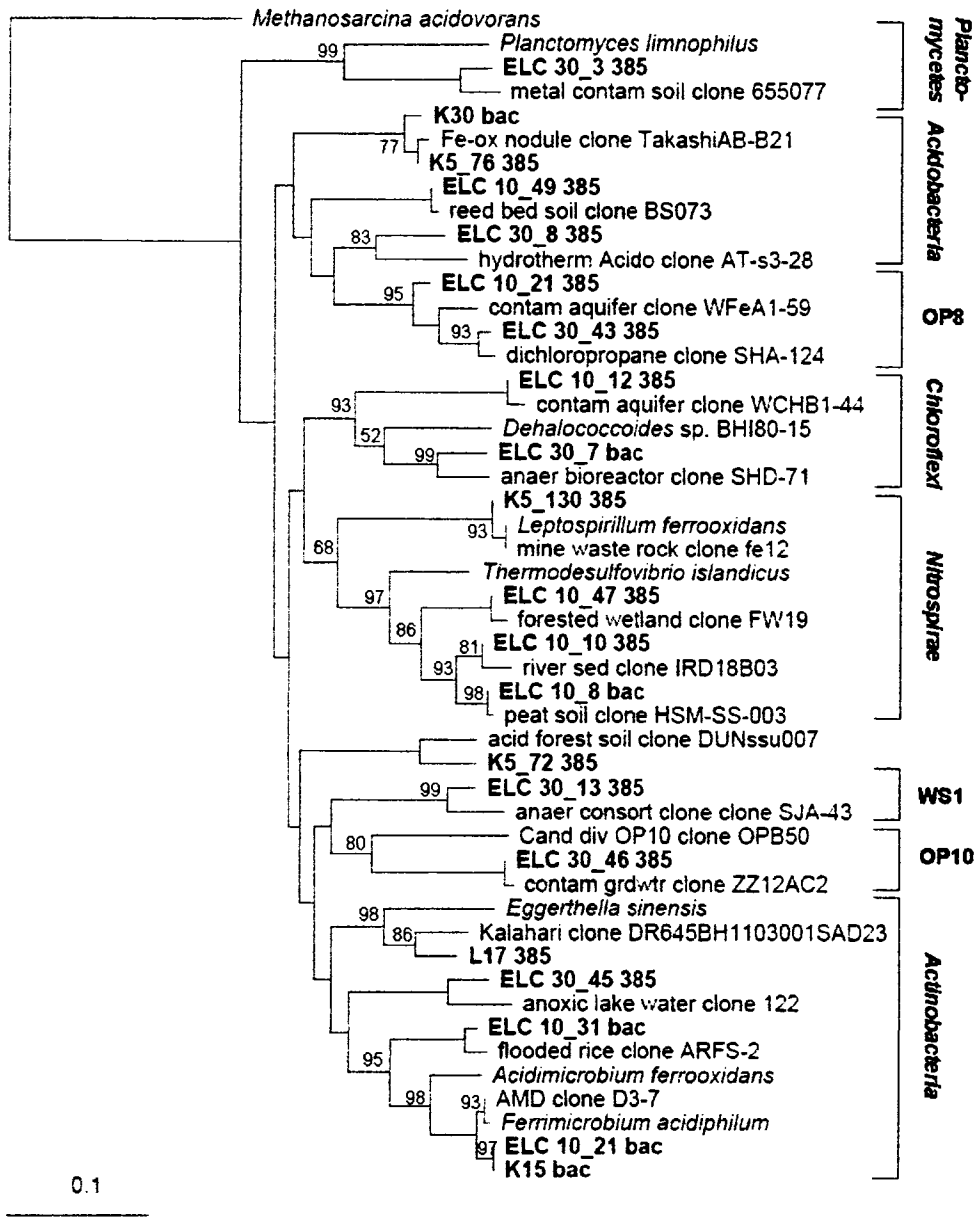

Figure 4-4. Neighbor-joining phylogenetic tree of sequences related to all other phyla than *Proteobacteria* and *Firmicutes*, incorporating a Jukes-Cantor distance correction, based on SSU rRNA genes from tailings and cultures from the Kidd Metsite (denoted K5, K), East Lake Catcha (ELC), ELC cultures amended with acetate (A) or lactate (L), and Kidd Metsite cultures amended with ethanol (E) or glucose (G). Sequences from this study and close relatives were aligned using the Fast Aligner algorithm, verified by hand, and compared to the *E. coli* SSU rRNA secondary structure using the ARB software package. Bootstrap analyses were conducted on 1,000 samples. *Methanosarcina acidovorans* was used as the outgroup. Bar, 0.10 change per nucleotide position.

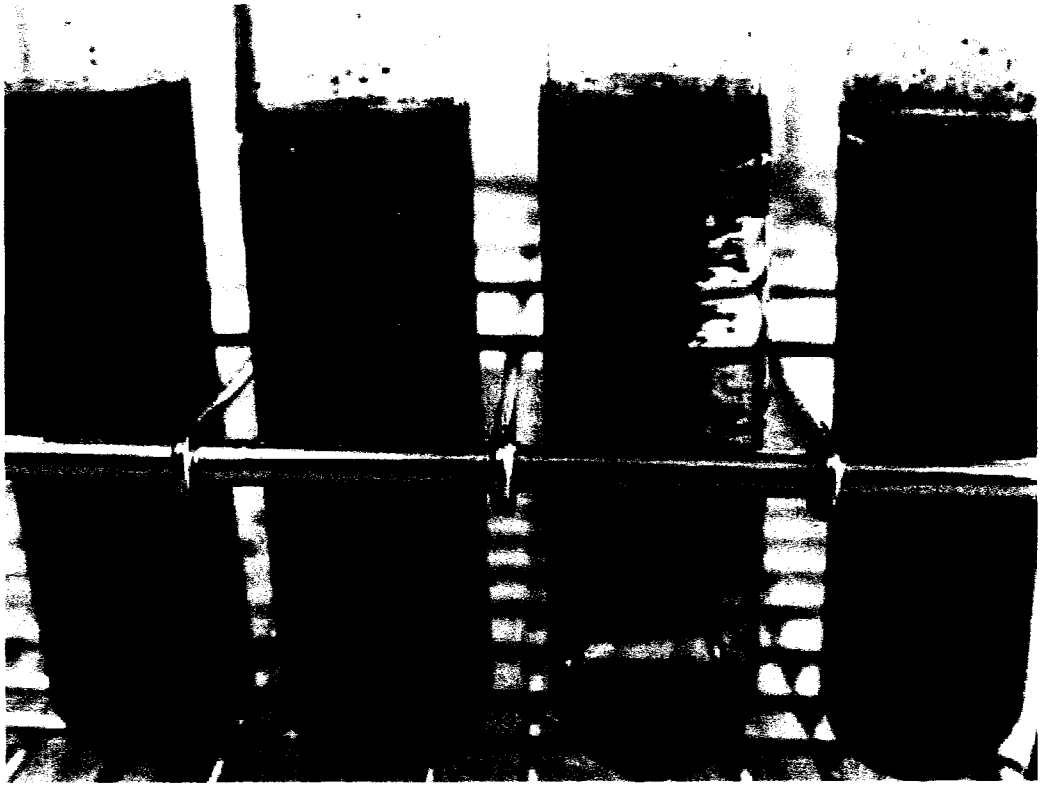

Figure 4-5. Photograph of SRB enrichment cultures in acidic medium (Sen and Johnson, 1999) showing silver-coloured precipitate adhering to culture tube walls.

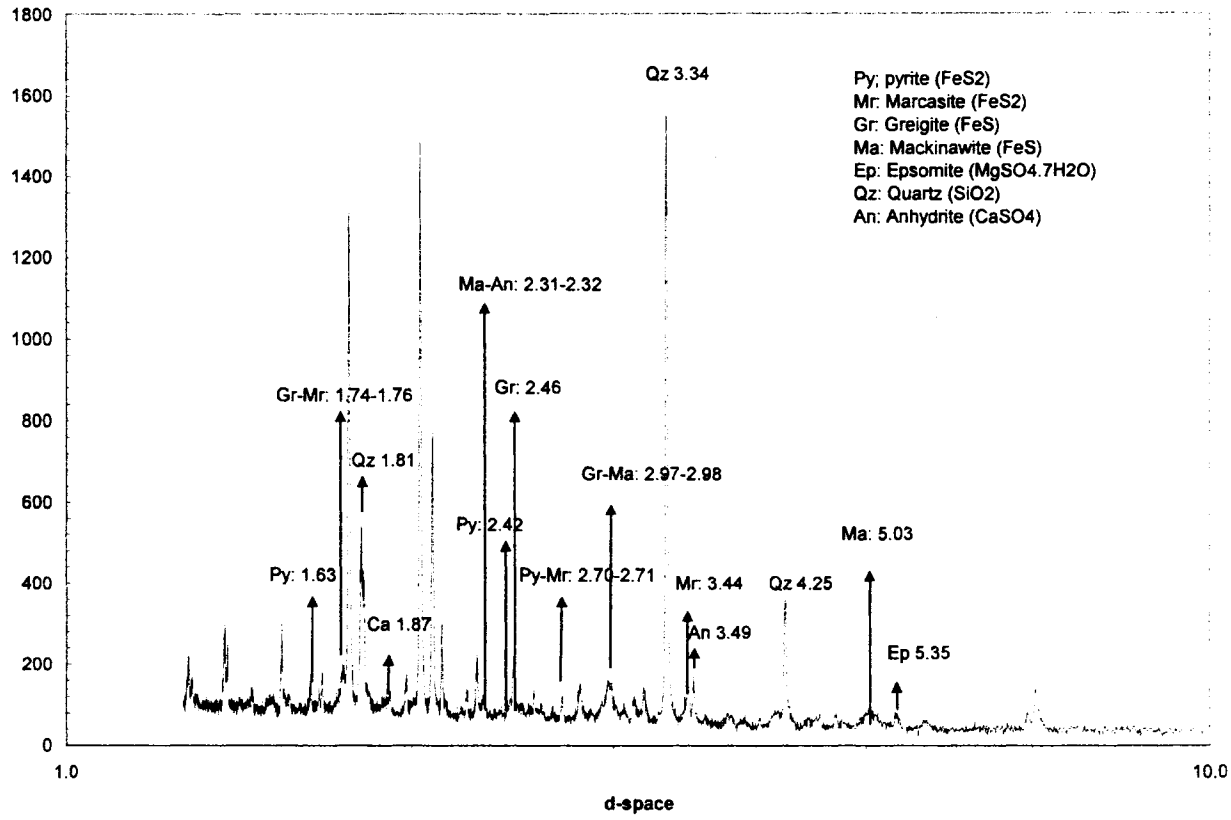

Figure 4-6. X-ray diffraction trace showing peaks corresponding to iron-sulfide minerals.

CHAPTER 5. SIGNIFICANCE OF RESEARCH AND FUTURE RESEARCH DIRECTIONS

5.1 IDENTIFICATION OF POTENTIAL METHYLMERCURY- CONTAMINATED MINE TAILINGS

This study examined mercury methylation in two contrasting mine tailings environments: acidic tailings from low-Hg sulfidic base-metal ores in northern Ontario, and high-Hg, pH-circumneutral tailings from historic gold mining in Nova Scotia. In each of these groups, one site featured a higher ratio of MeHg : Hg_T than any other comparable site, providing insights into the conditions under which MeHg might be present in environmentally significant concentrations. In the acidic tailings, MeHg contamination was most evident in the oxidizing, acidic upper layer of low-Hg_T tailings featuring extremely high sulfate concentrations, and colonized by a novel species of putatively acidophilic *Deltaproteobacteria*. In the gold mine tailings, MeHg contamination appeared to accumulate during the warmer months in high-Hg_T tailings found in a wet, revegetated, bog-type environment in which the microbial community was highly diverse.

Extrapolating from these results, one might envision a worst case scenario combining elements of these two situations, for example, oxidizing, acidic tailings with very high sulfate levels and SRR, but containing higher levels of Hg_T than the base-metal tailings from the Timmins area. Such environments might be found in situations where gold mine tailings have been co-located with acidic tailings. Although the Broulan site fits this description, the gold mine tailings at Broulan were not extracted by Hg amalgamation, but it is not uncommon for tailings from different ore bodies to be deposited in the same location. Alternately, tailings from pyritic Hg ores, as at the New Idrija mine in California (Boctor et

al., 1987), or Hg-amalgamation tailings from pyrite-rich gold ores might produce a similar situation. Demethylation processes might limit the amount of MeHg being produced in these situations once the threshold for induction of the *mer* operon was surpassed, but given the MeHg levels in the low-Hg_T Kidd Metsite tailings, that limit could be high enough to allow environmental MeHg concentrations warranting serious concern.

As the hazards of abandoned mine sites become the focus of increased government scrutiny in Canada and around the world, it will be important to identify situations where MeHg levels might need to be addressed. The results of this study can be used to narrow the search for potentially MeHg contaminated tailings.

5.2 POTENTIAL FOR METHYLMERCURY FORMATION RESULTING FROM ACID MINE DRAINAGE BIOREMEDIATION EFFORTS

Sulfate reduction, particularly by acidophilic SRB, produces alkalinity, raising pH, and this combined with the production of HS^- results in the removal of sulfate and precipitation of metals as metal sulfides (Hulshof et al., 2006; Saunders et al., 2005). Recently, the use of SRB has become widespread in the treatment of acid mine drainage and other acidic, metal-laden environments such as contaminated groundwater. Several approaches have been used to stimulate SRB activity, including injection of nutrients and electron donors directly into contaminated groundwater (Saunders et al., 2005), construction of permeable reactive barriers to treat mine leachate (Benner et al., 1999; Benner et al., 2002; Blowes et al., 2000), installment of constructed wetlands (Harmon et al., 2004), and treatment of AMD in

bioreactors (Tabak et al., 2003), all of which produce the desired result of precipitating and/or extracting metals. *In situ* treatments are more desirable than traditional pump-and-treat alternatives because of their use of naturally occurring bacteria to mitigate AMD in perpetuity with relatively little upkeep. Despite the very large number of papers in the literature that link sulfate reduction to methylmercury production, however, very little information is available regarding the production of methylmercury by sulfate reducers being stimulated by the various AMD treatment methods.

One study investigated the production of methylmercury in a constructed wetland designed to remove metals, particularly Cu and Hg, from industrial wastewater and storm runoff (Harmon et al., 2004). When sulfate concentration was increased to stimulate sulfate reduction, methylmercury concentrations in water increased up to threefold. These concentrations of sulfate (max. 482 uM) and total Hg (160 pM), however, were very low compared with AMD situations, where sulfate concentrations can reach > 1M and Hg_T can exceed 8 nM (e.g. at Broulan, Chapter 2). Methylmercury concentrations in constructed wetlands treating AMD might, therefore, be problematic. In light of the potential for Hg methylation in sulfate-reduction based systems designed to treat AMD, more work needs to be done to monitor MeHg in outflows from these systems, and molecular studies are needed to identify the sulfate reducers and *Deltaproteobacteria* that colonize them. This is particularly important in systems with high Hg_T and/or sulfate in the inflow. Currently, Hg is often not measured in AMD in Canada, as it is not required under the Metal Mining Effluent Regulations (MMER - Fisheries Act, 2002). If Hg and MeHg were routinely measured in mine wastes, data collected from these measurements could be included in models to help

understand and predict MeHg production in AMD treatment systems, and perhaps to modify existing treatment facilities and requirements for long-term monitoring plans.

5.3 RELEVANCE TO INTERNATIONAL MINING CONCERNS

Although gold extraction by Hg amalgamation has not been practiced in Canada for at least 60 years, unfortunately, this practice continues in many countries around the world. Most practitioners are poverty-stricken artisanal and small-scale miners (ASM) operating under unsafe conditions. Inexpensive metallic Hg is widely available for this purpose, and few ASM are able to protect themselves from exposure to Hg vapour, a situation that is especially hazardous to the many women and children involved in this sector.

The "Mining, Environment and Development" website, a joint effort by the United Nations, the International Labour Organization, the World Bank and other agencies, lists reports on artisanal and small-scale mining in the following countries: China, Niger, Peru, Philippines, Ghana, India, Papua New Guinea, Bolivia, Chile, Burkina Faso, Mali, Ecuador, Brazil, Zimbabwe, South Africa, Zambia and Indonesia, Venezuela, Namibia, Guinea, Madagascar, Viet Nam, United Republic of Tanzania, Pakistan, United States, Argentina, Chad, Chile, Colombia, Cuba, Guyana, Haiti, Jamaica, Kenya, Mexico, Mozambique, Mayanmar, Nepal, Thailand (http://www.natural-resources.org/minerals/ cd/ssm.htm#Country). A recent report from the World Bank and the International Development and Reconstruction Bank (IDRB) describes the environmental and social impacts of artisanal and small-scale mining in Mongolia (IBRD and World Bank, 2006).

The findings of the research presented in this thesis indicate that the hazards associated with Hg amalgamation tailings are long-term. Under favourable conditions, the *in situ* transformation of Hg^0 to MeHg in environmentally significant concentrations continues at least 60-100 years after the cessation of mining operations. Accordingly, as national governments and international organizations establish initiatives to address artisanal and small-scale mining, long-term plans will be needed for environmental monitoring and land reclamation, taking into account Hg concentrations and the potential for significant MeHg contamination.

5.4 CONCLUSIONS

In conclusion, this study provides insights into the biogeochemical conditions under which methylmercury concentrations may become hazardous in mine tailings environments. Further research is warranted into the cause and effect relationships between the situational factors affecting MeHg concentrations, identified in this study, and rates of MeHg production in mine tailings. This is a preliminary investigation, but the prevalence of acidic and high-Hg mine tailings make its findings pertinent worldwide. The results of this research should be taken into consideration in selecting tailings deposits potentially contaminated with MeHg for evaluation and research, and in the design of long-term monitoring and remediation plans for decommissioned mine sites.

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