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Linking Mining Wastewater Discharge to Methylmercury Production in a Sub-Arctic Peatland

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Abstract

It is well established that the addition of sulphate (SO_4^{2-}) to peatlands increases methylmercury (MeHg) concentrations in pore waters via microbial methylation. Less information exists about the effects of different concentrations and sources of SO_4^{2-} loading on MeHg production in remote, non- SO_4^{2-} impacted regions like Canada's north, where increased SO_4^{2-} loadings come not from the atmosphere, but often from mining waste water and rock tailings. A three year field study (two years of loading; one year of recovery) examined the effects of simulated wastewater (containing 27.2 mg/L SO_4^{2-}) on MeHg production. Methylmercury concentrations increased to concentrations > 4.0 ng/L (background average = 0.09 ng/L) by the end of each field season but during the recovery year decreased to < 0.80 ng/L - still above background. Changes in partitioning between pore waters and peat were observed in the experimental fen, suggesting that the SO_4^{2-} additions significantly impacted MeHg production in pore waters, and down-gradient movement. To evaluate different SO_4^{2-} loadings and sources, laboratory column experiments were conducted at a range of SO_4^{2-} concentrations in solution, as well as using mine tailings rock that leached SO_4^{2-}. All additions increased MeHg concentrations; the highest MeHg concentrations were seen in the intermediate 5 mg/L additions suggesting limits for SO_4^{2-} utilization by microbes. Results from this work indicate that even very small additions of SO_4^{2-} to these pristine peats will increase MeHg in pore waters. Potential downstream impacts of MeHg on biota will require careful consideration of both wastewater and waste rock management for SO_4^{2-}.

Keywords

Peatlands, Mercury, Methylmercury, Methylation, Mining, Wastewater, Sulphate, Waste Rock

List of Abbreviations

BrCl	Bromine monochloride
CVAF	Cold vapour atomic florescence
DGPS	Differential global positioning system
DOC	Dissolved organic carbon
MDL	Method detection limit
MeHg	Methylmercury
MRL	Method reporting limit
OFRI	Ontario Forest Research Institute
OPR	Ongoing precision recovery
PETG	Polyethylene terephthalate glycol-modified
PVC	Polyvinyl chloride
QA/QC	Quality assurance/quality control
SO_4^{2-}	Sulphate
SRB	Sulphate-reducing bacteria
THg	Total mercury
%MeHg	Percent total mercury as methylmercury
%TS	Percent total sulphur
U.S. EPA	United States Environmental Protection Agency

Co-Authorship Statement (where applicable)

I hereby declare that I am the sole author of this thesis, except where noted below. I understand that my thesis may be made electronically available to the public. In this thesis, I collected all field and lab samples as well as performed all the following analyses independently: MeHg (solid and aqueous), THg (solid and aqueous), DOC, and ions (anions and cations). %TS was the only analysis not performed by myself as these samples were sent to OFRI for analysis. Method development for lab-based column experiments was performed by myself with the assistance of our lab manager, Aaron Craig.

Exceptions to sole authorship:

For all chapters, Dr. Brian Branfireun acted as my advisor, editor and offered suggestions on scientific content, and the treatment and presentation of data in this thesis. He will be listed as co-author on any and all subsequent publications stemming from this work. Dr. Jonathan Price and Dr. Colin McCarter provided field infrastructure and the experimental design for the field work carried out in Chapter 2 of this thesis and will thus be co-author on any and all subsequent publications stemming from this work.

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1 Mercury Cycling and Biogeochemistry

1.1 Global Mercury Cycle

Mercury (Hg) is a naturally occurring element found in the lithosphere (Seneviratne, 2007). Elemental mercury (Hg(0)) has both liquid and gaseous phases at ambient temperatures and pressures. Ionic inorganic Hg has two cationic states: monovalent mercury (Hg(I)) and divalent mercury (Hg(II)). Divalent mercury (Hg(II)) is more stable and commonly associated with inorganic molecules such as sulphur (cinnabar/meta-cinnabar), chlorine (mercuric chloride), and oxygen and hydroxyl ions (Carpi, 1997; Seneviratne, 2007). Mercury can also form organic substances such as dimethylmercury (Me_2Hg) or methylmercury (MeHg) which are more toxic than the inorganic forms of Hg. Mercury has a relatively high vapour pressure which means it transforms into a colourless, odourless gas with relative ease (Seneviratne, 2007). Gaseous elemental Hg(0) (GEM) is found as a vapour which allows for its easy atmospheric transport and can have an atmospheric residence time of several months to a year which allows for hemispheric circulation in the stratosphere (Pirrone & Mason, 2009).

Both natural processes and anthropogenic activities emit Hg into the atmosphere as GEM, Hg(II) and particulate Hg (Pirrone *et al*., 2010). Natural emissions include those from crustal degassing, volcanoes, and Hg volatilization from geologically-enriched material (Rasmussen, 1994; Gustin *et al*., 2000; Rytuba, 2005). Forest fires, soils and oceans also re-release Hg from long-range transport of Hg from anthropogenic sources (Mason *et al*., 1994; Fitzgerald *et al*., 1998; Ebinghaus *et al*., 1999; Sunderland & Chmura, 2000; Friedli *et al*., 2003). Anthropogenic sources of Hg increased with the Industrial Revolution with these emissions clearly increasing between 1850 and 1890 (North American gold rush) and peaking in the 1970s due to increased reliance on coal combustion for power generation (Schuster *et al*., 2002; Streets *et al*., 2011; UNEP, 2013). Aside from coal combustion, other anthropogenic sources of Hg are artisanal goal mining, ore processing, production of consumer products (e.g. paint, electronics) and industrial scale chemical manufacturing (Pirrone *et al*., 2010; UNEP, 2013). Artisanal gold mining is

currently the largest contributor to anthropogenic emissions of Hg as Hg is used for amalgamation of gold then is subsequently burned off and released into the atmosphere (see Cordy *et al*., 2011; UNEP, 2013). Present (2013) estimates of Hg emissions range from 6500 – 8200 metric tonnes per year with the majority of these emissions classified as secondary emissions which are Hg re-emissions from previously deposition with primary emissions only contributing 30 – 35% of the total global emissions (Driscoll *et al*., 2013). Once GEM has been released into the atmosphere, it can be transported long distances in the stratosphere and will eventually re-enter the troposphere (Pirrone & Mason, 2009). Once GEM re-enters the troposphere, it can be oxidized by aerosols and halogens to form particulate bound mercury (Hg(II)) or stay as elemental Hg(0) (Pirrone & Mason, 2009). Once Hg has been deposited as Hg(II) species, it will either remain bound to soil or be hydrologically transported through the environment in both dissolved and particulate forms. With the presence of the right microbial community and/or environmental conditions, Hg(II) can be transformed into MeHg or reduced to Hg(0). Mercury can be transitioned within the environment by forming organic and inorganic complexes, Hg sulphide complexes, transformed to MeHg and bioaccumulated or evaporated from the system (Figure 1.1) (Skyllberg *et al*., 2000; Heyes *et al*., 2004).

1.1.1 Methylmercury

Methylmercury is a form of Hg that is an environmental toxin and contaminant of concern as it has the ability to bioaccumulate and biomagnify through the aquatic food web (Morel *et al*., 1998). Bioaccumulation means that a substance accumulates in organisms faster than biological processes are able to break it down or remove it. Biomagnification is the increase in contaminant concentration with increasing trophic level, ultimately resulting in concentrations of concern in higher tropic level fish that present a risk to consumers. This accumulation in higher trophic organisms such as birds or fish can have serious effects on these individuals such as behavioral, neurochemical, hormonal and reproductive changes (Scheuhammer *et al*., 2007).

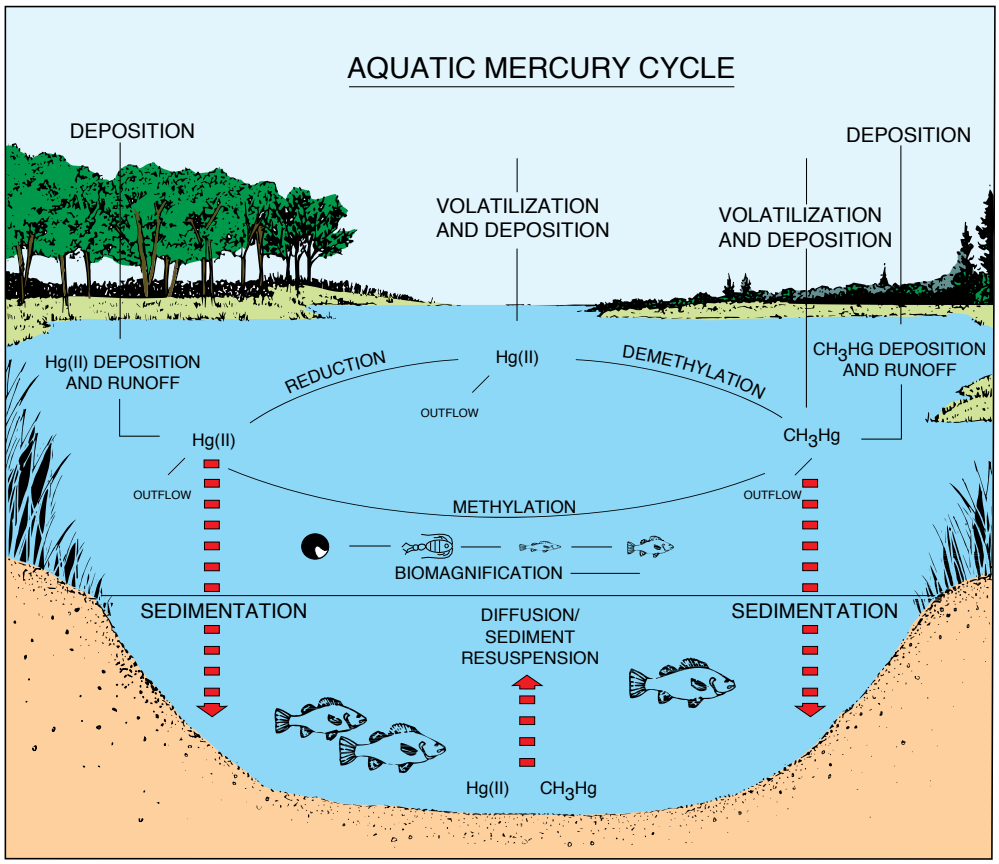

Figure 1.1: Mercury deposition, transport and cycling in the environment (from Mercury Pollution: Integration and Synthesis. Copyright Lewis Publishers, an imprint of CRC Press Krabbenhoft & Rickert, 2013).

Methylmercury can also cause health complications in humans as it is a neurotoxin that can cause emotional changes, headaches, tremors, muscle weakness and impaired cognitive function (Mergler *et al*., 2007). Minamata disease in humans is a severe form of Hg poisoning that originated from Minamata, Japan where industrial waste containing Hg and MeHg from the Chisso Corporation chemical factory was released into Minamata Bay from 1932 to 1968 (Harada, 1995). Mercury accumulated in the fish and shellfish that Minamata residents consumed caused serious Hg poisoning in local residents (Harada, 1995).

Mercury methylation is primarily a microbial process (Compeau & Bartha, 1985) performed by some anaerobic iron- and sulphate-reducing bacteria that have the ability to transform Hg(II) and produce MeHg as a by-product of their microbial metabolism (Fleming *et al*., 2006; Kerin *et al*., 2006). Some *Desulfobacterales*, *Geobacter*, and *Desulfuromonales* have been found to have the

ability to methylate Hg (Gilmour *et al*., 2011). Relatively recently it was discovered that at least one bacteria (*Desulfovibrio desulphuricans*) has the ability to methylate elemental Hg as well as Hg(II) (Colombo *et al*., 2013). Compeau & Bartha (1985) proposed that Hg methylation in this particular organism occurred through the transfer of a methyl group from methyl-tetrahydrofolate using methylcobalamin The methyl group was found to have originated from the C-3 from serine or formate using acetyl-coenzyme A pathway. This pathway is found outside of *D. desulfuricans* LS so it was suggested that the ability to methylate Hg is likely associated with the substrate specificity of its enzymes. Ekstrom *et al*. (2003) and Ekstrom & Morel (2008) determined that the ability to methylate was not simply tied to the acetyl-coenzyme A synthase pathway as some known methylators lack this pathway. Since there is a known difference in the pathway involved in methylation, this could possibly explain the differences in methylation rate. The most common theory for Hg uptake is diffusion of small neutrally charged Hg complexes. Mercury is able to enter bacterial and algal cells though the cell wall using passive diffusion of $HgCl_2$ and HgS (Mason *et al*., 1996; Benoit *et al*., 1999; Benoit *et al*., 2001). Golding *et al*. (2002) suggested that Hg uptake may actually occur via a facilitated transport mechanism. In all instances identified thus far, Hg is methylated in the cell and is then excreted by the microbe into the environment.

Parks *et al*. (2013) found two gene clusters (HgcA and HgcB) that encode for proteins that have the ability to carry methyl groups in known Hg methylators *Desulfovibrio desulfuricans* and *Geobacter sulferruducens*. Following this discovery, Gilmour *et al*. (2013) looked for the presence of these gene clusters in all microorganisms with their genomes sequenced, including those previously identified Hg methylators. The HgcA and HcgB gene clusters were found in known methylators as well as other microorganisms not yet known to be Hg methylators including methanotrophic, syntrophic, acetogenic and fermentive anaerobes from both Archaea and Bacteria domains (Gilmour *et al*., 2013). These microorganisms survive in a diverse range of environments including rice paddies, the animal gut, and environments of extreme pH and salinity (Gilmour *et al*., 2013). Though there is now a variety of identified Hg methylators, iron- and sulphate-reducing bacteria remain the primary methylators.

Methylmercury production is an anaerobic process that occurs in a saturated environment, at relatively low redox potentials and at lower pH. Methylmercury production can also be affected

by dissolved organic carbon (DOC) availability as DOC can complex with inorganic Hg and are generally too large to pass through the cell membranes (Miskimmin *et al*., 1992). At a lower pH, DOC is less negatively charged which makes it less likely to complex Hg and increases the available Hg for methylating bacteria (Miskimmin *et al*., 1992, Haitzer *et al.,* 2003, Kelly *et al*., 2003). Methylmercury can also be demethylated in the environment and thus the net MeHg concentration in soil, sediment and water is governed by both methylating and demethylating processes (see Marvin-DiPasquale *et al*., 2000). Demethylation of MeHg in the environment can occur biotically by mircoorganisms that have the *mer* operon (Marvin-DiPasquale *et al*., 2000; Barkay *et al*., 2006) and abiotically, through mechanisms like photodegradation (see Sellers *et al.,* 1996).

1.2 Peatlands as a Source of Methylmercury

St. Louis *et al*. (1994) found that the presence of wetlands in a catchment resulted in higher MeHg exported from these catchments. The degree of MeHg loading to downstream environment was also found to be related to wetland type (St. Louis *et al*., 1996). MeHg was found in high concentrations in peat soils of some wetlands suggesting that they are net sources of MeHg to the downstream environment (Krabbenhoft *et al*., 1995; Branfireun *et al*., 1996).

Methylmercury production in peatlands can vary widely both within a single peatland (Mitchell *et al*., 2008b) and between peatland types due to differences in nutrient status and hydrologic fluctuations (Tjerngren *et al*., 2012a, b). Provided sulphate (SO_4^{2-}), a labile carbon source and bioavailable Hg, are present, Hg methylation can occur in the reducing peat environment.

Divalent Hg has a high affinity for binding with dissolved organic matter and soil organic matter and in the natural environment, the majority of the soil Hg present is bound to organic matter (Skyllberg *et al*., 2000; Åkerblom *et al*., 2008). This means that the Hg mobility in soils is controlled by the complexation with organic matter (Kalbitz & Wennrich, 1998; Matilainen *et al*., 2001; Skyllberg *et al*., 2003). The organic compounds that Hg complexes strongly with contain reduced sulphur groups, such as thiols (Skyllberg *et al*., 2006) as well as other, weaker binding locations such as phenolics (Drexel *et al*., 2003). Drexel *et al*. (2003) was able to show

that Hg(II) showed a preference for thiols at lower Hg(II) concentrations and a preference for phenolic binding sites at higher Hg(II) concentrations.

Sulphide (S^-), generated by SO_4^{2-} reduction can impact Hg availability. Gilmour *et al*. (1998) determined that there is a SO_4^{2-} concentration producing a MeHg production optima in wetlands where there is sufficient SO_4^{2-} to support MeHg production but not so much that excess S^- is generated that would to inhibit methylation. Sulphide concentrations above 0.3 – 3.0 mg/L in different wetland ecosystems have been shown to inhibit methylation (Gilmour *et al*., 1998; Benoit *et al*., 2001; Langer *et al*., 2001; Jay *et al*., 2002; Drott *et al*., 2007). The formation of $HgS_{(s)}$ removes bioavailable Hg through precipitation (Björnberg, 1988; Benoit *et al*., 1999). In sulphidic environments, S^- can out-compete other ligands as the solubility constant for Hg(II) and $HgS_{(s)}$ is extremely low ($K_s = 10^{-52}$) meaning when S^- if present, virtually all Hg would be precipitated as $HgS_{(s)}$ (Björnberg, 1988; Dyrssen & Wedborg, 1991).

1.3 Sources of Sulphate to Peatlands

Sulphate-reducing bacteria control Hg methylation in peatlands which makes SO_4^{2-} concentration in peatlands important as it regulates their metabolic activity (Mitchell *et al*., 2008a; Stickman *et al*., 2016), and the deposition of SO_4^{2-} in rain and snow is a primary vector of SO_4^{2-} delivery to northern latitude ecosystems, including peatlands. Global sulphur/SO_4^{2-} emissions increased fairly consistently until the end of the 1980s (Lefohn *et al*., 1999) and emissions decreased from 1990 to 2000, with estimated peak global sulphur emissions between 70 and 80 Tg S/yr (Stern, 2006). In the past, the majority of sulphur dioxide was released to the atmosphere from coal-burning power plants. Sulphur dioxide interacts with water in the atmosphere to produce sulphuric acid (H_2SO_4), which delivers SO_4^{2-} to ecosystems in rain and snow. Sulphate deposition is highest near industrialized regions and decreases with distance from the emission sources (Singh & Agrawal, 2005). Sulphate atmospheric deposition in northern peatlands is lower due to their remote location, compared to more southern locations closer to SO_4^{2-} sources. This is well illustrated in the 2007 sulphur wet deposition map for North America in the following figure showing the lowest mass/year deposited in the more northern regions (Vet *et al*., 2014):

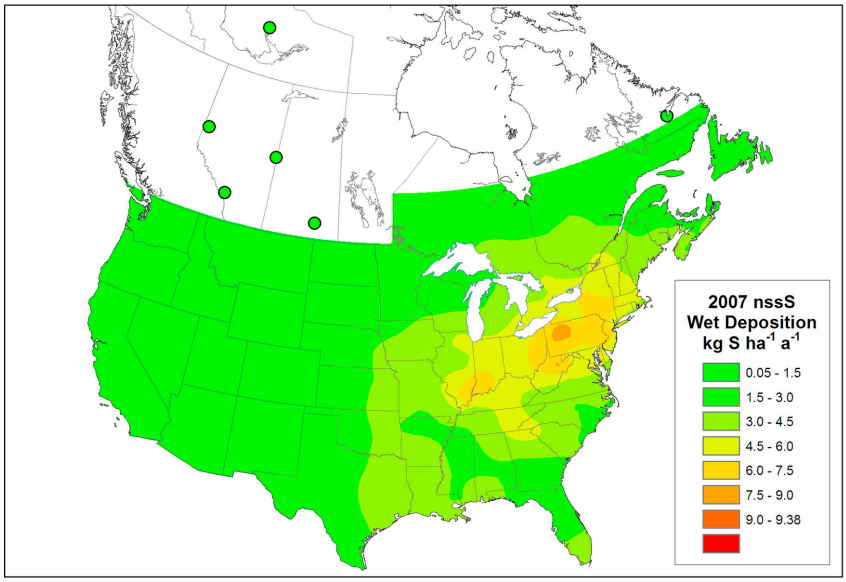

Figure 1.2: 2007 Annual Wet Deposition of SO_4^{2-} across Canada and the United States (Vet *et al*., 2007).

Once sulphur is deposited to peatlands, it can be partitioned to both inorganic (e.g. SO_4^{2-}, FeS, H_2S) and organic forms (e.g. carbon-bound S, ester S) (Chapman & Davidson, 2000) governing the amount of SO_4^{2-} available for Hg methylation (Novák & Wieder, 1992; Coleman-Wasik *et al*., 2015). In general, peatlands are SO_4^{2-} sinks, however it can be released from the peat stores with fluctuating water tables which can re-oxidize reduced and organic bound S to SO_4^{2-} (Devito & Hill, 1999; Dowrick *et al.*, 2005; Coleman-Wasik *et al*., 2015).

Regional hydrology can influence SO_4^{2-} supply to wetlands through surficial and sub-surface flow. Devito & Hill (1997) observed small SO_4^{2-} concentration peaks following water table rises above the wetland surface during storm runoff. Groundwater upwelling can provide SO_4^{2-} to wetlands (Devito & Hill, 1997; Branfireun *et al*., 2002) providing even SO_4^{2-}-limited wetlands with a SO_4^{2-} source. Periodic release of SO_4^{2-} has been observed in wetlands and peatlands following water table drawdown causing the re-oxidation of reduced sulphur during dry summers (Wieder, 1985; Bayley *et al*., 1986; Coleman-Wasik *et al*., 2015). Upon rewetting, SO_4^{2-} can be released into pore water allowing for sulphate-reducing bacteria to access this newly regenerated SO_4^{2-} pool (Coleman-Wasik *et al*., 2015).

Gold and base-metal mines have been known to have MeHg contamination problems (Grandjean *et al*., 1999; Winch *et al*., 2008; Winch *et al*., 2009). Sulphate-reducing bacteria has been found

in acidic mine tailings (Winch *et al*., 2009). Though these tailings can be very acidic, sulphate-reducing bacteria have been found to be active in environments with pH ~2 (eg. Praharaj & Fortin, 2004).

McCarter *et al*. (2017) found that after one year of simulated mining wastewater additions (27.2 mg/L SO_4^{2-}) to an Experimental Fen that there were increases in pore water MeHg, Total mercury (THg) and %MeHg (percent of THg found as MeHg). Despite these increases in SO_4^{2-}, not all SO_4^{2-}-contaminated sites exhibit enhanced methylation or MeHg contamination issues. Johnson *et al*. (2016) found that there was no significant difference in MeHg pore water and solid phase accumulation in a wetland with long term, high concentration (> 100 mg/L) mine SO_4^{2-} tailings additions. These findings are consistent with the observation that there are SO_4^{2-} and S^- optima for Hg bioavailability and methylation. Methylmercury production in wetlands with exposure to elevated SO_4^{2-} loading does not respond proportionally to SO_4^{2-} loading, and wetlands that are SO_4^{2-}-limited may exhibit a stronger methylation response than those with chronically elevated SO_4^{2-} (Branfireun *et al*., 1999; Jeremiason *et al*., 2006; Mitchell *et al*., 2008a; Johnson *et al*., 2016).

1.4 Thesis Objectives

Sulphate additions to peat has been shown to increase pore water MeHg concentrations through SO_4^{2-} reduction by sulphate-reducing bacteria (Branfireun *et al*., 1999; Mitchell *et al*., 2008a). As mining and industrial development in the north continues, wetlands as wastewater treatment options may become more prominent form of wastewater treatment, yet we have incomplete knowledge about how the SO_4^{2-}-limited peatlands found at higher latitudes will respond in terms of Hg methylation to increased SO_4^{2-} loading from both tailings-derived SO_4^{2-}, as well as the discharge of other SO_4^{2-}-containing waters.

To address these knowledge gaps, the objectives of this thesis are:

1. To experimentally determine the impacts of multi-year SO_4^{2-} addition on Hg methylation in a sub-arctic fen peatland (Chapter 2) and;

2. Assess the relative impacts of different levels of SO_4^{2-} loading on MeHg production in sub-arctic peats using laboratory experiments with natural peats, simple SO_4^{2-} solutions, and leached mine waste rock to simulate tailings-derived SO_4^{2-} (Chapter 3).

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Chapter 2

2 Effect on Timing and Magnitude of Point Source Sulphate Loading on Methylmercury Production in Northern Peatlands

2.1 Introduction

Mercury (Hg) is released or re-emitted into the atmosphere through natural and anthropogenic process, with natural processes including outgassing of mercuriferous rocks and soils, wild fires, volcanoes and erosion, and anthropogenic sources including coal burning, artisanal gold mining and waste burning (see Schroeder & Munthe, 1998; Taylor *et al*., 2005; Driscoll *et al*., 2013; U.S. EPA, 2015). Anthropogenic sources have increased the atmospheric concentration of Hg by approximately 3 times since the pre-industrial era (Lindberg *et al*., 2007) with the majority of the atmospheric Hg found as gaseous elemental Hg (GEM). The atmospheric residence time of Hg has been estimated to be from several months to a year (Pirrone & Mason, 2009) allowing it to travel long distances and be deposited in regions far from the original source (Jaffe *et al*., 2005; Durnford *et al*., 2010). Mercury is transported long distances in the atmosphere as GEM and is then oxidized and deposited largely as divalent inorganic Hg (Hg(II)) through wet (precipitation) and dry (particulate) deposition (Lindqvist & Rodhe, 1985). This Hg can then be converted into methylmercury (MeHg) by sulphate (SO_4^{2-}) and iron-reducing bacteria as a by product of their microbial metabolism (Compeau & Bartha, 1985; Kerin *et al*., 2006; Gilmour *et al*., 2013). Mercury enters bacterial and algal cells through the cell wall by passive diffusion of $HgCl_2$ and HgS (Mason *et al*., 1996; Benoit *et al*., 1999). Active Hg uptake by SO_4^{2-} and iron-reducing bacteria has also been identified and the type of uptake can depend on which thiol-containing compounds are present (Schaefer *et al.*, 2011). Mercury methylation occurs inside sulphate-reducing bacteria via enzyme-mediated methyl transfer from methylcobalamin (Choi & Bartha, 1993; Choi *et al*., 1994) and is then excreted from the cell as MeHg.

Much of the fundamental research on methylation and sulphate-reducing bacteria was conducted on lake sediments (Korthals & Winfrey, 1987; Gilmour *et al*., 1992) where it was determined that anaerobic conditions (Olson & Cooper, 1976; Callister & Winfrey, 1986) and pH are

important controls on Hg methylation rates (Ramlal *et al*., 1985). One aspect that pH can control is the fraction of available Hg for methylation through interactions with dissolved organic matter (DOM) where DOM is less negatively charged at lower pH levels and thus less likely to complex with Hg(II) (Haitzer *et al*., 2003; Kelly *et al*., 2003).

The reduction of SO_4^{2-} in SO_4^{2-}-rich environments can lead to a buildup of sulphide that can inhibit microbial activity (Compeau & Bartha, 1983; Benoit *et al*., 1999). Research in the Florida Everglades identified a SO_4^{2-} optima for MeHg production where there is enough SO_4^{2-} for sulphate-reducing bacteria to metabolize but not enough reduction that sulphide concentrations inhibit methylation (Gilmour *et al*., 1998; Benoit *et al*., 2001; Langer *et al*., 2001; Jay *et al*., 2002; Drott *et al*., 2007). Sulphide concentrations can play an important regulatory role in methylation as $HgS_{(s)}$ precipitates from solution, making this Hg less bioavailable (Björnberg, 1988; Benoit *et al*., 1999). In sulphidic environments, sulphide can out-compete other ligands for Hg complexation as the solubility constant for Hg(II) and $HgS_{(s)}$ is extremely low ($K_s = 10^{-52}$) indicating that in the presence of sulphide, essentially all Hg would precipitate to the solid phase (Björnberg, 1988; Dyrssen & Wedborg, 1991). In aquatic environments, it has been suggested that solid phase organic matter may control Hg partitioning between aqueous and solid phase (Hammerschmidt *et al*., 2008; Hammerschmidt & Fitzgerald, 2006). As sulphur species and DOM exert a large control over Hg speciation in wetlands, it is important to examine these variables to determine which may be contributing to changes in MeHg concentrations.

As sites of SO_4^{2-} reduction, wetlands (particularly peatlands) have been identified as sources of MeHg to downstream aquatic systems (St. Louis *et al*., 1994; Branfireun *et al*., 1996; Loseto *et al*., 2004). Mercury methylation does not occur uniformly in peatlands naturally with hot spots of methylation occurring at the peatland-upland interface (Mitchell *et al*., 2008b) or at locations of groundwater upwelling where in both cases, solutes (e.g. available carbon, SO_4^{2-}) are delivered to different areas where methylation can then occur. It is well established that the zone of highest MeHg concentrations is near the mean annual water table position where anoxia is maintained, and temperature and nutrient supply are optimal for sulphate-reducing bacterial growth (see Branfireun *et al*., 1996). Over a large array of watersheds, there has been a positive relationship observed between proportion of the watershed being wetland area and MeHg concentration and flux (Grigal, 2002) all indicative of peatlands as significant sources of MeHg to the environment.

Since the industrial period began, atmospheric SO_4^{2-} deposition from coal burning and other processes resulted in a considerable increase causing environmental acidification, especially in regions near heavily industrialized areas (Likens & Bormann, 1974). Successful regulations have since been put in place to minimize SO_4^{2-} release from industrial activity (Driscoll *et al*., 2001). The relationship between sulphur and Hg/MeHg in Boreal peatlands was not documented until the late 1990s and early 2000s (Branfireun *et al*., 1999, 1998; Branfireun & Roulet, 2002). Since these studies, most research has looked at the impact of atmospherically deposited SO_4^{2-} on MeHg production in peatlands (Jeremiason *et al*., 2006; Åkerblom *et al*., 2013) as this is the dominant source to catchments.

Sulphate additions have been found to increase SO_4^{2-} concentrations in peatlands (Branfireun *et al*., 1999; Coleman-Wasik *et al*., 2012) which are typically SO_4^{2-} limited. Mesocosm experiments were able to show that the combined additions of SO_4^{2-} and labile carbon was able to best stimulate Hg methylation rather than SO_4^{2-} alone (Branfireun *et al*., 1999; Mitchell *et al*., 2008a). Coleman-Wasik *et al*. (2012) found that after SO_4^{2-} additions ceased, MeHg concentrations were able to, relatively quickly, fall back to near-background concentrations. Drying and subsequent rewetting of the same SO_4^{2-} amended peatland resulted in SO_4^{2-} regeneration and increased MeHg concentrations (Coleman-Wasik *et al*., 2015) pointing to the potential for SO_4^{2-} additions to cause long term changes in MeHg cycling.

Methylmercury contamination has been associated with gold and base-metal mines (Grandjean *et al*., 1999; Winch *et al*., 2008; Winch *et al*., 2009) which can have very sulphur-rich geologies. Sulphate-reducing bacteria have been found in mine tailings (Winch *et al*., 2009) and even though these tailings can be very acidic, SO_4^{2-} reducers have been found to be metabolically active in environments with pH ~2 (e.g. Praharaj & Fortin, 2004). Johnson *et al*. (2016) demonstrated no significant difference in MeHg pore water and solid phase accumulation in a wetland with long term, high concentration (>100mg/L) mining SO_4^{2-} amendments and a non-amended wetland in the same area,, suggesting that wetlands with chronically elevated SO_4^{2-} additions may not have the same response to SO_4^{2-} loading as wetlands that are SO_4^{2-} limited (Johnson *et al*., 2016).

Much less is known about how SO_4^{2-} loading from mining processes may affect northern peatlands that naturally receive much lower SO_4^{2-} deposition. McCarter *et al*. (2017) found that after only a year of simulated wastewater additions (containing SO_4^{2-}), MeHg concentrations, THg concentrations, and %MeHg (percent of THg found as MeHg) all had multi-fold increases in an experimental fen in the James Bay Lowlands. Sulphate was found to have moved quickly through the peatland (McCarter *et al*., 2017) indicating the potential for large spatial variation in net MeHg production. As this study was only for one year of simulated wastewater additions, it was unknown what the implications would be for multi-year additions of SO_4^{2-} on MeHg production, nor what would happen to MeHg production after additions were stopped. Therefore, the objective of this study was to evaluate the impact of multi-year SO_4^{2-} additions and recovery on MeHg concentrations in a simulated wastewater treatment fen peatland.

To address this, we repeated wastewater (and subsequent SO_4^{2-}) loading as reported in McCarter *et al*. (2017), for one year (2015), and in a subsequent year (2016), resampled without any wastewater additions (recovery year). We repeated the SO_4^{2-} loading in order to determine if there was a cumulative impact on MeHg production and then sampled a subsequent recovery year without wastewater loading to assess carry over and rate of recovery.

2.2 Site Description

This study site (Experimental Fen) was instrumented in the summer of 2013 and nutrient additions, water sampling, and intensive hydrological monitoring began the following year. It is located in the Hudson/James Bay Lowlands (HJBL) in northern Ontario, Canada (52˚51’17 N, 83˚56’34 W) approximately 90km West of Attawapiskat, Ontario near the De Beers Group of Companies Victor Diamond Mine. The peatland complex that the Experimental Fen resides in is characterized by a 1.5–2.5m layer of peat over the Hudson Platform which consists of limestone, mudstone, dolostone and evaporites (Singer & Cheng, 2002). During the Quaternary period, the retreat of the Laurentide Ice Sheet, deglaciation and formation of the Tyrell Sea (~8,000 years ago) left behind glacial tills and marine silts and clays that are between 10 and 30 m thick (Glaser *et al*., 2004). Relatively low hydraulic conductivity (5.2×10^{-5} m/day) prevents enhanced groundwater recharge from the overlying fen and minimizes the effects of the water table drawdown from the nearby open pit mine (Whittington & Price, 2013). This peatland formed due

to the poor drainage in the area, continued isostatic rebound of the HJBL at 1 m/century (Hunter, 1970) and thus flattening of the landscape resulting in the accumulation of peat deposits and eventually leading to a peatland-dominated region over the HJBL (Riley, 2011).

The Experimental Fen (*Figure 2.1*) is approximately 225 m long and shows a pool-ridge-pool pattern with the direction of the water flow perpendicular to the peat ridges. To the East and West of the Experimental Fen are bogs, to the North lies a large pool (used to draw water for hydrologic loading) and to the South is a North branch of North Granny Creek (McCarter & Price, 2017).

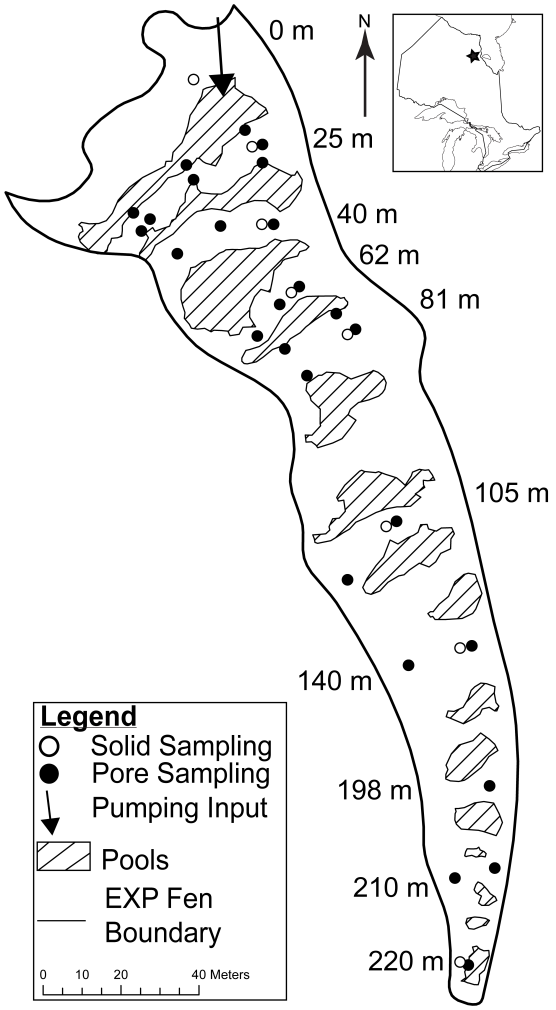

Figure 2.1: Map of the Experimental Fen with SO_4^{2-} added at the arrow at the top of the fen (2014 and 2015) and flowing down the length of the fen with pore water sampling locations denoted by the filled circles and 2016 solid sample locations denoted by the open circles (Modified from McCarter *et al*., 2017).

Ridges were divided into three different sections based on similar MeHg concentrations and ancillary water chemistry data. The 25m ridge which was 25m away from the wastewater additions as the upper ridge, the 40 m, 62 m and 81 m ridges which were 40 m to 81 m away as the middle ridges and the 105 m, 140 m, 198 m, 210 m and 225 m ridges which were called the lower ridges. Each sampling section had approximately the same number of sample locations (*Figure 2.1*) with the most dense sampling occurring in the 25m ridge where we expected to see the largest changes.

Two reference fens (52˚47'01 N, 83˚53'12 W and 52˚47'00 N, 83˚53'19 W) located near the Experiment Fen were chosen and sampled periodically (THg, MeHg, DOC, SO_4^{2-}) to be used as a baseline for changes seen in the Experimental Fen. These two locations have similar topography, vegetation and peat depth to the Experimental Fen. Three small well transects were set up in the pools and ridges, as well as two pond wells at each site, to monitor natural water chemistry and hydrology changes.

2.3 Methods

During the 2014 and 2015 summer field season (May-August), simulated wastewater was added to an Experimental Fen in the James Bay Lowlands. Over 38,000 L and 30,000 L of water was pumped into the Experimental Fen each day during the summer field season in 2014 (July 11 – August 31) and 2015 (July 4 – August 14), respectively. The simulated wastewater contained 27.2 mg/L SO_4^{2-}, 27.2 mg/L nitrate, 9.1 mg/L ammonium, 7.4 mg/L phosphate and 47.2 mg/L chloride, similar to actual wastewater measured at the mine site (McCarter *et al*., 2017). Water for the experiment was pumped in from a nearby pond where it was then mixed with concentrated fertilizer and added as a point source to the top of the fen (Arrow at the top of *Figure 2.1*). For a full description of the experimental design see McCarter *et al*. (2017). Field hydrologic data was collected as well as various water and solid samples throughout the three field seasons.

All %MeHg values were calculated on an individual sample basis then those values were used to provide averages for %MeHg. Distribution coefficients for THg and MeHg were also calculated [$\log K_d = \log ([Hg]_{solid}/[Hg]_{porewater})$; µg/L] for each solid sample location with corresponding

pore water samples. Solid samples were taken at 3 (10 – 15cm, 20 – 25 cm, 30 – 35 cm below surface) depths but average MeHg and THg concentrations over all depths at each sample location were used to calculate distribution coefficients for both the reference site and Experimental Fen. All in text values are (± one standard deviation).

2.3.1 Precipitation and Air Temperature

The local meteorological station located near the De Beers Group of Companies Victor Diamond Mine has 9 years of temperature data available (2006-2015). Summer precipitation data was collected at the site using a tipping bucket rain gauge (Texas Instruments TE525M-L tipping bucket rain gauge) with values being totaled every 20 minutes. Longer term climate data was retrieved from Environment Canada (http://climate.weather.gc.ca/climate_normals) Moosonee (~250km Southeast of the field site) which were both used to collect meteorological data for this site.

2.3.2 Water Sample Collection and Analysis

Every ~7-10 days the wells and piezometers were purged the day before sampling. The pH was recorded using a YSI 650-01 Series Handheld with 600XL-B-0 YSI Sonde equipped with pH probe by rinsing the YSI sample cup and probe 3 times with water from each well then filling the cup and screwing in the probe to take the final reading. The probes were calibrated the morning before every sampling event and the pH probe used pH 4, 7 and 10 standards to calibrate.

Mercury samples were collected in double-bagged 250mL PETG bottles using a peristaltic pump with Teflon tubing and only taken from certain wells throughout the fen. Between each Hg sample the lines were rinsed with 18.2 MOhm/cm DI water to eliminate cross-contamination of samples and sample lines were rinsed with sample prior to collection. Surface (pond) water samples were collected using a ~3 m long PVC pipe with a three prong extension clamp attached to the end to allow reaching to the center of large ponds. This clamp was outfitted with a nitrile glove that was changed between samples. All sample bottles were environmentalized three times prior to filling and all Hg samples were collected using the “clean hands, dirty hands” method (EPA Method 1669) for ultra-trace sampling. Field duplicates were collected every ~10 samples and field and lab blanks were collected for QA/QC. Samples were vacuum filtered in a PTFE-

outfitted modified desiccator using Macherney-Nagel 0.45 μm glass microfiber filters within 36 hours of collection. After filtering, samples were preserved using OmniTrace Ultra EMD Millipore HCl to 1% v/v. Before and after filtering samples were stored in a dark refrigerator (4-6˚C).

Water samples for all analytes other than Hg were collected into sterile 50 mL Environmental Express Flipmate® filtration bottles using a peristaltic pump with C-Flex and Teflon tubing. Environmental Express Flipmate® bottles come with two caps, one for storage and one for filtration with the filtration cap accepting two threaded sample bottles – one empty and one with unfiltered sample. A port on the side of the filtration cap allows for vacuum pressure to be applied and the sample is pulled through a 0.45 μm filter in the filter cap into the empty cup. Between sampling events, all sample lines were rinsed with 10% HCl for 20 minutes and 18.2 MOhms DI water for 20 minutes to prevent contamination. Prior to sampling, sample was run through the Teflon lines to minimize sample cross-contamination. Field duplicates were collected every ~10 samples and field blanks were collected periodically for QA/QC. Samples were stored in a cooler with icepacks in the field then transferred to a refrigerator (4-6˚C) in the on site laboratory until shipment back to Western University for analysis. All water samples were filtered within 36 hours of collection using Macherney-Nagel 0.45 μm glass fiber filters.

2.3.2.1 Water Chemical Analyses

Ions and Dissolved Organic Carbon: Waters were analyzed for anions (only SO_4^{2-} is reported here) on a Dionex ICS-1600 Ion Chromatograph in accordance with EPA Method 300.0 using 0.5 mL sample aliquots and diluted using 18.2 MOhm deionized water when required (sample concentration > 100 mg/L). Analytical duplicates were run every 10 samples and field duplicates were collected every ~10 samples and were both expected to fall within ±15% of each other. Matrix spikes and check standards were run every 10 samples and were also expected to fall within ±15% of the expected value. Samples were rerun if they did not meet proper QA/QC. The instrument was calibrated to analyze samples between 0.5 mg/L and 100 mg/L and the reporting limit was 0.05 mg/L.

DOC samples were analyzed on an OI Analytical Aurora 1030W Combustion TOC Analyzer using a wet oxidation method (minimum detection limit = 0.2 mg/L). A minimum 7 mL of sample was used for analysis. Analytical duplicates were completed on 10% of samples, matrix spikes were done on 20% of the samples and a set of three check standards were completed in each run for both DOC and ions and were all expected to fall within ±15% of their expected values. Samples that failed to meet QA/QC were rerun. The instrument was calibrated to analyze samples with concentrations between 0.5 mg/L and 50 mg/L, with a reporting limit of 0.5mg/L.

Methylmercury: MeHg was analysed in accordance with EPA Method 1630 (U.S. EPA, 1998), briefly those methods included the following: 40 mL aliquots with 1% ammonium pyrrolidine dithiocarbamate, distilled for 3 hours using nitrogen gas at 125˚C for ~3 hours from Teflon distillation vessels, through polyfluorinated plastic tubing into glass vials. Ascorbic acid was added to 30 mL sample aliquots, samples were shaken and left uncapped for 10 minutes to allow for removal of free halogens. 2M acetate buffer was added to adjust the sample pH to ~4.5 and sodium tetraethyl borate ($NaBEt_4$) was added to ethylated MeHg and samples were capped. Samples were purged with argon gas and analyzed for MeHg on a Tekran© Model 2700 Automated Methyl Mercury Analysis System by gas chromatography and detection by cold vapour atomic fluorescence spectrometry (CVAFS). The Biotron Analytical Services Laboratory method detection limit (MDL) for MeHg analysis was 0.006 ng/L and the method reporting level (MRL) was 0.18 ng/L. Recovery of MeHg matrix spikes (mean ± standard deviation) was 106 ± 1.53% (n = 43) in 2014, 95.3 ± 1.43% (n = 38) in 2015 and 95.3 ± 2.00% (n = 40) in 2016 and sample duplicate recovery was 100 ± 1.80% (n = 24) in 2014, 101 ± 0.81% (n = 22) in 2015 and 98.4 ± 1.14% (n = 17) in 2016.

Total Mercury: To analyze THg in water samples, bromine monochloride (BrCl) was added to 25 mL sample aliquots, shaken, left uncapped for 10 minutes then stored overnight for BrCl oxidation. The following day, hydroxylamine hydrochloride (HA) was added, samples were shaken and left uncapped for 30 minutes to allow for free halogen removal. Finally, stannous chloride was added to convert all Hg species present in the sample to GEM. Gaseous elemental Hg was purged with high purity nitrogen gas from aqueous solution, captured on a gold trap, thermally desorbed and quantified using CVAFS on a Tekran© 2600 Mercury Detector according to EPA Method 1631 (U.S. EPA, 1999) for THg. The Biotron Analytical Services

Laboratory reports a MDL of 0.048 ng/L and an MRL of 0.144 ng/L for water samples run for THg. Recovery of THg matrix spikes (mean ± standard error) was 96.1 ± 2.30% (n = 30) in 2014, 98.6 ± 1.27% (n = 41) in 2015 and 103 ± 1.22% (n = 26) in 2016 and sample duplicate recovery was 99.1 ± 3.33% (n = 18) in 2014, 101 ± 1.83% (n = 22) in 2015 and 102 ± 2.03% (n = 12) in 2016.

For both Hg methods, method blanks and quality control standards were acidified to 1% v/v instead of 0.5% v/v. 20% of the samples run were analytical duplicates, 5% matrix spikes and quality control standards were run periodically throughout the run.

2.3.3 Peat Sampling and Analysis

Peat field samples were collected at 10-15 cm, 20-25 cm and 30-35 cm below the surface and approximately 90 g wet weight of peat was collected in a transect down the fen. Samples were collected in 10.16 cm x 15.24 cm plastic bags and duplicates were collected every ~10 samples for QA/QC. Samples were stored in a cooler with ice packs while in the field then frozen in the on-site lab and kept in the dark for shipment back to Western University. Samples were freeze-dried for 4 days. The peat was then ground and homogenized using a KitchenAid coffee grinder by splitting the sample and pulse grinding the peat 10 times for 1 second each time and placing the ground sample in a new bag.

Total Sulphur: Total sulphur samples were sent to the Ontario Forest Research Institute (OFRI) in Sault Ste. Marie, Ontario. The method used for percent total sulphur analysis is combustion of the peat at 1350˚C on an Eltra Helios C/S Analyzer. OFRI reports a MDL of 0.004%.

Methylmercury: Solid sample MeHg data was obtained by digesting ~100 mg of peat in 2 mL of 25% KOH in methanol for 4 hours at 82˚C in Teflon bombs. Samples were left to cool for 1 hour before being diluted with 8 mL of 18.2 MOhm deionized water and vortexed for 10 seconds to ensure the samples were homogenous. Samples were then transferred to 15 mL falcon tubes and stored in the fridge overnight for analysis the following day (sample digestion timing does not allow for instrument start up and calibration curve building on the same day). The following day samples were brought to room temperature, centrifuged, diluted with distilled water and made up to 30 mL with 500 µL 2M buffer and 30 µL $NaBEt_4$. Samples were shaken and left to

react for 30 minutes with $NaBEt_4$ before being run on a Tekran© Model 2700 Automated Methyl Mercury Analysis System with 10% analytical duplicates (101.81 ± 6.72%, n = 10) and 10% matrix spikes (97.53 ± 5.52%, n = 5).

Total Mercury: The samples were then run on the Milestone DMA-80 direct Hg analyzer according to EPA Method 7473 (U.S. EPA, 2007). The calibration detection limit was 1 ng. Every 10 samples, analytical duplicates were analyzed (105.66 ± 6.80%, n = 5) and were required to fall within ± 15% of each other. A certified reference material (CRM), in this case MESS-3 (0.091 ± 0.009 mg/kg Hg) and IAEA 158 (0.132 ± 0.014 mg/kg Hg), was run at the beginning and end of each run. Blanks were analyzed at the start of each run and following every CRM (mean: 0.022 ng (± 0.019), n = 10).

Distribution coefficients ($LogK_d$) were calculated [$logK_d = log([Hg]_{solid}/[Hg]_{porewater})$; µg/L] using the solid phase Hg concentrations and dissolved Hg concentrations for each individual sample. Average distribution coefficients were averaged over the 3 replicates of each addition type.

2.4 Results

2.4.1 Precipitation and Temperature

The average temperature and precipitation during July and August was 15.6˚C and 154 mm calculated from the near site 9-year data collection. The average temperature and precipitation for Moosonee (July and August between 2014 and 2016) was 14.9˚C and 89 mm (Environment Canada, 2016) which agrees relatively well with the values from the near site meteorological station. During 2015, however there was above average precipitation at the site (~300 mm vs. long term average of ~120 mm over the summer) (McCarter & Price, 2017).

2.4.2 Pore Water Chemistry

pH: pH at the reference site averaged 5.17 (± 0.04) in 2014, 5.12 (± 0.57) in 2015 and 5.40 (± 0.34) in 2016 and remained consistent over the summer field season. pH values fluctuated between year at the Experimental Fen with a value of 4.89 (± 0.54) in 2014 (before the additions started) and dropped to 4.64 (± 0.45) at the end of the additions in 2014. Before wastewater

additions began in 2015, the average pH value over the entire fen was 5.15 (± 0.46) and at the end of additions in 2015, this value had dropped to 4.83 (± 0.40). pH values in 2016 rose to an average of 5.60 (± 0.31) at the beginning of 2016 and dropped down to 4.89 ± (0.43) by the end of the summer.

Methylmercury: Pre-addition MeHg concentrations in the experimental fen ranged from 0.035 – 1.88 ng/L. This is slightly higher than the average MeHg concentration found at the reference fens that were 0.10 ng/L (± 0.03), 0.075 ng/L (± 0.018), and 0.082 ng/L (± 0.037) in 2014, 2015 and 2016, respectively.

After the first simulated wastewater additions in 2014, mean MeHg concentrations in the upper ridge (25 m from addition source) was 5.20 ng/L (± 2.57) by the end of the 2014 additions; an increase of 40 times the pre-addition MeHg concentration (*Figure 2.2*). The middle ridges also showed an increase in MeHg concentration to an average of 1.35 ng/L (± 0.96) from a pre-addition average of 0.11 ng/L (± 0.03). The furthest sampling locations down the Experimental Fen showed the least change in MeHg concentration at 0.20 ng/L (± 0.12) but were still higher than pre-addition (0.14 ng/L ± 0.06) and reference fen concentrations.

The 2015 pre-addition MeHg concentration in the upper ridge averaged 0.92 ng/L (± 0.40) which was more than 7 times higher than the 2014 pre-addition MeHg concentrations (0.13 ± 0.03), and higher than reference fen concentrations (*Figure 2.2*). Methylmercury concentrations increased the most in the upper ridge, ending the 2015 field season at an average MeHg concentration of 4.15 ng/L (± 3.38) but showed a large range in concentration (1.46 ng/L – 13.93 ng/L) throughout the ridge at the end of the season. The middle ridges had the second largest increase in MeHg concentration during the 2015 additions, increasing from 0.62 ng/L (± 0.23) pre-2015 additions to 3.23 ng/L (± 2.14) by the end of the season. The smallest increase in MeHg concentration in 2015 was in the lower ridges where the MeHg concentrations increased from 0.16 ng/L (± 0.12) pre-addition to 0.28 ng/L (± 0.19) post additions.

The MeHg concentrations in 2016 all decrease from the end of 2015 field season values and remain relatively consistent throughout the field season (*Figure 2.2*). These MeHg concentrations decrease but do not fall back to pre-addition values or reference site values. The largest MeHg concentrations remain in the upper ridge with an average of 0.72 ng/L (± 0.39)

over the entire field season. The middle ridges and lower ridges averaged 0.64 ng/L (± 0.26) and 0.20 ng/L (± 0.11) over the 2016 field season, respectively.

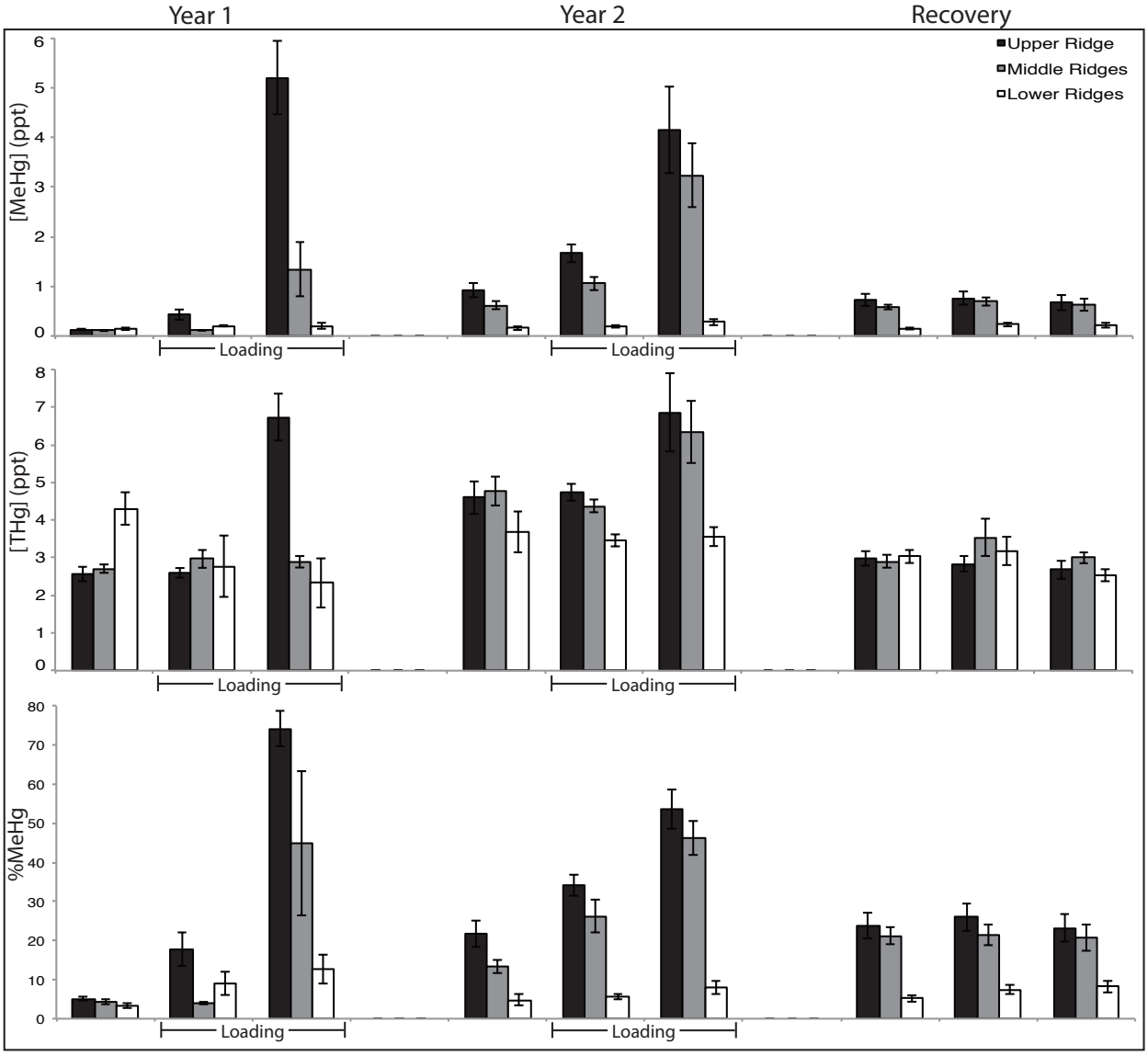

Figure 2.2: Mean MeHg concentration (top), THg concentration (middle) and % of THg as MeHg (bottom) in peat pore waters before, during and after additions over the three year (2014 - 2016) experiment broken down into the upper ridge (25m), middle ridges (40m – 81m) and lower ridges (105m – 220m). Year 1 and 2 sampling split into June (before additions), July and August sampling events. Recovery Year sampling split into June, July and October sampling events.

Total Mercury: Total mercury at the reference sites averaged 1.83 ng/L (± 0.51), 3.04 ng/L (± 0.88) and 2.28 ng/L (± 0.38) during the 2014, 2015 and 2016 field seasons respectively. After the

first wastewater additions in 2014, THg concentrations more than doubled from 2.56 ng/L (± 0.51) (pre-addition) to 6.73 ng/L (± 2.13) (end of the field season) in the upper ridge. THg in the middle ridges remained relatively unchanged increasing only slightly (2.71 ng/L (± 0.28) to 2.89 ng/L (± 0.26)) over the course of the 2014 addition whereas THg in the lower ridge decreased from 4.30 ng/L (± 1.14) to 2.34 ng/L (± 1.32) over the course of the experimental addition.

Total mercury pre-addition concentrations increase from 2014 to 2015 in the upper ridges and the middle ridges averaged 4.60 ng/L (± 1.24) and 4.77 ng/L (± 1.17) in 2014 and 2015 respectively, averaging 2 ng/L higher in 2015. Total Hg concentrations in the upper ridge increased over the course of the additions ending with a final concentration of 6.86 ng/L (± 4.05), similar to the THg concentration at the end of the 2014 field season. The middle ridges, however, increased much more in 2015 than 2014, with the end of season THg concentration in 2015 (6.34 ng/L (± 2.74)) more than doubling the 2014 end-of-season concentration. Lower ridges THg concentrations were consistent throughout the 2015 field season (mean: 3.57 ng/L (± 0.96)).

In 2016, THg concentrations were relatively consistent at all locations throughout the field season. The upper ridge and middle ridges started the 2016 field season at concentrations similar to those from pre-addition 2014 with the upper ridge at 2.99 ng/L (± 0.56) and the middle ridges at 2.90 ng/L (± 0.49). The lower ridges started with the highest THg concentration in 2016 at 3.03 ng/L (± 0.46). All three of the locations ended the 2016 field season with THg concentrations similar to that from the 2014 pre-addition sampling averaging 2.68 ng/L (±0.71), 3.00 ng/L (± 0.46) and 2.54 ng/L (± 0.48) at for 2014, 2015 and 2016 respectively.

Percent Methylmercury: Percent MeHg at the reference sites averaged 6.07% (± 3.36), 2.77% (± 1.19) and 3.70% (± 1.64) over the 2014, 2015 and 2016 field seasons respectively. Percent MeHg was highest in the upper ridge at 5.02% and increases by almost 15 times over the 2014 field season resulting in the highest %MeHg of all the sample locations (~74% average %MeHg in the upper ridge) followed by the middle ridges which averaged a little more than half the upper ridges final 2014 value (averaging >44% %MeHg). The lower ridges tripled over the 2014 field season. The 2015 field season follows a similar pattern to the 2014 field season, with the largest increases seen in the closest two locations and little change seen in the farthest locations.

The middle ridges in 2015 reached a higher %MeHg once the additions had started than 2014 at 3.97% (± 0.50) compared to 26.21% (± 16.60) in 2015. %MeHg in 2016 remained consistent throughout the season and does not show an increasing pattern as seen in the previous two field seasons.

Sulphate: Pore water SO_4^{2-} concentrations varied throughout the fen but generally the highest concentrations were at the top of the fen closest to the addition site. Average pore water SO_4^{2-} concentration was 9.51 mg/L (± 8.61) and 1.74 mg/L (± 3.01) during the 2014 and 2015 Experimental Fen wastewater additions, respectively. In 2016, the average SO_4^{2-} concentration at the Experimental Fen was 0.43 mg/L (± 0.65) compared to the reference sites average of 0.20 mg/L (± 0.18) in 2016. Sulphate concentrations in the upper ridge averaged 13.42 mg/L (± 8.66), 4.18 mg/L (± 3.96) and 0.62 mg/L (± 0.52) in 2014, 2015 and 2016, respectively with the 2016 value being much lower without the additions of SO_4^{2-}. The large variability as seen in the standard deviation is due to the presence of non-uniform preferential flow paths which deliver more SO_4^{2-} to some locations over others (McCarter & Price, 2016). Lower SO_4^{2-} concentrations were measured in the lower ridges of the fen in 2015 (0.65 mg/L (± 0.94)) compared to 2014 (0.86 mg/L (± 2.05)), and even lower in 2016 (0.17 mg/L (± 0.11)). Average SO_4^{2-} concentrations at the reference site were 0.28 mg/L (± 1.10), 0.41 mg/L (± 0.27), and 0.20 mg/L (± 0.18) in 2014, 2015 and 2016, respectively.

Dissolved Organic Carbon: Pore water dissolved organic carbon concentrations were similar among sites and relatively invariant over the years and averaged 32.5 mg/L (± 13.7) in 2014, 36.3 mg/L (± 12.4) in 2015 and 34.4 mg/L (± 8.1) in 2016 over the entire site. Average DOC concentrations at the reference sites for each year were 39.4 mg/L (± 7.6) in 2014, 33.0 mg/L (± 11.1) in 2015 and 33.4 mg/L (± 8.0) in 2016 respectively.

2.4.3 Solid-Phase Peat Chemistry

Methylmercury: Methylmercury concentrations in the 0m and 25m ridges were elevated above background though relatively low compared to the 40m and 62m ridge concentrations. Methylmercury concentrations in the 0 m and 25 m ridges were elevated above background but relatively low compared to the 40 m and 62 m ridges. The highest MeHg concentration was 12.8

ng/kg$_{dw}$ (10cm – 15cm below surface) and occurred 40m from the wastewater additions followed closely by 10.8 ng/kg$_{dw}$ (10cm – 15cm below surface) and 10.5 ng/kg$_{dw}$ (20cm – 25cm below surface) at the 62m location (*Figure 2.3*). After the 62m ridge the concentrations are more similar to that seen in the 0m and 25m ridges. The average MeHg concentration of all the depths combined was highest at the 62m location, followed by the 40m location. Methylmercury concentrations were lowest at the top and bottom of the fen, peaking in the middle. There did not seem to be any consistent pattern with depth. The reference site average peat MeHg concentration in 2016 was 0.65 ± 0.06 µg/L.

Total Mercury: Total mercury concentrations did not show any consistent pattern throughout the length of the fen with the highest average concentration at 81m and the lowest at 140m (Figure 2.3). Reference sites average peat THg concentration was 104.9 ± 25.7 ng/kg$_{dw}$.

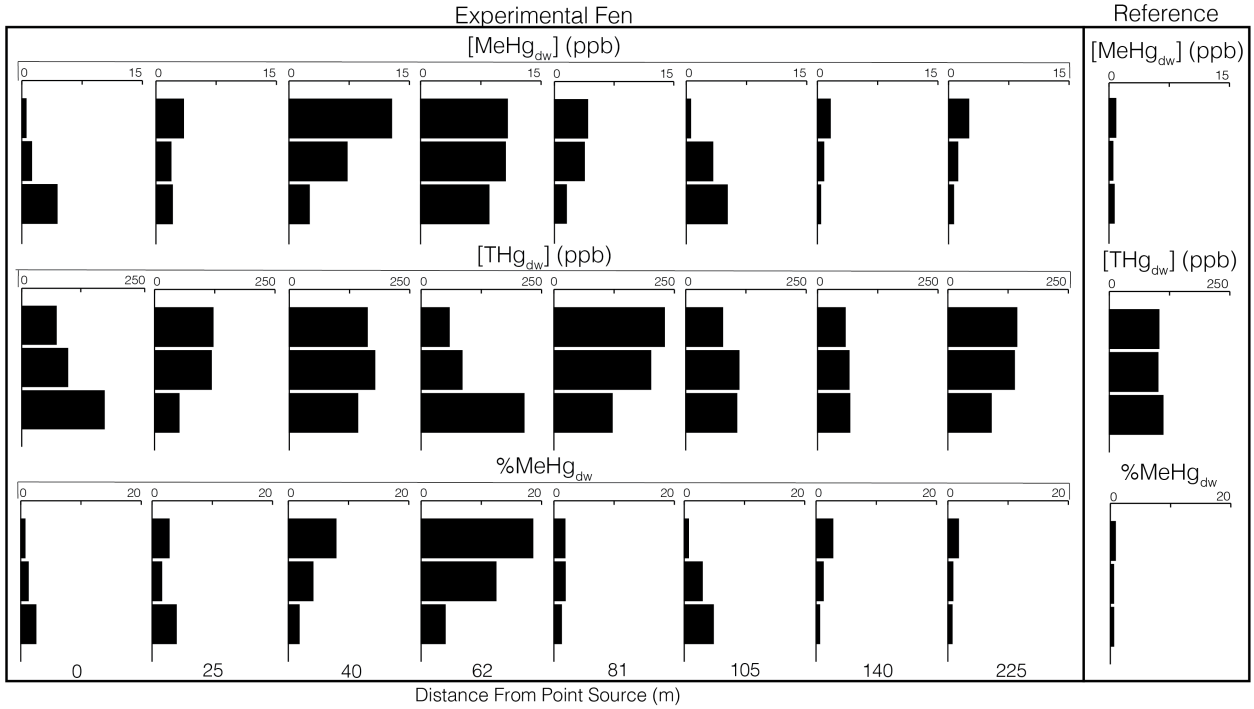

Figure 2.3: Solid mean MeHg concentration (top), THg concentration (middle) and percent THg as MeHg (bottom) for solid peat samples taken during the 2016 recovery field season ordered on the y-axis from shallow to deep (10 cm – 15 cm, 20 cm – 25 cm, 30 cm – 35 cm below surface). Reference values (at right) for mean MeHg$_{dw}$, THg$_{dw}$, and %MeHg$_{dw}$.

Percent Methylmercury: The %MeHg remained low ranging from 0.51% to 4.37% in the first two ridges (0 m and 25 m). The 62 m location had the highest %MeHg value at 18.5% in the

upper 10 cm – 15 cm. The 62 m sample location had the highest average increase in %MeHg. The average %MeHg value at the reference sites in 2016 was 0.62 % (± 0.17).

Total Sulphur: Percent total sulphur (%TS) from the solid peat samples increased in various locations throughout the Experimental Fen above reference site values. The 140m sample location was also the lowest for %TS as well as MeHg concentration, THg concentration, and %MeHg. Reference site %TS averaged 0.142% (± 0.050) in 2016 with the majority of the sample locations at the Experimental Fen having higher %TS than the reference site. In some of the sample depths in the locations closer to the SO_4^{2-} additions, such as the 25 m ridge (mean: 0.242% (± 0.066)) and 40 m ridge (mean: 0.269% (± 0.019)), the %TS was almost double the average from the reference site.

Distribution Coefficients: The 25 m and 40 m MeHg $LogK_d$ values were the lowest values from the Experimental Fen at 0.2 – 0.3 lower than the average for the Experimental Fen and 0.3 – 0.4 lower than the average for the reference sites (Table 2.4). The 62 m value was the highest as seen in Table 2.4 and was 0.4 higher than the Experimental Fen average. The variation in $LogK_d$ values for the Experimental Fen was approximately two times that of the reference sites. Total mercury $LogK_d$ was relatively consistent throughout the Experimental Fen and similar to the average value calculated from the reference sites.

Table 2.4: Distribution coefficients calculated for each 2016 solid sample location using corresponding pore water and solid phase MeHg concentrations and THg for both the Experimental Fen and average reference site value (± 1 standard deviation).

	Distance (m)	**Methylmercury** ($LogK_d$)	**Total Mercury** ($LogK_d$)
Upper Ridge	25	3.6	4.6
Middle Ridges	40	3.7	4.7
	62	4.5	4.6
	81	4.2	4.5
Lower Ridges	105	3.8	4.8
	140	3.9	4.7
	225	4.0	4.4
Exp Fen Average		3.9 (± 0.29)	4.6 (± 0.12)
Reference Sites		4.0 (± 0.14)	4.6 (± 0.16)

2.5 Discussion

2.5.1 Porewater Mercury

Response to Multi-Year Sulphate Loading: Methylmercury concentrations and %MeHg increased in the Experimental Fen in both 2014 and 2015 in response to the simulated wastewater additions to values well above those reported in the literature for natural peatlands (Heyes *et al*., 2000; Branfireun *et al*., 2002), as well as the unimpacted reference fens studied here. There is evidence that the addition of SO_4^{2-} in the simulated wastewater that was added to the Experimental Fen in 2014 and 2015 stimulated the activity of sulphate-reducing bacteria which, in turn, resulted in a net increase in MeHg production through biotic methylation though sulphate-reducing bacteria were not investigated in these experiments.

Moreover, MeHg concentrations and %MeHg at peat ridge locations relatively close to the point of SO_4^{2-} addition were above those reported in other short and long-term peatland SO_4^{2-} addition experiments (Branfireun *et al*., 1999; Mitchell *et al*., 2008a; Coleman-Wasik *et al*., 2012; Åkerblom *et al*., 2013). This suggests that despite the low Hg and SO_4^{2-} deposition in this pristine, high latitude peatland, methylation potential is very high. This is reinforced by the observations reported in McCarter *et al*. (2017) that showed a sharp increase in MeHg concentrations and %MeHg only days after the initiation of the first wastewater addition.

The highest MeHg concentrations were spatially constrained to the uppermost ridges of the Experimental Fen. This is likely due to the rapid reduction of other terminal electron acceptors (e.g. nitrate) in the wastewater and the activation of SO_4^{2-} reduction due to the presence of excess SO_4^{2-} but, at least initially, without the inhibitory effects of the presence of free sulphide. Despite an expected increase in sulphide both within and across years due to the addition of large amounts of SO_4^{2-}, there was no clear indication of complete sulphide inhibition of Hg methylation. The significant addition of water during the experimental additions (~35 000L/day) would have decreased water residence time and increased pore water turnover, potentially flushing excess sulphide from the fen, though no direct sulphide measurements were part of this study. 2015 was also a much wetter than average year, contributing to this pore water flushing.

Moreover, the low loading of SO_4^{2-} at this location historically may afford more opportunity for solid-phase interactions and incorporation into the organic sulphur pool.

A shift in the balance of the activity of Hg methylators and demethylators (Marvin-Dipasquale & Oremland, 1998) as SO_4^{2-} was consumed along the hydrological flow path is a possible explanation for lower net MeHg concentrations down-gradient, however no direct measurements of these processes were made as part of this study.

Higher pre-addition MeHg concentrations in 2015 than in 2014 was an indication of the carry over of MeHg that was produced in the previous year, the continued availability of the previous year's excess SO_4^{2-} for methylation, the re-oxidation of the previous years reduced sulphur to SO_4^{2-}, making it available for methylation (Coleman-Wasik *et al*., 2016), or some combination of all three. Mitchell *et al*. (2008c) measured elevated MeHg concentrations and %MeHg in peat porewaters early in the spring during snowmelt. They conclude that this MeHg was produced in the previous fall, and preserved in pore waters. We suggest a similar mechanism here.

Total mercury concentrations also increased during the wastewater additions over time in both 2014 and 2015. This can be accounted for by the production of MeHg contributing to the overall increase in THg concentration. Changes in partitioning from the solid phase was not evident in changes in THg $LogK_d$ values from samples taken in 2016 (post-addition). There was ~5% of the increase in THg that was not accounted for by changes in the MeHg concentration which could simply be analytical variability and acceptable method-based cumulative error, and cannot be overinterpreted.

Pore water SO_4^{2-} concentrations increased across the Experimental Fen during the additions in 2014 and 2015. The largest increase was in the ridges closest to the wastewater additions (25 m and 40 m). Dissolved organic carbon fluctuated between years but did not show any significant change between years. Sulphate additions do not seem to have affected DOC concentration after or during the two years of additions.

Response in Post-Addition Recovery Year: Methylmercury concentrations in the post-addition year were also elevated similar to second year pre-addition values, reinforcing the idea of carry-over of MeHg produced in the previous season. Importantly, MeHg concentrations

remained similar at all of the sampling locations throughout the entire field season, with only the lower ridges returning to approximately pre-addition background concentrations. Although samples were only taken at three times, there was no evidence of temperature-driven seasonality, and no evidence of enhanced methylation at the upper or middle sampling locations in the absence of fresh SO_4^{2-} loading, suggesting that the recovery year MeHg concentrations are a legacy of the previous two years of SO_4^{2-} addition, rather than a continued increase in net MeHg production. Coleman-Wasik *et al*. (2012) showed a similar pattern of recovery after the cessation of multi-year peatland SO_4^{2-} additions with an initial decrease and then a maintenance of lower but still elevated concentrations two years later.

2.5.2 Solid Phase Sulphur

After the 2 years of wastewater additions, solid phase sulphur (measured as %TS) increased in the Experimental Fen. This increase was seen most clearly at the 25m and 40m sample locations that were closest to the additions. Changes to the amount of solid sulphur present in the Experimental Fen can impact dissolved MeHg concentrations in the future. As Coleman-Wasik *et al*. (2015) found, under future drought conditions, SO_4^{2-} regeneration from reduced sulphur pools can stimulate MeHg methylation. So although the wastewater additions have stopped, fluctuations in water table could cause SO_4^{2-} release, stimulating SO_4^{2-}-reduction and Hg methylation.

2.5.3 Aqueous-Solid Phase Distribution

We observed increases in both the solid and dissolved phase MeHg concentration indicating that indeed, pore water increases in MeHg were the result of a net production of MeHg, rather than a shift in partitioning (Skyllberg, 2008). The pattern of increased MeHg in peat with the highest concentrations in the middle ridges after 3 years suggests that enhanced methylation of dissolved inorganic Hg closer to the wastewater discharge point increased MeHg in porewaters, which were then transported down the hydrological gradient and subsequently sorbed to the solid peat. The solid peat appears to serve as an effective sink for the excess MeHg produced as a result of the wastewater additions. This is supported by the observation that there was no change in the MeHg concentration at the surface water discharge point of the fen, indicating that despite

significantly enhanced methylation in the upper locations in the fen, this MeHg did not travel more than a few 10s of meters before it was sorbed to the solid phase.

Distribution coefficients ($LogK_d$) calculated for these sub-arctic fens are within the range of those reported for THg in the Great Lakes and northern Ontario (Rolfhus *et al*., 2003; Branfireun *et al*., 2005) and MeHg in peatlands (Heyes *et al*., 2000). Lower values of $LogK_d$ for MeHg from the upper ridge suggests a disequilibrium between the dissolved and solid phase, which is consistent with active MeHg production and higher pore water concentrations at this location. . Conversely, The 62m ridge had the highest $LogK_d$ for MeHg supporting the conclusion that these mid-fen locations were a sink for MeHg. The lower variability in THg $LogK_d$ indicates that the increase in pore water THg was smaller than for MeHg relative to the large pool in solid phase. Although site-wide averages were the same between the experimental fen and the reference fens, the variability in $LogK_d$ for both THg and MeHg in the experimental fen clearly reflect spatially heterogeneous disequilibria. Within-site processes, flowpaths, and biogeochemical 'hot spots' must be carefully considered in any study such as this.

2.5.4 Conclusions

In this three year, field-based experiment we were able to study how a relatively nutrient poor fen responded to increased hydrologic and nutrient loading and monitor the changes in MeHg concentration and monitored a subsequent recovery year to determine if there were any legacy effects. Relatively nutrient poor, northern fens have the ability to produce large amounts of MeHg when stimulated with SO_4^{2-}. Northern fens have the ability to buffer the transport of the MeHg they produce through phase partitioning while active with potentially long term changes to $LogK_d$ for MeHg. Years following any kind of wastewater or SO_4^{2-} addition have the potential to continue to produce elevated MeHg concentrations. Solid phase MeHg and sulphur content are also affected by these nutrient additions, both remaining elevated a year after waste water additions ceased. Providing enough interaction time between the peat and pore water before discharge into a large body of water should minimize MeHg export.

2.6 References

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Chapter 3

3 Evaluating the Impact of Sulphate Additions on Net Methylmercury Production in Pristine Sub-Arctic Peats

3.1 Introduction

Mercury (Hg) is an atmospherically transported contaminant that has increased an estimated 2 to 3-fold in the atmosphere since the industrial revolution (~1850) (Lindberg *et al*., 2007). Mercury is released into the atmosphere primarily as gaseous elemental Hg (GEM) and can travel hemispherically with an estimated residence time of ~6 months to 2 years (Lindqvist & Rodhe, 1985; Schroeder & Munthe, 1998). Once Hg is deposited into the environment, it can enter food chains leading to health complications in higher trophic level species, including humans (Mergler *et al*., 2007).

Once Hg is deposited as Hg(II) it can be methylated by sulphate (SO_4^{2-}) and/or iron reducing bacteria in the aquatic environment (Compeau & Bartha, 1985; Kerin *et al*., 2006; Gilmour *et al*., 2013). Methylmercury is produced as a byproduct of the metabolism of sulphate-reducing bacteria. It has been proposed that Hg enters the bacterial cell through passive transport of lipophilic Hg species like $HgCl_2$ and HgS through the lipid membrane (Mason *et al*., 1996; Benoit *et al*., 1999). Active Hg uptake has also been identified by SO_4^{2-} and iron reducing bacteria and depends on the thiol chemistry present in the uptake media (Schaefer *et al*., 2011). Once MeHg has been formed, it is then released from the cell into the environment where it can then be transported or partitioned to the solid phase.

Although there are ecosystem differences, the literature indicates that SO_4^{2-} regulates the activity of sulphate-reducing bacteria and in turn, MeHg production, in the vast majority of freshwater systems (Mitchell *et al*., 2008; Stickman *et al*., 2016). Sulphate-reducing bacteria are obligate anaerobes, and are naturally found in wetlands where the reducing conditions that they require are found. Mercury methylation requires the presence of specific nutrients and substrates (SO_4^{2-}), bioavailable Hg and a labile carbon source which are present in varying quantities in northern

peatlands. In peatlands, SO_4^{2-} availability has been found to limit MeHg production more than presence of labile carbon substrates and are thus an important regulator for MeHg production in wetlands (Mitchell *et al*., 2008). Significant spatial variation in SO_4^{2-} distribution and loads as well as redox processes within wetlands contribute to variant and transient Hg methylation capabilities across individual wetlands and wetland types (Mitchell & Gilmour, 2008; Tjerngren *et al*., 2012).

Sulphate reducing bacteria reduce SO_4^{2-} to sulphide, that can inhibit Hg methylation. Sulphide regulates Hg availability by binding to Hg molecules as $HgS_{(s)}$ and precipitating from solution, thus removing available Hg (Björnberg, 1988). When sulphide concentrations are high enough, the majority of Hg can be found as $HgS_{(s)}$ (Björnberg, 1988; Dyrssen & Wedborg, 1991) therefore limiting the supply of Hg for methylation by sulphate-reducing bacteria. Gilmour *et al*. (1998) found that across a nutrient gradient in the Florida Everglades, MeHg increased most in the more pristine regions. When specifically examining sulphur, a similar pattern was found by Johnson *et al*. (2016) in wetlands that were heavily impacted by high concentrations of SO_4^{2-}; the normally positive relationship between SO_4^{2-} loading and MeHg concentrations did not hold.

Although it has been demonstrated that simulated wastewater containing SO_4^{2-} (McCarter *et al*., 2017) and simulated atmospheric SO_4^{2-} deposition (e.g. Jeremiason *et al*., 2006) increases Hg methylation, it is not known exactly how varying levels of additions, nor SO_4^{2-} from other sources may impact methylation. Mine wasterock tailings can contain sulphide-bearing minerals (e.g. iron sulphide) which when dissolved in water can generate high concentrations of SO_4^{2-} and lead to acid mine drainage problems (see Akcil & Koldas, 2006). Fresh sulphur-bearing wasterock can deliver a pulse of SO_4^{2-} to a system during rainfall events leading to a cyclic pattern of SO_4^{2-} additions in the surrounding environments. Sulphate additions from mining wastewater, wasterock and/or pit dewatering can vary in sulphur concentration, anywhere from ~100 mg/L to over 1000 mg/L (Wiessner *et al*., 2005; Kaldec & Wallance, 2009; Steinback, 2012 Wu *et al*., 2013; Johnson *et al*., 2016). Mining SO_4^{2-} impacts on MeHg production in waters and sediments have been examined in various water bodies (e.g. streams, lakes) (Berndt & Bavin, 2012; Bailey & Johnson, 2015) but minimal information has been published on mining impacts on wetland-specific MeHg production. Where some treatment wetlands in the past have been exposed to high SO_4^{2-} concentrations from acid mine drainage sites (Sheoran & Sheoran,

2006), the concentrations used in these experiments are much lower which are less common in the literature.

Therefore the objectives of this study were to:

(1) Experimentally determine the relationship between SO_4^{2-} and MeHg production in pristine northern peats across a range of SO_4^{2-} concentrations, and;

(2) Evaluate the impact of SO_4^{2-} derived from sulphur-bearing mining waste rock on Hg methylation in pristine northern peats.

To address these objectives, two laboratory column experiments were undertaken. Experiment 1 added SO_4^{2-} to anaerobic peat columns at a range concentrations (1 mg/L, 5 mg/L and 30 mg/L) in a simple solution to assess relative net MeHg production in pristine subarctic peats that, under natural conditions, have very low MeHg concentrations, but high rates of net MeHg production under simulated waste water SO_4^{2-} additions (McCarter *et al*., 2017; Chapter 2, this thesis). These SO_4^{2-} concentrations were chosen based on the field results from Chapter 2 of this thesis. Experiment 2 was designed to more closely mimic field conditions that have been observed at the mine site of interest. Sulphate solutions were generated from a column packed with crushed mine waste rock that is known to leach SO_4^{2-} (T. Ternes, Pers. Comm.), which was then delivered to anaerobic peat columns to assess the potential net increase in MeHg due to the delivery of runoff and leachate from mine waste rock stockpiles in peatland-dominated landscapes.

3.2 Methods

3.2.1 Peat Sampling and Preparation

Approximately 60 kg of wet peat was shipped back from the reference site (52˚47’01 N, 83˚53’12 W) at the end of the 2016 field season (September 30, 2016). To collect the peat, the top 10cm of living vegetation and near surface peat was removed and the next 40 cm (10-50cm below the surface) was sampled, as this is the zone of water table fluctuation, more persistent anaerobic conditions, and known zone of Hg methylation (Branfireun *et al*., 1996). This peat was

kept fully saturated in a sealed bag in a cooler that was stored overnight in a fridge at ~4˚C and then shipped to the Biotron Facility at the University of Western Ontario where it was immediately put into a walk-in fridge (~4-6˚C)

Peat was manually homogenized in a nitrogen glove bag and stored in lab-grade plastic bags in at ~4˚C until the columns were packed. Columns were packed to the same approximate bulk density as calculated in the field. The field bulk density value used to pack the columns was 0.08 g/cm^3 which was chosen as it fell within reported bulk density values reported previous for the Experimental Fen (McCarter & Price, 2017) and other research sites located nearby (Whittington & Price, 2006).

Waste rock is rock that is removed from a mining area that does not contain the mineral(s) of interest. Waste rock piles at the De Beers Victor Diamond Mine are located a short distance from the open pit mine where it is piled and layered over time. For Experiment 2, the Environment Department at the De Beers Victor Diamond mine provided large rocks from the open pit mine (these rocks had not sat in a waste rock pile). This wasterock was dominantly limestone/dolostone and had been found to leach SO_4^{2-} over time (Pers. Comm. Brian Steinback). The large rocks that were sent were then ground and homogenized to be used to fill waste rock columns. Waste rock columns were filled with ground waste rock in a nitrogen glove bag to ensure columns were anaerobic.

3.2.2 Experimental Design

The three SO_4^{2-} concentrations (1 mg/L, 5 mg/L, 30 mg/L) were chosen based on field data from Chapter 2 at different locations throughout the fen to show a range of MeHg responses to SO_4^{2-} loading. The 1 mg/L concentration was chosen based on low MeHg production in locations furthest from the field SO_4^{2-} loading location where average SO_4^{2-} concentrations were below 1 mg/L. 5 mg/L SO_4^{2-} was chosen since the highest MeHg concentrations were in the upper ridge (~25m from the SO_4^{2-} additions) and were close to averaging a 5 mg/L SO_4^{2-} concentration. Finally, 30 mg/L SO_4^{2-} was chosen as the concentration that was added to the fen was 27.2 mg/L SO_4^{2-} so this was rounded to 30 mg/L SO_4^{2-} for the column experiments.

12 Kontes® Chromaflex™ glass chromatography columns (30 cm x 4.8 cm) with Teflon fittings were used in these experiments. For the first SO_4^{2-} addition experiment, 12 individual columns were fed three different SO_4^{2-} concentrations (1 mg/L, 5 mg/L, 30 mg/L) from 20 L pre-prepared carboys. These carboys were periodically sampled to ensure there was no shift in concentration from their original values. 0.16 cm i.d. Teflon tubing was connected to the pump Masterflex platinum silicone tubing (1.42 mm i.d.) using Teflon barbed lure locks on either side. Pumps were turned on to fill the tubing with solution before they were attached to the bottom of the columns as to not push air into the columns. Teflon tubing was then connected to the bottom of the chromatography column using Teflon fittings. Teflon tubing was also connected to the top of the column and directed into a 15 L waste bucket emptied during every sampling event. Each column had a cover made of dark fabric to block light into the column during the additions.

A twelve channel Carter Manostat® digital peristaltic pump was used to deliver the SO_4^{2-} solutions to the columns. The flow through the columns was set to a realistic rate based on hydraulic conductivity and hydraulic gradient measured in the first ridge of the field site (Colin McCarter, Pers. Comm.), producing a solution delivery volume of 15 mL/hour (0.83 cm/hour).

Column outlet samples were collected on varying 24 hour intervals (every 24 hours for first 3 days, then every 48-168 hours for duration) in sterile 250 mL PETG bottles taking approximately 4 hours to collect 60mL of water required for analyses. Sample bottles were bagged and kept dark in closed, small coolers on ice while the samples were being collected. After collection samples were filtered through ashed 0.45µm glass filters (Macherney-Nagel). A 2.2 mL aliquot of filtered sample was set aside for SO_4^{2-} and DOC analyses, and the remainder was acidified with EMD Millipore OmniTrace® HCl to 1% v/v within 2 hours of being collected and stored in the dark at ~4˚C until analyzed for THg and MeHg.

3.2.3 Water Chemical Analyses

Ions and Dissolved Organic Carbon: Water samples were analyzed for SO_4^{2-} on a Dionex ICS-1600 Ion Chromatograph following U.S. EPA Method 300.0 using 0.5 mL sample aliquots and diluted using 18.2 MOhm deionized water, when required (sample concentration > 50 mg/L). Analytical duplicates were run every 10 samples and field duplicates were collected every ~10 samples and both analytical and field duplicates were required to fall within ± 15% of each other.

Matrix spikes and check standards were run every 10 samples and were also expected to fall within ±15% of the expected value. Samples were rerun if they failed to meet the proper QA/QC. The instrument was calibrated to analyze samples between 0.5 mg/L and 50 mg/L and the reporting limit was 0.05 mg/L.

Dissolved organic carbon was measured on an OI Analytical Aurora 1030W Combustion TOC Analyzer using a wet oxidation method (minimum detection limit = 0.2 mg/L). A 2 mL sample aliquot was used and diluted to 8 mL total volume due to small sample volume. Analytical duplicates, matrix spikes and check standards were run every 10 samples and were expected to fall within ± 15% of expected value. Field duplicates were also expected to fall within ± 15% of each other. If samples failed to meet QA/QC, samples were rerun. The instrument was calibrated to analyze samples with concentrations between 0.5 mg/L and 50 mg/L, with a reporting limit of 0.5 mg/L.

Methylmercury: Methylmercury concentrations were measured by cold vapour atomic fluorescence spectroscopy (CVAFS) using U.S. EPA Method 1630 (U.S. EPA, 1998). 20mL of sample was diluted with 20 mL of 18.2 MOhm deionized reagent water. 1% ammonium pyrrolidine dithiocarbamate (APDC) was added and the sample distilled in Teflon® distillation vessels for 3 hours at 125˚C while being purged with Ultra High Purity 5.0 nitrogen gas. Distillate was collected in glass receiving vials. Ascorbic acid was added to 30mL of distillate, samples were shaken and left uncapped for 10 minutes to remove Cl. 2M acetate buffer was added to adjust the sample pH to ~4.5 and sodium tetraethyl borate ($NaBEt_4$) was added to ethylated MeHg and samples were capped. Samples were purged with argon gas and analyzed for MeHg on a Tekran© Model 2700 Automated Methyl Mercury Analysis System by gas chromatography and detection by cold vapour atomic fluorescence spectrometry (CVAFS). The Biotron Analytical Services Laboratory method detection limit (MDL) for MeHg analysis was 0.006 ng/L and the method reporting level (MRL) was 0.18 ng/L. Recovery of MeHg matrix spikes (mean ± standard deviation) was 90.5 % (± 7.3, n = 15) and sample duplicate recovery was 102.1% (± 6.9, n = 15).

Total Mercury: Total Hg concentrations were also determined by CVAFS in accordance with U.S. EPA Method 1631 (U.S. EPA, 1999). Bromine monochloride was added to 15 mL of

sample diluted with 10 mL of 18.2MOhm deionized water), shaken, left uncapped for 10 minutes then stored overnight for bromine monochloride oxidation. The following day, hydroxylamine hydrochloride was added to neutralize the bromine monochloride, samples were shaken and left uncapped for 30 minutes to allow for free halogen removal. Finally, 20% stannous chloride was added to convert Hg(II) to Hg(0). Gaseous Hg(0) was purged with high purity nitrogen gas from aqueous solution, captured on a gold trap, thermally desorbed and quantified using CVAFS on a Tekran© 2600 Mercury Detector. The Biotron Analytical Services Laboratory reports a MDL of 0.048 ng/L and an MRL of 0.144 ng/L for water samples run for THg. Recovery of THg matrix spikes (mean ± standard deviation) was 93.1% (± 7.0, n = 13) and sample duplicate recovery was 101.4% (± 6.5, n = 16).

For both Hg analytical methods, method blanks and quality control standards were acidified to 1% v/v instead of 0.5% v/v. 20% of the samples run were analytical duplicates, 5% matrix spikes and quality control standards were run periodically throughout the run.

3.2.4 Column Peat Sampling and Analysis

After the final water sampling event, columns were extruded intact using an acid washed, long glass stir stick with a 250mL PETG bottle cap attached to the end which was acid rinsed between samples to eliminate cross-contamination. The peat was stored in sealed plastic bags at -25˚C. Entire peat samples from the first experiment were homogenized, and peat samples from the second experiment were sub-sectioned into three 10cm sections (upper, middle, lower). All peat samples were then freeze-dried and homogenized for analyses of and THg, MeHg and total sulphur.

Total Sulphur: Total sulphur samples were sent to the Ontario Forest Research Institute (OFRI) in Sault Ste. Marie, Ontario. The method used for percent total sulphur analysis is combustion of the peat at 1350^{o}C on an Eltra Helios C/S Analyzer. OFRI reports a method detection limit of 0.004%.

Methylmercury: Methylmercury in peat was determined by digesting ~100mg of peat in 2mL of 25% KOH in methanol for 4 hours at 82˚C in 60 mL sealed Teflon digestion vessels (Savillex®). Samples were left to cool for 1 hour before being diluted with 8mL of DI water and

vortexed for 10 seconds to ensure the samples were homogenous. Samples were then transferred to 15mL falcon tubes and stored in the fridge overnight for analysis the following day (samples are not run on same day as the digestion process does not allow enough time for instrument start up and calibration curve building within the same day). The following day samples were brought to room temperature, centrifuged, diluted with distilled water and made up to a total volume of 30mL with 500µL 2M acetate buffer and 30µL $NaBEt_4$. Samples were shaken and left to react for 30 minutes with $NaBEt_4$ before being run on a Tekran© Model 2700 Automated Methyl Mercury Analysis System with 10% duplicates (96.98 ± 7.93%, n = 7) and 10% matrix spikes (85.28 ± 7.79%, n = 8).

Total Mercury: Solid peat samples were analyzed for THg on a Milestone DMA-80 direct Hg analyzer according to U.S. EPA Method 7473 (U.S. EPA, 2007). The calibration detection limit was 1 ng. Every 10 samples, analytical duplicates were analyzed (100.00 ± 8.93%, n = 6) and were required to fall within ± 15% of each other. A certified reference material (CRM), in this case MESS-3 (0.091 ± 0.009 mg/kg Hg) and IAEA 158 (0.132 ± 0.014 mg/kg Hg), was run at the beginning and end of each run. Blanks were analyzed at the start of each run (mean: 0.022 ± 0.019 ng, n = 10) as well as following every CRM.

Distribution coefficients were calculated [$\log K_d = \log([Hg]_{solid}/[Hg]_{porewater})$] using the solid phase Hg concentrations and dissolved Hg concentrations for each individual sample. Average distribution coefficients were averaged over the 3 replicates of each addition type.

3.3 Results

3.3.1 Experiment 1 – Continuous Sulphate Addition

3.3.2 Water Chemistry

All replicated experimental results are presented as means with ± Standard Deviation unless otherwise indicated.

Methylmercury: Over the first ~120 hours, MeHg in the the 1 mg/L SO_4^{2-} treatment almost doubled with average values increasing from 0.28 ± 0.04 ng/L to 0.55 ± 0.04 ng/L (*Figure 3.1*). For the remainder of the experiment (120 hours to 216 hours) the 1 mg/L addition only increased

0.07 ng/L with a final MeHg concentration of 0.62 ± 0.06. MeHg concentrations in the 5 mg/L SO_4^{2-} additions increased from 0.25 ± 0.01 ng/L to 0.60 ± 0.06 ng/L in the first 120 hours. After the 120 hour sampling point, the MeHg concentrations increased more rapidly than the previous 120 hours to 1.02 ± 0.10 ng/L at 168 hours then 1.82 ± 0.18 ng/L at 216 hours with the final MeHg concentration being more than seven times the first sampling event concentration. The 5 mg/L SO_4^{2-} addition had the largest increase in MeHg concentration out of all the SO_4^{2-} additions. MeHg concentrations in the 30 mg/L SO_4^{2-} treatment increased from 0.25 ± 0.03 ng/L to 0.47 ± 0.04 ng/L over the first 120 hours of SO_4^{2-} additions. After the 120 hour sample event, the MeHg concentrations of the 30 mg/L SO_4^{2-} addition increased more rapidly. The 216 hour sampling event was the highest MeHg concentration from the 30 mg/L SO_4^{2-} additions and ended with a MeHg concentration of 1.23 ± 0.11 ng/L. The 5 mg/L and 30 mg/L SO_4^{2-} additions displayed a similar pattern in MeHg concentration over time with larger increases seen in the 5 mg/L additions. The control columns MeHg concentrations remained relatively similar throughout the additions with an average concentration of 0.23 ± 0.04 ng/L over the 216 hours of additions. The control columns did not display a similar concentration to either the 5 mg/L/30 mg/L or 1 mg/L SO_4^{2-} additions as the concentration remained steady over the duration of the additions. The mass of SO_4^{2-} added over the course of the 1 mg/L additions produced a mass of MeHg of 1.72 ng. The other continuous additions of 5 mg/L and 30 mg/L produced 2.83 ng and 2.03 ng of MeHg, respectively.

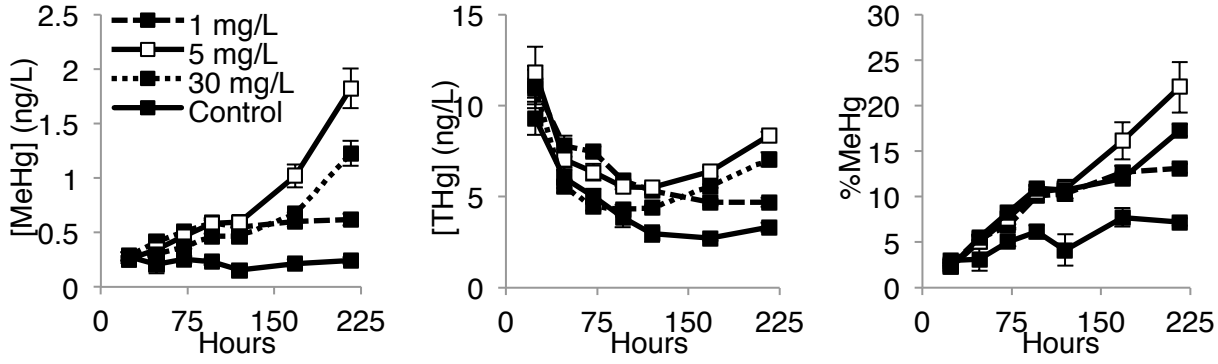

Figure 3.1: Mean MeHg concentration, THg concentration and %MeHg for pore waters from the first column experiment with control, 1 mg/L, 5 mg/L and 30 mg/L SO42- (all values are means of 3 replicate columns ± Standard Error).

Total Mercury: Concentrations of THg for all experimental treatments during the first 120 hours decreased as depicted in *Figure 3.1*. At 24 hours, THg concentrations were similar among

all treatments, averaging 10.79 ng/L (± 0.93) for all treatments. Over the first 120 hours, all treatment THg concentrations decrease by at least 50%. After the first 120 hours, THg concentration for the 5 mg/L and 30 mg/L treatments increase, with the 5 mg/L SO_4^{2-} treatment producing the largest THg concentration, followed by the 5 mg/L treatment then the 1 mg/L treatment. Total mercury concentration for 1 mg/L SO_4^{2-} addition remained low. The THg concentrations for the control columns decrease over the duration of the additions with the final concentration being 3.33 ng/L (± 0.25), approximately a third of the 24 hour THg concentration.

Percent Methylmercury: All experimental additions had similar %MeHg values for the first 120 hours but control peat columns was lower during this time, never reaching over 10% MeHg over the course of the experiment. There is a two step increasing pattern seen for the experimental treatments (controls not included) where the %MeHg values stabilize between the 96 and 120 hour sampling times then continue to increase after this point at varying paces. Patterns for %MeHg mirrored the previous results with 5 mg/L being the highest followed by 30 mg/L and finally 1 mg/L (*Figure 3.1*).

Sulphate: After the first 24 hours, none of the columns had 100% breakthrough of SO_4^{2-} where breakthrough is the percent of SO_4^{2-} passing through the columns. By 48 hours, the 30 mg/L addition had 100% breakthrough of SO_4^{2-}, while the 1 mg/L treatment averaged 61% (± 17) and the 5 mg/L treatment averaged 93% (± 3). Over the course of the experimental additions, the 30 mg/L SO_4^{2-} would remain at ~100% breakthrough while the other additions fluctuated and generally SO_4^{2-} breakthrough decreased over time. Sulphate breakthrough at the end of the SO_4^{2-} additions were 16% (± 1) and 52% (± 3) for the 1 mg/L and 5 mg/L treatments, respectively. During Experiment 1, 3.6 mg, 18 mg and 108 mg were added during the 1 mg/L, 5 mg/L and 30 mg/L treatments respectively over the 10 day experiment. The mass of SO_4^{2-} that passes through the columns was 1.29 mg (35.8% of SO_4^{2-} added), 11.84 mg (65.8% of SO_4^{2-} added) and 91.21 mg (84.5% of SO_4^{2-} added) for the 1 mg/L, 5 mg/L and 30 mg/L additions respectively.

Dissolved Organic Carbon: Dissolved organic carbon concentrations averaged 63.60 mg/L (± 4.3) at the 24 hour sampling point at all the experimental treatments. Concentrations of DOC were highest at the beginning of the SO_4^{2-} additions and dropped down to between 16.5 mg/L and 20.8 mg/L at 120 hours and remained relatively constant after that point. Dissolved organic

carbon concentrations did not differ greatly between treatments though columns with SO_4^{2-} additions had slightly higher DOC concentrations by the end of the additions with the control columns averaging 14.7 mg/L (± 0.7) DOC at 216 hours and 1 mg/L, 5 mg/L and 30 mg/L averaging 17.7 mg/L (± 1.3), 17.7 mg/L (± 1.8), and 16.2 mg/L (± 0.2), respectively.

3.3.3 Peat Chemistry

Methylmercury: All of the experimental SO_4^{2-} additions increased in solid phase MeHg above the control columns after 10 days of additions. Methylmercury concentrations in solid peat samples averaged 0.77 $\mu g/kg_{dw}$ and 0.78 $\mu g/kg_{dw}$ higher than the control columns which averaged 0.77 $\mu g/kg_{dw}$ after 10 days of additions for the 1 mg/L and 5 mg/L additions, respectively (Figure 3.2). The 30 mg/L SO_4^{2-} addition increased the least over the 10 days of additions ending at 0.55 $\mu g/kg_{dw}$ higher than the control columns.

Total Mercury: Average THg concentrations were 73.3 $\mu g/kg_{dw}$ (± 4.8), 76.8 $\mu g/kg_{dw}$ (± 13.4) and 66.7 $\mu g/kg_{dw}$ (± 5.4) for 1 mg/L, 5 mg/L and 30 mg/L SO_4^{2-} additions respectively, while peat control columns averaged 63.8 $\mu g/kg_{dw}$ (± 4.8) (Figure 3.2).

Percent Methylmercury: Percent MeHg in the peat almost doubled in all experimental treatments, with the largest average %MeHg in the 1 mg/L SO_4^{2-} treatment at 2.09% (± 0.24). The 5 mg/L and 30 mg/L SO_4^{2-} treatments increased the %MeHg to 2.04% (± 0.17) and 1.97% (± 0.18) after the 10 days of additions. The control columns %MeHg averaged just 1.20% (± 0.13) after the 10 days of additions.

Sulphur: Peat average %TS were 0.109% (± 0.004), 0.114% (± 0.003), and 0.111% (± 0.003) after the 10 days of 1 mg/L, 5 mg/L and 30 mg/L SO_4^{2-} additions, respectively. The control columns averaged 0.103% (± 0.003) after the 10 days of additions. All experimental treatments showed slight increases in %TS after 10 days of additions compared to the control columns.

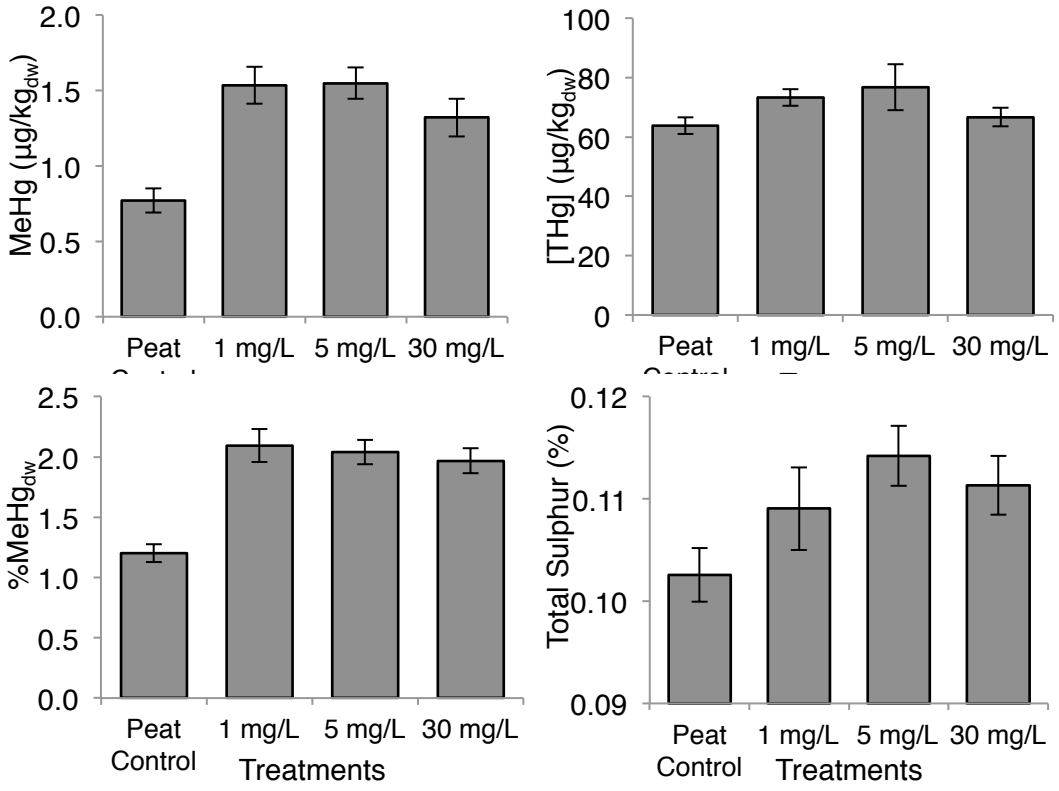

Figure 3.2: Mean MeHg concentration, THg concentration, %MeHg and %Total Sulphur (%TS) of solid peat samples from column Experiment 1 after 10 days of varying SO_4^{2-} additions presented as a bulk core average (all values ± Standard Error).

3.3.4 Experiment 2 – Waste Rock Sulphate Additions

3.3.5 Water Chemistry

Methylmercury: Aqueous MeHg concentration during waste rock additions was highest coming from the waste rock into peat treatment over the 20 days of additions (Figure 3.3). During the first 120 hours of additions, the wasterock additions columns have a small increase around 48 hours that then drops off until 120 hours. After the first ~120 hours of additions the MeHg concentration in the waste rock addition columns increased from 0.56 ng/L (± 0.06) at 120 hours to 1.09 ng/L (± 0.08) at 192 hours to approximately double the average concentration compared to the peat control columns (0.50 ng/L (± 0.01)). Both the peat control columns and waste rock control column's MeHg concentrations remained relatively consistent and distinctly lower than the experimental treatment throughout the 20 days of additions. The wasterock additions which

delivered a pulse of SO_4^{2-} produced 2.85 ng MeHg over the first 8 days, 3.63 ng MeHg over the first 11 days of additions and 7.50 ng total.

Total Mercury: Total mercury concentrations for the experimental and control treatments decreased over time. Total mercury concentration in the experimental treatment (waste rock + peat) decreases the least over the 20 days of additions dropping from 9.82 ng/L (± 0.45) at 24 hours to 3.56 ng/L (± 0.16) at 480 hours.

Percent Methylmercury: Percent MeHg increases the most in the waste rock additions column but is followed very closely by the peat only control column. After 20 days of additions, the waste rock additions columns reach a %MeHg value of 32.71% (± 2.67) while the peat control column, although elevated, only reached 26.65% (± 5.45) at the 20 day mark (Figure 3.3). The peat only control columns were not actually producing much MeHg, it remains relatively consistent, but the THg concentrations drop causing the perception of increase %MeHg values.

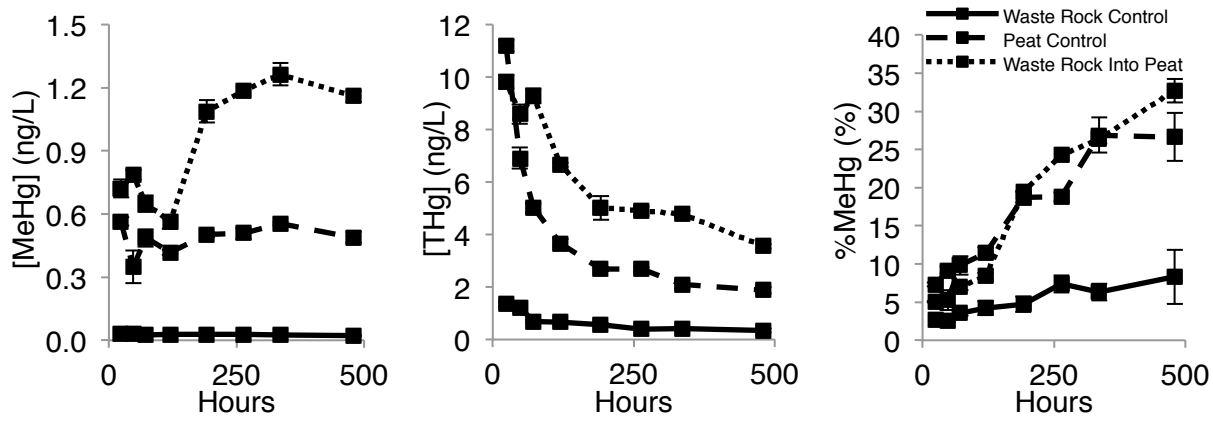

Figure 3.3: Mean MeHg concentration, THg concentration and %MeHg for pore waters from the Experiment 2 with waste rock SO_4^{2-} additions to peat columns plus MeHg and THg controls (all values ± Standard Error).

Sulphur: Sulphate concentrations rapidly decreased over the course of the SO_4^{2-} additions, unlike the continual additions from Experiment 1, the SO_4^{2-} additions from the waste rock decrease over time (Figure 3.4). After 24 hours, SO_4^{2-} concentrations remained below 5 mg/L for the duration of the 20 days of additions. Based on the samples taken, the mass of SO_4^{2-} leaving the columns, 31.06 mg was lost in the first 11 days and 31.49 mg after the entire 20 days.

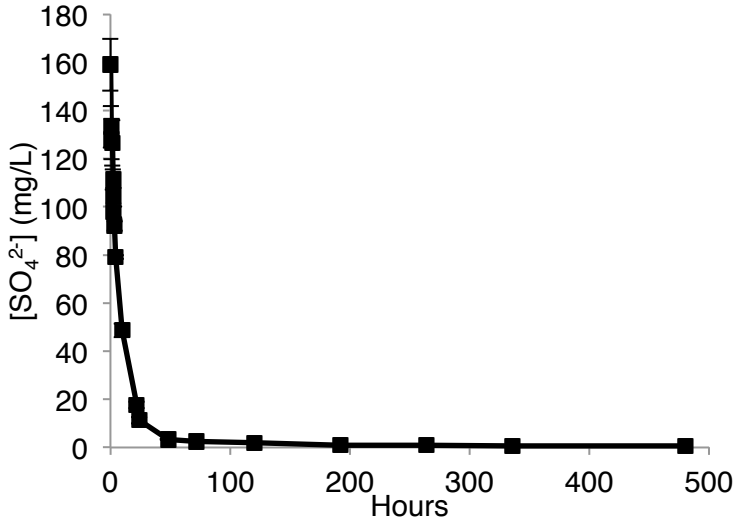

Figure 3.4: Experiment 2 Mean SO_4^{2-} Concentrations from Waste Rock Flowing into Peat Columns during 20 Days of Additions (all values ± Standard Error).

3.3.6 Peat Chemistry

Methylmercury: For Experiment 2, the solid peat columns were broken down into ~10cm blocks as the inlet (closest to the additions), middle and outlet (farthest from the additions) as seen in *Figure 3.5*. Methylmercury concentrations were higher in peat with the waste rock SO_4^{2-} additions in all instances with average concentrations of 1.36 $\mu g/kg_{dw}$ (± 0.07), 2.12 $\mu g/kg_{dw}$ (± 0.13) and 1.95 $\mu g/kg_{dw}$ (± 0.15) at the inlet, middle and outlet of the columns, respectively with the largest concentration in the middle 10 cm section. The average concentrations of MeHg in the inlet, middle and outlet of the peat only columns was 1.06 ng/kg_{dw} (± 0.02), 1.41 $\mu g/kg_{dw}$ (± 0.04) and 1.35 $\mu g/kg_{dw}$ (± 0.07), respectively, with the highest concentration again in the middle section.

Total Mercury: Total mercury concentrations were higher in the peat only control columns than in the waste rock addition columns at the inlet (65.02 $\mu g/kg_{dw}$ (± 2.64) vs. 58.90 $\mu g/kg_{dw}$ (± 8.67)) and in the middle (67.78 $\mu g/kg_{dw}$ (± 6.54) vs. 64.67 $\mu g/kg_{dw}$ (± 4.67)) of the column. The waste rock addition columns, however, averaged higher at the outlet of the columns at 73.38 $\mu g/kg_{dw}$ (± 8.41) compared to the peat only control columns outlet average of 59.65 $\mu g/kg_{dw}$ (± 2.62).

Percent Methylmercury: %MeHg increased in all experimental columns at the inlet, middle and outlet sections with average %MeHg values of 2.34% (± 0.22), 3.28% (± 0.27) and 2.68% (± 0.22), respectively. The highest average %MeHg value was in the middle section of the columns.

Total Sulphur: %TS in peat was highest at the outlet of the experimental columns (0.115% (± 0.004)) with the middle (0.108 (± 0.004)) and inlet (0.106% (± 0.001)) coming in at similar values. The middle section of the columns was the only location to have higher %TS in the peat only control columns (0.113% (± 0.001)) than the waste rock treatments.

3.4 Discussion

3.4.1 Methylmercury and Sulphate

All SO_4^{2-} additions to these pristine peats resulted in an increase in MeHg concentration in peat pore waters as well as solid phase accumulation in as little as 10 days of additions, similar to previous SO_4^{2-} addition experiments (Jeremiason *et al*., 2006; Mitchell *et al*., 2008). Methylmercury concentrations for the 5 mg/L and 30 mg/L SO_4^{2-} additions continued to increase to the end of Experiment 1 suggesting a lack of complete inhibition of methylation by either changes in inorganic Hg bioavailability through sulphide complexation (Björnberg, 1988; Dyrssen & Wedborg, 1991) or sulphide toxicity to sulphate-reducing bacteria (Reis *et al*., 1992). This, combined with the 100% breakthrough of added SO_4^{2-} in the 30 mg/L experiment suggests that the sulphate-reducing bacteria community were unable to utilize all of the added SO_4^{2-} due to limits on total sulphate-reducing bacteria biomass, metabolism, or both. In fact, the two peaks in MeHg concentrations observed over Experiment 2 which was twice as long as Experiment 1 suggests that with additional time, the sulphate-reducing bacteria community responded to the increase in available SO_4^{2-} by moving into growth phase (Zwietering *et al*., 1990). Given the almost complete absence of available SO_4^{2-} in these peats under field conditions (see Ulanowski & Branfireun, 2013), it is not surprising that there would be a lagged response in SO_4^{2-} reduction and Hg methylation as these microbial communities grow in response to these additions (Rolfe *et al*., 2012).

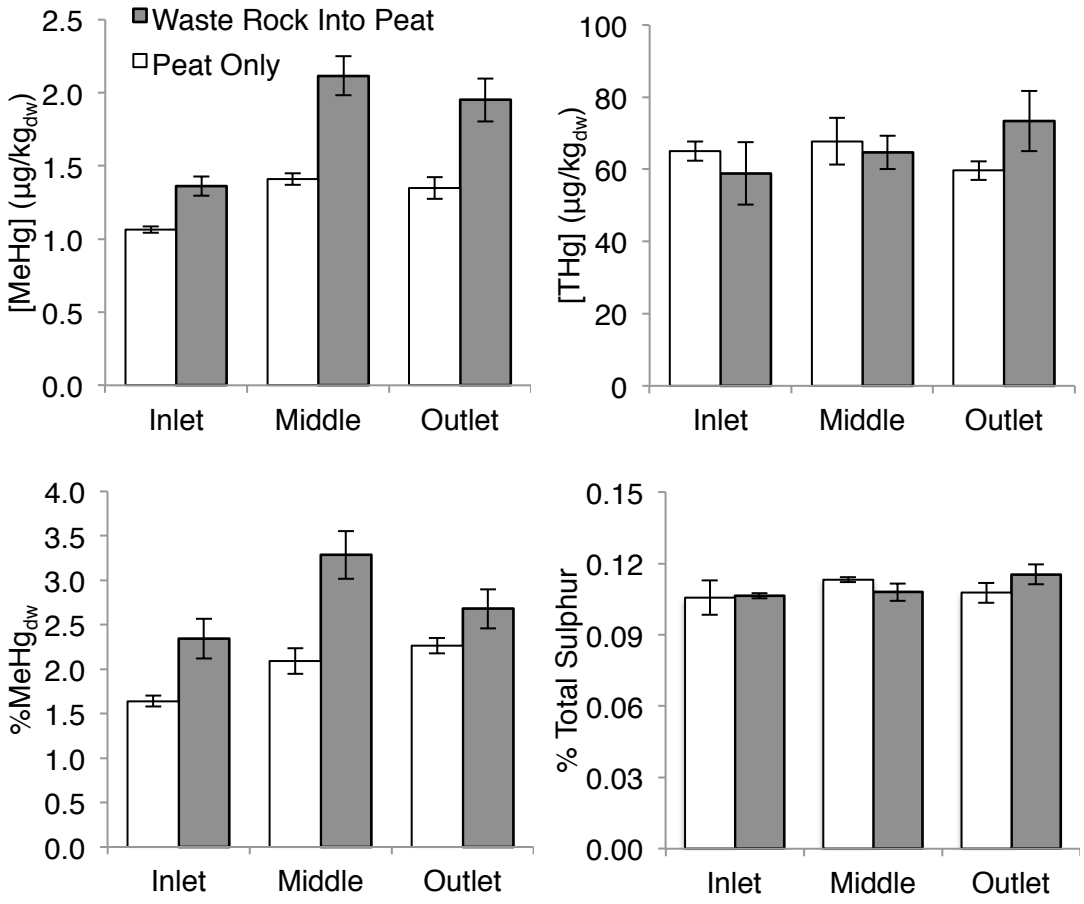

Figure 3.5: Mean MeHg concentration, THg concentration, %MeHg and %Total Sulphur (%TS) of solid peat samples from column Experiment 2 after 10 days of varying waste rock SO_4^{2-} additions and control peat columns (all values ± Standard Error).

Another explanation for this lag could be the lagged growth in the bacterial community due to the SO_4^{2-} addition waste water being oxygenated. Bacteria using oxygen may have thrived at the start of the additions with the oxygen being removed at the inlet of the column then the sulphate-reducing bacteria were able to start growth once the oxygen was removed and reducing conditions moved up the column. Total microbial biomass and metabolism were not measured as part of this study, and would lend support to this contention.

The total mass of MeHg produced from each of the experimental treatments varied. The largest mass of MeHg was produced from the 20 day wasterock addition followed by the 5 mg/L addition. When comparing the 9 days of 5 mg/L SO_4^{2-} additions vs. the 8 days/11 days of

wasterock additions, the MeHg is higher at 9 days of 5 mg/L additions than the 8 days of wasterock additions but the 11 days of wasterock additions generates a larger mass of MeHg than the 9 days of 5 mg/L. These two additions are similar in mass after the 8-11 days of SO_4^{2-} additions though the SO_4^{2-} delivery methods are quite different. Although up to this point, these two additions have had different SO_4^{2-} deliveries (continuous vs. pulse) the output of MeHg mass was quite similar.

Total mercury (and DOC) both started at high concentrations and decreased as the additions continued which was due to the disturbance caused by homogenizing the peat and packing the column. Total Hg concentrations in pore waters also increased near the end of the SO_4^{2-} additions as MeHg concentrations increased while DOC concentrations remained relatively consistent after the 120 hour decrease. Similar initial flushing patterns in solutes such as DOC have been observed in other experiments (see Dieleman *et al*., 2016).

The SO_4^{2-} breakthrough that was seen at the beginning of the additions for both of the experiments was important as it meant the area affected by the SO_4^{2-} additions now becomes much larger. When the SO_4^{2-} that breaks through at the beginning of the additions makes contact with anaerobic peat layers, there would then be potential for Hg methylation to occur in areas much larger than the site of the additions. With increased SO_4^{2-} concentrations/masses being added, more SO_4^{2-} was passing through the columns meaning that with increasing concentration of SO_4^{2-} there is an increase in area affected as more SO_4^{2-} passes through the column to move through an area. The lowest concentration of SO_4^{2-} (1 mg/L) was able to remove 64.19% of the SO_4^{2-} being added in a 30 cm column which is the most efficient SO_4^{2-} removal out of all of the experiments. Increasing the SO_4^{2-} concentration up to 5 mg/L meant that only 34.2% of the SO_4^{2-} added was getting removed by the column. The large jump from 1 mg/L to 5 mg/L and even larger difference between 1 mg/L and 30 mg/L (15.6% removed) indicates that the peat is not very efficient at removing the higher masses of SO_4^{2-}.

3.4.2 Changes in Solid-Phase Methylmercury and Sulphur Accumulation

Increases in MeHg concentrations in peat were found in all experimental treatment columns, which indicates rapid partitioning of newly formed MeHg in pore waters to the solid phase. Increases in both pore water and in solid phase MeHg are indicative of net MeHg production rather than just changes in partitioning due to shifts in pH or porewater chemistry (Skyllberg, 2008).

From Experiment 2, more detailed information on the distribution of MeHg and sulphur was revealed by dividing the column up into 3 equal 10 cm sections at the end of the experiment. The lowest MeHg concentration in the columns was found in the 10 cm at the inlet where oxygenated water was being added to the columns. This addition of low ionic strength water to the column likely affected the binding kinetics of the MeHg already present in the column as the peat only controls also had a decrease in MeHg at the inlet (*Figure 3.5*). This decrease affected the MeHg in this section of the column as the MeHg was likely surface bound (more loosely bound) and would have more interaction with the SO_4^{2-} additions. Again, as the SO_4^{2-} additions water was oxygenated there was likely a redox gradient somewhere within that first 10cm near the inlet as in the other two sections of the column the MeHg accumulation is greater. A similar advection and partitioning pattern was seen in the field scale experiment (Chapter 2) though at a much larger scale where the MeHg was produced in the upper ridges and then partitioned to the solid peat in the lower ridges. This is similar to what was seen in Experiment 2 as the higher peat MeHg concentrations are in the lower two sections of the column.

%TS in Experiment 1 was higher in the experimental treatments than in the peat controls but in Experiment 2, %TS was similar in both the wasterock addition as well as peat controls with no clear pattern shown. The total mass of SO_4^{2-} added to the waste rock columns was not properly sampled so the peaks in both the initial SO_4^{2-} moving coming off of the waste rock columns was missed due to not sampling early enough and the peak breakthrough in the peat column was missed as the breakthrough peak should have been observed at 36.1 hours and samples were taken at 24 hours and 48 hours. Based on the concentrations of SO_4^{2-} coming out of the peat columns we can derived a minimum value of SO_4^{2-} coming off the waste rock but have no exact

value. This then makes the mass entering the columns difficult to compare as the wasterock mass seems most similar to the 5 mg/L addition but is actually underestimated.

3.5 Conclusions

Pristine peats from relatively SO_4^{2-} limited locations such as this high latitude location have substantial methylation potential when supplied with SO_4^{2-}, rapidly increasing pore water MeHg concentrations. Higher concentrations of SO_4^{2-} do not correspond to proportionally higher MeHg concentrations. In these pristine peats, the largest MeHg concentrations corresponded to the 5 mg/L SO_4^{2-} additions and similar to previous work (Gilmour *et al*., 1998), the highest SO_4^{2-} concentrations did not correspond to the highest MeHg concentration. Given increases in MeHg concentrations and SO_4^{2-} breakthrough observed at higher concentrations in particular, the range of concentrations of SO_4^{2-} presented in this data set will have the ability to promote Hg methylation as well as deliver SO_4^{2-} downgradient possibly enhancing methylation well beyond the point of discharge. We also saw increased %TS in solid peat leading to the potential for SO_4^{2-} regeneration and more long-term enhanced MeHg production even after SO_4^{2-} releases are stopped.

Investigations into possible bacterial community shifts and/or biomass changes would help explain the mechanism behind the increase in MeHg more than just SO_4^{2-} reducing bacteria activity. Sulphate source and delivery also seemed to affect methylation as slight different responses in MeHg concentrations. This was shown by the different delivery methods (continuous vs. pulse) though more data from Experiment 1 would be required to make any hard conclusions about absolute MeHg mass produced as the experiment was cut short. By comparing the first 10-11 days of both experiments, it is clear that SO_4^{2-} from wasterock has the ability to produce MeHg, similar to simple SO_4^{2-} solutions. Future work should include longer SO_4^{2-} additions of varying concentrations to try to further elucidate the effects different SO_4^{2-} additions have on both peat and peat pore waters.

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Chapter 4

4 Conclusions

4.1 General Conclusions

The peatland-dominated Hudson-James Bay Lowlands (HJBL) in northern Canada covers over 300 000 km^2 of northern Manitoba, Ontario and Quebec. With both climate change and land use change pressures increasing in the north and even in these remote wetland dominated regions of Canada, it is important to consider how these changes may affect MeHg production including the loading of nutrients like SO_4^{2-} to previously 'pristine' peatlands and peat soils. The lack of research in this particular area is not necessarily due to lack of interest or concern but largely due to the logistical and operational constraints associated with remote research. The purpose of the research presented in this thesis was to improve our understanding of how lower SO_4^{2-} concentrations and SO_4^{2-} from waste rock runoff affects MeHg production in these pristine northern peatlands. To accomplish this, both field and lab based experiments were used to attempt to tease out how different concentrations and sources of SO_4^{2-} affect MeHg production and recovery.

The findings of this thesis indicate that MeHg production is stimulated over a large range of SO_4^{2-} concentrations as well as two different delivery methods (pulse and continuous) and sources (simple solutions, and waste rock leachate). Current research in SO_4^{2-}-impacted mining sites have concentrations of SO_4^{2-} above 100 mg/L being added with little to no current impact on MeHg production. The research presented here indicates that SO_4^{2-} concentrations may indeed exceed that which can be reduced immediately by bacteria causing an increase in MeHg concentration, but that excess SO_4^{2-} may continue to move down the hydrologic gradient and then stimulate methylation at locations that are further from the SO_4^{2-} source.

4.2 Implications

During SO_4^{2-} additions, MeHg production can be high in certain areas and multi-year SO_4^{2-} additions can leading to a movement of the area of greatest methylation away from the source of

SO_4^{2-}. After SO_4^{2-} additions cease, MeHg concentrations are still elevated, possibly through SO_4^{2-} regeneration in wetting and drying peat. Under future climate change conditions, it is predicted that temperatures in the north will increase and along with this temperature increase, there is a predicted increase in decomposition (see Davidson & Janssens, 2006). Increasing decomposition can mean that not only the sulphur that has been incorporated into the solid peat can be released but that the MeHg that has been partitioned to the solid phase can also be released. Moreover, increased temperatures will also lead to a first-order increase in microbial metabolism, and thus, methylation. This multi-pronged effect may increase MeHg production by re-introducing legacy sulphur, increasing pore water DOC, bioavailable Hg, and MeHg concentrations that can be transported downstream to be bioaccumulated in the aquatic food web.

4.3 Limitations

The lab-based experiments had quite a few limitations as this was the first attempt at column experiments using this specific approach. For both the field and lab based experiments, examining the bacterial community composition before and after SO_4^{2-} could have provided interesting insight into bacterial community composition and metabolism. From the column experiments measuring for microbial community composition and biomass changes from before and after SO_4^{2-} additions would help determine if the increased MeHg response was from increased efficiency of already present bacteria or community growth of certain sulphate-reducing bacteria. Sulphide sampling at the outflow of the columns over time would also provide interesting insight into how the microbial communities were responding (with an increase in sulphide corresponding to increased sulphur reducer activity). Sampling for dissolved organic carbon (DOC) was done in all of the above experiments but aside from flushing, no significant DOC changes were identified as carbon quality was not analyzed. Changes in carbon quality may have been occurring during these additions even if significant changes in concentration weren't identified, and measures of carbon quality such as lability, would be of value.

Increasing sampling frequency of the column experiments for ions would allow for the capture of SO_4^{2-} break though as that was missed in Experiment 2 (Chapter 3). Increasing the sampling frequency as well as the duration of the sampling for the column experiments would also be suggested as to better capture the trend in MeHg production. In the future a minimum of 3 weeks

(21 days) would be recommended to hopefully see the columns reach steady state (e.g. wasterock additions, Chapter 3, Experiment 2).

4.4 Future Work

Future work should focus on the potential for SO_4^{2-} priming effects from SO_4^{2-} additions and their effect on MeHg production. Peat from previously SO_4^{2-} impacted sites with the sample additions from Experiment 1 (Chapter 2). If the increase in MeHg that was seen in Chapter 2 were from increased bacterial community growth from the SO_4^{2-} additions then with the community already present, there may be a more efficient Hg methylating community present. By using peats from other, more southern peatlands, with greater atmospheric SO_4^{2-} deposition, there may also be a natural difference in MeHg response simply due to location and access to SO_4^{2-} that may be identified. By using the same SO_4^{2-} additions on more southerly peats, it may be found that this MeHg response would be even more pronounced in these peats. To examine the impacts of increased temperature from climate change, peat columns could be warmed prior to and/or during SO_4^{2-} additions to simulate climate change temperature increases. Increasing temperature may not only increase the release of MeHg and sulphur from decomposition but also increase the methylation rates.

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