Assessing Mercury and Methylmercury levels in the Wabigoon River with special attention on mercury methylation

A Thesis Submitted to the Committee on Graduate Studies in

Partial Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

in the Faculty of Arts and Science

TRENT UNIVERSITY

Peterborough, Ontario, Canada

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Environmental & Life Sciences Ph.D. Graduate Program

May 2024

Abstract

Assessing Mercury and Methylmercury levels in the Wabigoon River

with special attention on mercury methylation

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The Wabigoon River is known for an historic mercury (Hg) pollution source, caused by a chlor-alkali facility operating in the 1960s. As legacy Hg contamination continues to cause serious adverse health effects to the local communities living in the Wabigoon River region, it is imperative to undertake additional research to understand the deposition and transport of historical mercury in this system and more importantly, its conversion into methylmercury (MMHg) which renders it bioavailable for ongoing bioaccumulation. The aim of this dissertation was to evaluate the transport and accumulation of Hg species by doing a spatial and temporal analysis of concentrations of mercury and methylmercury along the Wabigoon River, as well as assessing rates of methylation and demethylation, identifying areas of higher methylmercury production. Results show that locations downstream from the pollution source still show elevated mercury concentrations, with levels at least five times higher in water and up to 134 times higher in sediments compared to background levels. Among selected study sites, the Hydroelectric dam, the Wabigoon Rapids wetland and Clay Lake were identified to have high capacity for methylmercury production in the system, with notably Clay Lake presenting a higher potential for methylmercury accumulation due to the observed lower methylmercury demethylation rate. Furthermore, the impact of wetting and drying cycles on Hg methylation in riverbed and wetland

locations within the Wabigoon River system was investigated through a laboratory simulation. Findings indicated increased susceptibility of riverbed locations to wetting and drying cycles.

Keywords: *Wabigoon River; mercury; methylmercury; water; sediments; methylation; demethylation; wetting cycles*

Preface

This dissertation is written in the manuscript format and consists of three research chapters to be submitted for publication in suitable peer reviewed journals. Due to mercury and methylmercury in the Wabigoon River being the focus in all four chapters, there will be some content replication and overlap among individual method sections. The research presented in this thesis was carried out under the supervision of Dr. Holger Hintelmann and involved laboratory work as well as two field sampling campaigns in the Wabigoon River. I was responsible for planning, organizing, and conducting the field sampling, as well as executing all mercury and complementary analyses, except for sample collection during the 2018 field season, which was conducted by Stephen McGovarin, and the DOC analysis performed by Tyler Roy. I, Beatriz Bento, am first author on all chapters. Additionally, I am acknowledging and attributing co-authorship credit to individuals who have made significant contributions to the manuscripts that will stem from this thesis (see list of publications below).

Chapter 2: Bento, Beatriz; McGovarin, Stephen; Hintelmann, Holger: Temporal and spatial analysis of mercury and methylmercury along the Wabigoon River.

Chapter 3: Bento, Beatriz and Hintelmann, Holger: Assessment of Mercury Methylation and MMHg Demethylation Potentials in water and sediments along the Wabigoon River system.

Chapter 4: Bento, Beatriz and Hintelmann, Holger: Wet and Dry Cycle simulation and influence on Hg Methylation.

Acknowledgements

I would like to express my deepest appreciation to my supervisor, Dr. Holger Hintelmann for his patience, constructive feedback and the time he dedicated to sharing his invaluable expertise with me. As well as to my supervisory committee, Dr. Julian Aherne and Dr. Alexandre Poulain for their advice, and to my examining committee, Dr. Mary-Claire Buell and Dr. Brian Branfireun for an intellectually stimulating defence and suggestions that helped improve the final version of this work.

I am very grateful for the scholarships that supported me while completing this work: a PhD Research Studentship funded by the Portuguese Foundation for Science and Technology (FCT), Dean's PhD Scholarship award, International Graduate Scholarship and Research Fellowship Award. As well as a NSERC Discovery grant awarded to Dr. Holger Hintelmann, which provided support for the research conducted in this thesis.

The field seasons would not have been possible without insightful discussions with Stephen McGovarin about sampling logistics that helped select sampling locations and plan the field sampling. Additionally, I would like to thank all the boat drivers that assisted with the sampling of lakes and wetland locations; the IISD Experimental Lakes Area for accommodation and use of a field lab during the 2022 field season; the Trent School of the Environment for lending sampling equipment and finally, Andrew Williams and Adelheid Willsie for assistance in field sampling.

The laboratory analysis could not have been achieved without numerous departments and members at Trent University. I would like to particularly acknowledge the Water Quality Center for the use of the ICP-MS; Dr. Karla Newman for analytical training on the ICP-MS; Dr.

Doug Evans and Dr. Huy Dang for the use of the DMA; Wei Wang for analytical training on the DMA and Tyler Roy for the DOC analysis.

I am also thankful to the all the members that passed through the Hintelmann Lab during the 4 years of my PhD giving me a community to share ideas with. Specially to Shengliu Yuan for sharing his knowledge on mercury analysis, to Beatriz Martins for assistance in the laboratory and Una Jermilova for her extensive help with Rstudio.

I would also like to recognize all my friends in Peterborough and abroad that kept me sane during these 4 years, providing much needed socialization and emotional support.

Last but not least, I would like to thank my parents for supporting my decision to move across the world to pursue a scientific career and give strength through all the hardships that doing a PhD abroad entails.

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List of Abbreviations and Symbols

BOT – Bottom

CL – Clay Lake

CL I – Clay Lake Inflow

CL W – Clay Lake West

DL – Detection Limit

DMA – Direct Mercury Analyzer

DMHg - Dimethylmercury

DO – Dissolved Oxygen

DOC – Dissolved Organic Carbon

DOM – Dissolved Organic Matter

ELA - Experimental Lakes Area

EPA - U.S. Environmental Protection Agency

GIS - Geographic Information System

HCl - Hydrochloric Acid

HD – Hydroelectric Dam

Hg(II) – Divalent Inorganic Mercury

Hg^0 – Elemental Mercury

ICP-MS – Inductively coupled mass spectrometry

IRB - Iron-reducing Bacteria

Kd – Specific MMHg demethylation rate

Km – Specific Hg methylation rate

LOI – Lost on Ignition

MB – Minnitaki Bridge

MMHg – Monomethylmercury

OM – Organic Matter

PS – Pollution Source

PVC - Polyvinyl chloride

Rep – Replicate

Sal – Salinity

SD – Standard deviation

SRB - Sulfate-reducing Bacteria

THg – Total Mercury

WF – Wabigoon Falls

WL – Wabigoon Lake

WLWT – Wabigoon Lake Wetland

WR – Wabigoon Rapids

WRWT – Wabigoon Rapids Wetland

Chapter 1: Introduction

1.1. Mercury in the environment

Mercury (Hg) exists in the environment in several different physical and chemical forms exhibiting a wide range of properties. The most important mercury species are elemental mercury (Hg^0), divalent inorganic mercury (Hg(II)), monomethylmercury –or methylmercury- (MMHg) and dimethylmercury (DMHg).

Elemental mercury can stay in the atmosphere in gaseous form for as long as a year, making this species very important in the mercury cycle because it can be transported over long distances (Lambertsson, 2005). In aquatic environments, inorganic Hg is converted to methylmercury (Morel et al., 1998), which can easily penetrate the membranes of organisms and bioaccumulate in the food chain, attaining highest concentrations in high trophic fish (Lee & Fisher, 2016) (Figure 1.1).

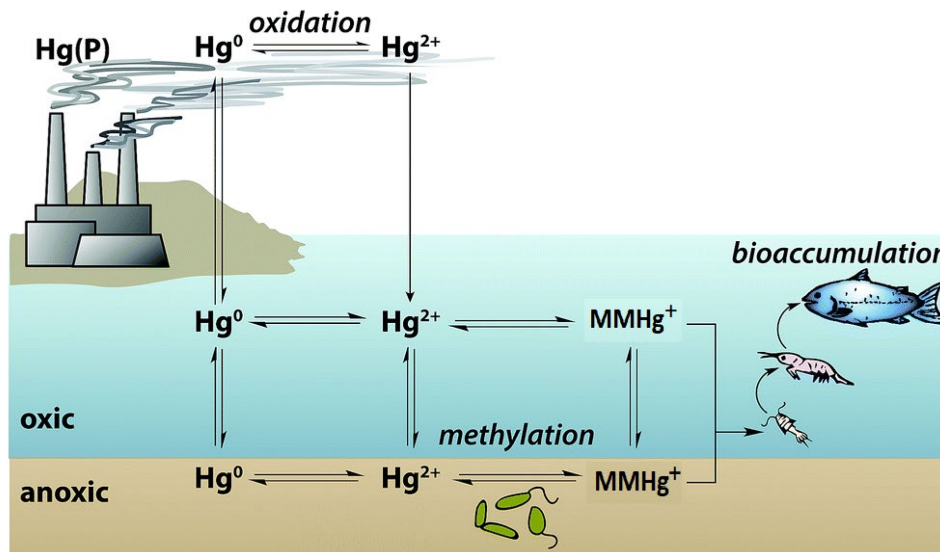

Figure 1.1 : Mercury cycle including aquatic Hg methylation adapted from (Lin & Erickson, 2015).

Mercury toxicity is highly dependent on its chemical form, with methylmercury being one of the most toxic mercury species. Once it is formed, methylmercury enters the lower food chain by rapid diffusion and tight binding to proteins in aquatic biota. It bioaccumulates in the food chain, attaining its highest concentrations in the tissues of top predatory fish due to biomagnifications through the trophic levels (Morel et al., 1998). The uptake efficiency of MMHg (near 100%) is much higher than for inorganic Hg (less than 10%) (Horvat, 1997), while the excretion processes of MMHg is slower by 3-fold (Trudel & Rasmussen, 1997).

Sources of Hg in the environment can have both natural (i.e., erosion, volcanic activity) and anthropogenic causes, such as coal combustion, mining, and chlor-alkali production (Pacyna & Pacyna, 2002). In aquatic systems, due to the complex Hg cycle, particularly Hg(II) binding to dissolved organic matter (DOM), Hg can be transported as far as 100 km downstream from its source (Nasr & Arp, 2017). Meaning that the noxious effects of Hg pollution can impact even distant areas from the pollution source.

1.1.1. Hg methylation

Due to the capacity of methylmercury to build up in aquatic food chains and display biomagnification, methylation of mercury in the aquatic environment is a critical step toward accumulation of this toxic metal in the aquatic food chain.

Inorganic Hg can be converted to MMHg by both abiotic and biotic mechanisms. Abiotic methylation of mercury can occur only in the presence of suitable methyl donors, where the primary reactants are believed to include small organic molecules like methyliodide and dimethylsulfide, along with larger organic constituents of dissolved organic matter such as fulvic and humic acids (Celo et al., 2006). However, it is generally considered

that abiotic methylation is less significant in comparison to the biotic pathway (Ullrich et al., 2001).

The identification of the hgcAB gene cluster essential for Hg methylation (Parks et al., 2013) has revealed numerous microorganisms across diverse taxonomic groups capable of methylating mercury. However, Hg methylation is thought to be mainly an anaerobic process primarily carried out by microorganisms such as sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB) and methanogens (Barkay & Wagner-Döbler, 2005; Grégoire & Poulain, 2018; Regnell & Watras, 2019). Consequently, anaerobic aquatic environments such as freshwater sediments (Gilmour et al., 1998; Schäfer et al., 2010) and hypolimnetic waters (Eckley & Hintelmann, 2006; Branfireun et al., 2020) are the main sites of Hg methylation. Additionally, wetlands have also been identified as important sources of MMHg to downstream ecosystems (Hall et al., 2008; Tjerngren et al., 2012; Branfireun et al., 2020).

Studies on Hg methylation in the environment often use ambient MMHg concentrations and % MMHg as proxies for Hg methylation activity (Gilmour et al., 1998; St.Louis et al., 2004; Eckley et al., 2017). However, advances of mass spectrometric methods permitted the determination of methylation rate potentials (*km*) in sediment and water samples using isotopically enriched Hg as a tracer (Hintelmann et al., 2000), and since then the use of an enriched Hg spike has become common practice (Eckley & Hintelmann, 2006; Heyes et al., 2006; Schäfer et al., 2010; Millard et al., 2023). The calculation of *km* values deepened our understanding of the mechanisms governing methylmercury (MMHg) concentrations. For example, it enabled the identification of preferential sites for methylmercury production, such as the anoxic hypolimnion of lakes (Eckley & Hintelmann, 2006) and the uppermost layer of sediments at the water-sediment interface (Schäfer et al.,

2010). As well, the application of *km* in conjunction with modelling tolls has been employed to understand the relative importance of methylation in sediment versus water column (Millard et al. 2023), bringing important implications for the management of MMHg production.

1.1.2. MMHg demethylation

The concentration of methylmercury in the aquatic environment is controlled by the balance between methylation and demethylation processes that occur simultaneously in the environment, with both biotic and abiotic mechanisms being recognized as important for MMHg demethylation (Barkay & Gu, 2022).

Microbial demethylation of MMHg has been observed to occur by two mechanisms distinguished by their volatile carbon product: reductive demethylation, in which the end products are CH_4 and Hg^0, and oxidative demethylation, that results in the production of CO_2 and Hg(II) (Barkay & Wagner-Döbler, 2005). Demethylation seems to be ubiquitous with both pathways occurring equally in aerobic and anaerobic environments (Merritt & Amirbahman, 2009). Several different strains of bacteria have been identified to participate in demethylation processes (Grégoire & Poulain, 2018), with microorganisms involved in reductive demethylation being mainly aerobic, while microorganisms involved in oxidative demethylation are mostly anaerobic, such as SRB, methanogens and IRB (Du et al., 2019). In terms of abiotic demethylation processes, light has a central role with photodegradation being the main pathway (Barkay & Wagner-Döbler, 2005; Barkay & Gu, 2022).

In general, MMHg demethylation in sediments is thought to be mainly a biotic process with microorganisms like SRB and methanogens being primarily involved (Du et al.,

2019), while in the water column the abiotic photochemical process is reported to be the most important pathway (Celo et al., 2006).

1.1.3. Factors influencing Hg methylation and MMHg demethylation

It is widely agreed that the efficiency of Hg methylation is controlled by microbial activity and Hg(II) bioavailability (Bravo & Cosio, 2020) which in turn depend on several environmental factors such as temperature, pH, redox conditions, and organic carbon and sulfate concentrations (Merritt & Amirbahman, 2009; Lehnherr, 2014), with high temperature and low pH generally thought to stimulate Hg methylation (Bigham et al., 2017). Whereas abiotic methylation is mainly affected by parameters that influence Hg speciation (Celo et al., 2006). However, many of these factors have opposing effects on Hg methylation, for example, high (> 10 µM) sulfide concentrations tend to inhibit methylation, through the formation of charged HgS complexes that decrease Hg(II) availability, while low levels of sulfide promote Hg methylation (Hammerschmidt et al., 2008) through the formation of neutral Hg-S complexes that penetrate SRB membranes diffusively (Benoit et al., 2001). As well, organic matter can either enhance Hg methylation by stimulating microbial activity (Bravo et al., 2017) and providing a carbon source for Hg methylators (Abdelhafiz et al., 2023) or hinder Hg methylation by reducing Hg bioavailability through complexation with Hg(II) (Hammerschmidt et al., 2008), with the molecular composition of organic matter being a critical parameter in determining Hg(II) availability for methylation (Bravo et al., 2017; Abdelhafiz et al., 2023).

Similar to methylation, demethylation is also influenced by similar factors such as pH, temperature, redox potential and organic matter (Compeau & Bartha, 1984; Li & Cai, 2013). As demethylation can be mediated by microorganisms, factors influencing microbial activity

and growth also influence MMHg demethylation (Miskimmin et al., 1992). In the case of the abiotic pathway of photodemethylation in water its efficiency is mainly related to light intensity and water depth, with factors such as pH and concentration and character of DOM also playing an important role (Li & Cai, 2013; Barkay & Gu, 2022). Similar to controls on Hg methylation, effects of variables on MMHg decomposition are also complex and sometimes contradictory (Du et al., 2019).

1.1.4. Influence of wetting and drying cycles on MMHg production

One of the variables that is deemed to impact MMHg concentrations is the occurrence of wetting and drying cycles, that simultaneously influence MMHg formation and degradation.

The influence of wetting and drying cycles on Hg methylation has previously been studied. It is generally assumed that MMHg production increases after inundation, resulting in higher MMHg levels in water, sediments and fish (Bodaly et al., 1984; Hecky et al., 1991; Gilmour et al., 2004; Feng et al., 2014; Coleman Wasik et al., 2015; Oswald & Carey, 2016). It is thought that the flooding of vegetation and soils induces oxidative releases of absorbed inorganic Hg (Ullrich et al., 2001), making newly inundated soils short-term sources of Hg to downstream systems, where it is now available to be methylated. In addition, inundating a terrain causes the flooded organic carbon in soils and plants to decompose, releasing large amounts of organic matter and nutrients that stimulate microbial methylation activity (St.Louis et al., 2004). Lastly, inundation may release sulfate, making it available to stimulate in-situ SRB activity and Hg methylation (Gilmour et al., 2004; Coleman Wasik et al., 2015). Additionally, flooding is not only thought to intensify Hg methylation but also result in the release of MMHg from the sediments to the water column (Gustin et al., 2006), increasing

the net MMHg concentrations. Repeated rewetting cycles (Gustin et al., 2006) as well as longer drying periods (Gilmour et al., 2004; Singer et al., 2016) seem to further enhance net MMHg production. However, some studies (Strickman & Mitchell, 2017) also observed that drying-rewetting cycles in surface-flow artificial wetlands did not enhance MMHg accumulation at the wetland margins, suggesting that responses to flooding may vary among different sites. As well, flooding was reported to enhance MMHg degradation, especially in the presence of annite (Xie et al., 2020), resulting in lower net MMHg productivity.

1.2. Wabigoon River System

The Wabigoon River is located in the northwest Ontario. It originates at Wabigoon Lake and flows through the town of Dryden, reaching Clay Lake around 100 km downstream, later entering Ball Lake where it joins the English River (Jackson et al., 1980) (Figure 1.2). This river system stretches across a sparsely populated land of boreal forest, low relief, and Precambrian granitic and greenstone belts overlain by patches of clay, silt and sandy till, which results in high turbidity of waters in the system (Parks 1976). However, due to discharges from a paper mill in Dryden, sediments up until the inflow of Eagle River (38 km), are largely covered with wood fibre (Parks 1976), where suspended debris are also seen in the water (Jackson et al., 1980).

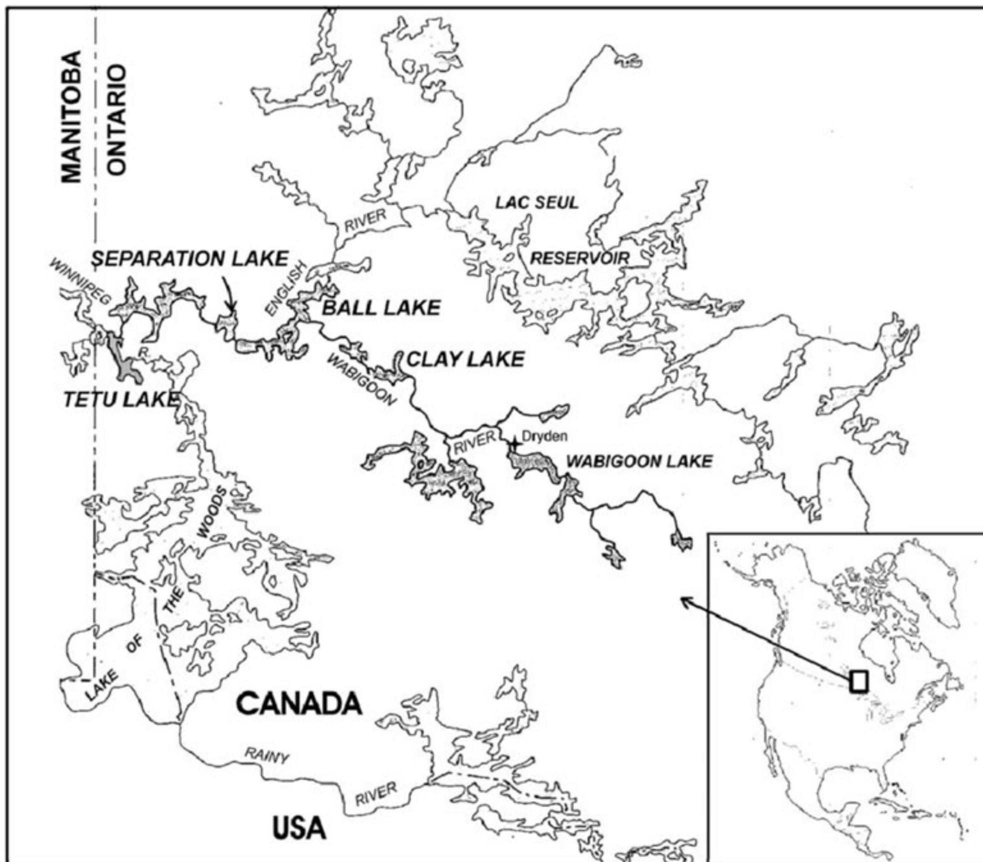

Figure 1.2 : Map of the English-Wabigoon River system showing the location of the pollution source (Dryden), taken from Neff et al. (2012).

The Wabigoon River is known for severe Hg contamination caused by a chlor-alkali facility operating in the 1960s in Dryden that used Hg cells for chlorine production, with a total of more than 10 tonnes of Hg being discharged into the local terrestrial and aquatic environments (Parks, 1976). Due to the Hg contamination, a former commercial fishery at Clay Lake, responsible for 8,000 lbs of fish per year, has shut down (German, 1969). This impacted not only the commercial use but also the local consumption of fish for the local communities of Asubpeeschoseewagong Netum Anishinabek (Grassy Narrows) and Wabaseemoong (White Dog) First Nations. Besides that, the agricultural use of the land around the Wabigoon River has ceased, highly influencing the culture and diets of these two communities. Although the commercial fishery has stopped, to this day traditional foods (including local fish) continue to be consumed among the local communities (Sellers, 2014).

Most importantly, these fish contaminated with Hg pose a severe health hazard to people that consume them as a large part of their diet, which carries in extreme situations the risk of mercury poisoning, commonly named “Minamata disease”, a neurological disease with symptoms such as numbness in the hands and feet, constriction of the visual field, damage to hearing and speech, and in severe cases it can lead to death (Harada, 1995).

Even though the industrial use of Hg stopped after installation of diaphragm cells for chlorine production in 1975 (Parks, 1976), effluents from the plant still contained substantial quantities of Hg (approximately ~1% of the uncontrolled discharges) (Jackson et al., 1980). While the exact date at which Hg release completely stopped is unknown, no evidence of ongoing discharges from the plant site to the river water was observed in 2017 (Rudd et al., 2021). As of 2018 (McGovarin, 2020; Rudd et al., 2021) Hg concentrations in water, sediments and fish remain elevated between Dryden and Clay Lake, with Clay Lake surface sediments showing concentrations more than 16 times higher than background levels. As the legacy Hg contamination continues to cause serious adverse health effects for the First Nation Communities (Philibert et al., 2022), more studies to understand the fate and transportation of Hg in this system are extremely important.

1.3. Objectives

The objectives of this study were to investigate the accumulation and transport of mercury and methylmercury along the Wabigoon River system, focusing on locations between Wabigoon Lake and Clay Lake. A spatial variation of both total mercury and methylmercury levels in water and sediments as well as a temporal analysis of mercury and methylmercury was established by analysing water, surface sediment and sediment cores (Chapter 2). Additionally, due to the ability of MMHg to bioaccumulate in biota, posing a

health concern to humans, this work also intended to identify areas of MMHg production potential by determining Hg methylation and MMHg demethylation potentials in several ecosystems across the Wabigoon River system (Chapter 3). Finally, as the intensification of the hydrological cycle is thought to increase due to climate change (Bapiri et al., 2010), a study on the influence of wetting and drying cycles on the Hg methylation on riverbed and wetland locations in the Wabigoon River system was also performed through a laboratory simulation (Chapter 4).

1.4. References

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Chapter 2: Temporal and spatial analysis of mercury and methylmercury along the Wabigoon River

Abstract

The Wabigoon River is infamous for the severe historical mercury pollution caused by a chlor-alkali facility in the 1960s. Once mercury contamination was discovered, intense studies were performed in this system during the 1970s and 1980s. However, there was a subsequent lack of systematic reporting on mercury levels in water and sediments until late 2010s. Most recent studies in the area show that the system remains contaminated with mercury, suggesting that current levels may be due to remobilization of legacy inorganic mercury from riverbank erosion. This study aims to assess spatial and temporal trends in mercury and methylmercury species. Results from this study suggest a very slow return to ambient background concentrations that would be typical for such ecosystems, as surface sediments are still on average 44x higher than background concentrations upstream of the pollution source. Additionally, present data from selected sampling locations indicate net methylmercury production within the system. For example, contaminated areas such as the wetland near Wabigoon Rapids pose a higher potential for methylmercury formation. Other locations along the river could also be sinks for methylmercury, reducing methylmercury transport to downstream locations.

Keywords: *Mercury; methylmercury; Wabigoon; River; sediments; water; cores; temporal; spatial*

2.1. Introduction

The Wabigoon River located in Northwest Ontario, originates at the Wabigoon Lake, running through the City of Dryden and reaching Clay Lake approximately 100 km downstream (German, 1969). This river is known for severe Hg contamination caused by a chlor-alkali facility in Dryden operating in the 1960s, with a total of more than 10 tonnes of Hg being discharged into local terrestrial and aquatic environments (Parks, 1976).

Studies carried out between 1971 and 1975 showed that the highest total Hg (THg) concentrations were located just downstream of the paper factory and decreased with increasing distance downstream (Parks, 1976), consistent with point source contamination.

After the discovery of the mercury contamination there were extensive studies in the area (German, 1969; Parks, 1976; Jackson, 1980; Jackson et al., 1982; Denison, 1982; Rudd & Turner, 1983), mostly focussing on possible remediation measures, while more recent studies (Kinghorn et al., 2007; Neff et al., 2012) focused on mercury fish concentrations in the system. Since the initial studies in the 1970s and 80s, most of the studies on mercury concentrations in the system seemed to focus specifically on sediments of Clay Lake, with repeated cores being collected in 1995 (Lockhart et al., 2000), 1999 (Jackson, 2016), 2004 (Sellers, 2005) and finally in 2018 (Rudd et al., 2021). Recent literature (McGovarin, 2020; Rudd et al., 2021) confirmed that mercury concentrations in this system remain elevated in sediments, water, and biota. However, while those studies have analysed total mercury concentrations in sediments and fish (McGovarin, 2020) and total mercury and methylmercury in water (Rudd et al., 2021) along the Wabigoon River system, methylmercury concentrations in sediments have not been reported in recent peer reviewed

papers, although they are important to assess the bioavailability of mercury to fish and hence affect human health.

Given that the point source mercury contamination is thought to have stopped in 1975 with the installation of diaphragm cells for chlorine production (Parks, 1976) and there should be longer ongoing Hg discharges from the plant site to the river water (Rudd et al., 2021), it was suggested that the Wabigoon River itself, especially upstream from Clay Lake, is still a source of mercury to downstream sites (Sellers, 2014). This is attributed to the remobilization of mercury-laden particles from contaminated riverbanks and floodplains (Rudd et al., 2021). Hence, a systematic investigation on mercury and methylmercury levels on locations upstream from Clay Lake is critically needed to fully understand mercury transportation and accumulation in this system.

This study aims to assess the accumulation and transport of mercury and methylmercury along the Wabigoon River system, focusing on locations between Wabigoon Lake and Clay Lake, by establishing a spatial overview of current levels of both total mercury and methylmercury in water and sediments as well as a temporal analysis of mercury and methylmercury through analysis of sediment cores.

2.2. Methods

2.2.1. Study area

Several locations along the Wabigoon River between Wabigoon Lake and Clay Lake were selected for sampling, including different types of ecosystems such as lakes, wetlands, and riverbank. Wabigoon Lake (WL) and a wetland next to it (WLWT) are located upstream from the pollution source and serve as reference (uncontaminated) locations in this river

system. Several locations were sampled downstream from the pollution source, starting with the Hydroelectric dam (HD), located 5 km away from the pollution source, followed by Minnitaki Bridge (MB) at 30 km, Wabigoon Rapids (WR) at 62 km, a wetland next to Wabigoon Rapids (WRWT) at 60 km, Wabigoon Falls (WF) at 71 km and finally Clay Lake Inflow (CL I) at 85 km (Figure 2.1).

2.2.2. Sampling collection

Sediment cores between 14 and 44 cm long were collected in Fall 2018 at eight different locations along the Wabigoon River system (identified in Figure 2.1). Replicate core samples were collected within 15 m of each other to assess location variability, with replicates being collected at Wabigoon Lake (n = 2), Wabigoon Lake Wetland (n = 3), Hydroelectric dam (n = 3), Wabigoon Rapids wetland (n = 2) and Clay Lake (n = 2). Coordinates for core samples and replicates can be found in the Appendix (Table A.1).

Cores from lake basins were collected using a gravity corer while cores from riverine and wetland locations were collected using a hand corer in locations that were submerged at the time of collection. Cores were sliced in the field at 1 cm intervals and frozen until analysis. Note that locations with high wood debris, such as the Hydroelectric dam, have irregular core slices, because it was not possible to cut cores every 1 cm. Prior to analysis sediment cores were dried over night at 60 ºC and homogenized using a mortar and pestle.

To establish present THg and MMHg levels in the Wabigoon River, a surface water sample (August 2022) and a surface sediment sample (July 2022) were also collected at each location (except MB and WF), with an additional sediment sample collected at the deepest part of the west basin of Clay Lake (CL W) (Figure 2.1). The top layer of sediments from lake locations (WL and CL) were sampled using an Ekman style dredge and transferred into

ziplock bags. Sediments from riverine and wetland locations were collected by pushing 5 cm diameter polyvinyl chloride (PVC) tubes by hand where the top 6 cm of cores were dried over night at 60 ºC and homogenized with a mortar and pestle. Unfiltered surface water samples were collected using the clean hands/dirty hands protocol according to EPA method 1669 (EPA, 1996) into certified 250 mL fluorinated polyethylene wide mouth bottles (Brooks Rand Instruments; < 0.4 ng/L Hg), except for bottom waters at the Wabigoon Lake (WL BOT) where a peristaltic pump and a Teflon line was used for collection.

2.2.3. Laboratory analysis

Total Hg in core sediment samples was measured using a direct mercury analyzer (DMA 80, Millestone). Approximately 40 mg of homogenized dry sediment were directly analysed without requiring additional pre-treatment. In the DMA the samples are thermally decomposed in a furnace and Hg is trapped and determined by atomic absorbance spectrometry in compliance with EPA method 7473 (EPA, 1998).

Total Hg in water and surface sediments was measured using an Inductively Coupled Plasma Mass Spectrometer - ICP-MS (Agilient 8800). Approximately 200 mg of dry sediment were digested using a mixture of sulfuric/nitric acid (7:3, v/v) overnight at around 90 °C, while 20 mL of unfiltered water was treated with a strong oxidant solution (150 µL of 0.2 N Bromine monochloride– BrCl) overnight at room temperature to oxidize all forms of Hg into Hg^{2+} before analysis by ICP-MS. To correct for procedural recoveries, an internal ^{199}Hg enriched standard solution (Trace Sciences International, see Table A.2 for isotopic abundances) was added to both water and sediment samples at the time of the digestion.

The measurement of MMHg in both water and sediment samples followed the EPA method 1630 (EPA, 1998), where a water vapour distillation was used to separate MMHg

from the sample matrix. Around 200 mg of homogenized dry sediment or 50 mL of unfiltered water were used to perform the distillation where 500 µL of H_2SO_4 (9 M) and 200 µL of KCl (20 %) were added to all samples. Additionally, $MM^{199}Hg$ synthesised from ^{199}HgO (Trace Sciences International, see Table A.2 for isotopic abundances) was added as an internal standard as a way of correcting for procedural recoveries. The samples were distilled at 115 °C with a mercury free nitrogen gas flow of 60 ml/min until approximately 90 % of the sample was transferred to the receiving vessel, which took approximately 4 hours for water samples and 2 hours for sediment samples. After addition of 225 µL of sodium acetate buffer (2 M, pH = 4) and 30 µL of sodium tetraethylborate reagent (1 %) to ethylate all Hg species present, the distillate was measured using an Automated Methyl Mercury Analyzer (Tekran® 2700) coupled to ICP-MS (Agilent 8800).

2.2.4. QA/QC

Quality assurance was performed by analysis of THg in CRM PACS-2 (3.04 ± 0.20 mg/kg) Marine Sediment using DMA, and SRM 1944 (3.4 ± 0.5 mg/kg) Marine Sediment using ICP-MS analysis. In addition, CRM IAEA-475 ($0.199 \pm 0.034 \times 10^{-3}$ mg/kg) Marine Sediment was analyzed for MMHg by ICP-MS. A concentration of 3.01 ± 0.39 mg/kg (n = 34) was obtained for PACS-2, 3.5 ± 0.4 mg/kg (n = 4) for SRM 1944, and $0.154 \pm 0.076 \times 10^{-3}$ mg/kg (n = 20) for IAEA-475. The method detection limit for MMHg was 0.09 ng/g in sediments and 0.11 ng/L in water. The DMA method detection limit for THg in sediments was 0.015 mg/kg, based on 40 mg used to perform the analysis, while the THg detection limit for ICP-MS analysis was 0.04 ng/g in sediments and 0.10 ng/L in water. All stated detection limits are based on 3 standard deviations of the mean of the method blank.

2.3. Results & Discussion

2.3.1. Total mercury in water and surface sediments

Uncontaminated freshwaters generally contain less than 5 ng/L of total Hg (Ullrich et al., 2001), which matches the concentrations found our reference locations, with Wabigoon Lake showing 3.60 ng/L of THg, while it´s wetland showed 6.41 ng/L. The slightly higher THg level found in the wetland can be explained by the fact that wetlands are often sinks of inorganic mercury (Tjerngren et al., 2012).

The reference locations also showed low THg concentrations in sediments (Table 2.1), with 0.07 mg/kg THg in surface samples from both Wabigoon Lake and its wetland, while the top 5 cm of core samples showed 0.04 and 0.12 mg/kg, respectively, which is consistent with other lakes from west Ontario (Arp et al., 2016). Locations downstream from the pollution source all showed THg concentrations that are at least 8x higher in water (28.1 to 122 ng/L), and more than 5x higher in surface sediments (0.37 to 0.79 mg/kg), while the top 5 cm of core samples show concentrations up to 134 times higher than Wabigoon Lake (0.13 to 5.5 mg/kg), with the exception of Minnitaki Bridge, where concentrations were similar to background levels (0.032 mg/kg).

The differences in THg observed for surface grab sediments and the top 5 cm of core samples is likely due to the high THg variability within the system (Section 2.3.5) combined with differences in the exact sampling location. Despite those differences, locations downstream of the pollution source are clearly elevated in mercury, showing concentrations consistently higher than the reference locations (0.08 vs 1.1 mg/kg average of THg).

Mercury concentrations reported for Clay Lake here are obtained from sediments in the west basin (CL W) of the lake and in the inflow (CL I), showing lower levels (0.34 to 0.52 mg/kg) than values reported in an earlier study (1.01 mg/kg, McGovarin, 2020). This is consistent with previous observations that the west basin is not a THg deposition zone and therefore exhibits lower concentrations than the northern and eastern basins (Parks, 1976; Jackson, 1980; Rudd & Turner 1983). Similarly, sedimentation at the inflow (CL I) is low or absent resulting in low THg, not fully reflecting the Lake's capability of THg deposition.

Total mercury concentrations in water appeared to increase over distance from the pollution source (Figure 2.2). This suggests that THg which was deposited to sediments is slowly being mobilized and being transported in water along the river. Similar results were found by Rudd et al. (2021), who suggested that inorganic particles (fine clay) are being remobilized from the contaminated riverbanks, transporting THg downstream.

2.3.2. Methylmercury in water and surface sediments

Similar to total mercury, methylmercury concentrations in the reference locations were also low, with 0.21 ng/L in surface waters of Wabigoon Lake, while MMHg in the wetland was 0.11 ng/L. The bottom waters of Wabigoon Lake show a slightly higher MMHg concentration (0.31 ng/L), which could be explained by low oxygen levels (0.16 mg/L) found at the bottom of the lake, which are known to enhance Hg methylation by anaerobic bacteria (Olson & Cooper, 1976).

Both surface and bottom waters showed higher MMHg concentrations than values reported in 2017 for the outflow of Wabigoon Lake (Rudd et al., 2021). Concentrations of MMHg in the Wabigoon Lake outflow were consistent during different months, while other locations sampled changed seasonally (Rudd et al., 2021), suggesting that the outflow is not

subjected to seasonal changes, creating a spatial variation within the lake, with higher concentrations found at its middle, most probably due to seasonal active methylation in the hypolimnion (Eckley & Hintelmann, 2006).

The reference locations also showed low concentrations of MMHg in surface sediments (Table 2.1), with Wabigoon Lake having 0.16 ng/g and its wetland having 1.46 ng/g MMHg, while the top 5 cm of core samples showed 0.59 and 0.65 ng/g, respectively, which is consistent with MMHg levels found in uncontaminated sediments (Mikac et al., 1999). The higher MMHg level found in wetland sediments can be explained by the fact that wetlands are known to have favourable conditions for methylation (Hall et al., 2008), suggesting that MMHg production could be a factor at this location.

Locations downstream from the pollution source show MMHg concentrations which are at least 5x higher in water (0.96 to 4.70 ng/L) and more than 4x higher in surface sediments (0.66 to 34.1 ng/g), while the top 5 cm of core samples show MMHg concentrations up to 89 times higher than Wabigoon Lake (0.93 to 53.0 ng/g), with the exception of Minnitaki Bridge that shows MMHg levels similar to the reference location (0.56 ng/g) (Table 2.1).

The high concentrations at Wabigoon Rapids (between 10.2 and 34.1 ng/g), followed by lower concentrations in downstream locations (0.52 to 6.42 ng/g) indicates that WR is not only a producer of MMHg but could also be a sink. However, MMHg concentrations in water (Figure 2.3) increased even past WR, suggesting that some fraction of the produced MMHg is being mobilized to the water column and transported to locations downstream.

Methylmercury concentrations in water along the Wabigoon River (Figure 2.3) seem to increase with distance from the pollution source, indicating ongoing methylation

processes in riverbed and wetland locations along the river. This trend is consistent with results from Jackson et al. (1982) where dissolved MMHg levels in the Wabigoon River increased in the downstream direction, indicating that MMHg transport and production along the river was ongoing since the 1980s until present days.

2.3.3. Total mercury profiles in sediment cores

Total Hg concentrations in the Wabigoon Lake core show consistently low THg concentrations (between 0.025 and 0.057 mg/kg, average of 0.038 ± 0.007 mg/kg) which is in accordance with historical data where this lake exhibited an average of 0.04 mg/kg of THg from 1973 to 1975 (Parks, 1976). The THg concentration along the depth of Wabigoon Lake core is consistently low and comparable to values from the 1970s, suggesting that this location has not been impacted by Hg pollution, and thereby making Wabigoon Lake a suitable reference location.

Most locations downstream from the pollution source show a peak in THg in their mid to low depths (Figure 2.4), with peaks of 60.9 mg/kg THg at 30.5 cm for HD, 2.74 mg/kg at 28.5 cm for MB, 15.05 mg/kg at 6.5 cm for WR, and 5.12 mg/kg at 12.5 cm for the WRWT core. Total Hg concentrations seem to be slowly decreasing below these concentration peaks. However, surface concentrations are still 14 to 77 times above background levels, with the exception of Minnitaki Bridge which shows surface THg concentrations comparable to the reference location. This location seems to have a higher sedimentation rate compared to other locations sampled, evidenced by the peak being buried deeper (28.5 cm depth) and having surface THg concentrations near background levels (0.03 mg/kg). Additionally, surface sediments at this location are mostly sand and gravel (S. McGovarin, personal

communication), which are known to be poor-mercury sediments (Bongers & Khattak, 1972), explaining the low surface concentrations.

Total Hg concentrations at Wabigoon Falls are fairly uniform with depth, without displaying a peak, however the very top layer shows concentrations that are 37 times higher than in its bottom sediments. This odd THg profile may be explained by the existence of high velocity and turbulent waters at this location, causing irregular or very low sedimentation.

The Clay Lake core also does not show a peak in THg concentration. Possibly, the collected core of 21 cm depth was too short to reach the peak in contamination. Several long cores collected at this location between 1971 and 2017, show that there was an increased rate of burial between 2004 and 2017, with the peak THg in Clay Lake in 2017 being found at the 19-20 cm core segment (Rudd et al., 2021). The non-existence of this peak in the 21 cm core sampled in our work suggests that Clay Lake maintains an elevated rate of sediment accumulation, resulting in the peak being buried below the here sampled 21 cm of sediment. Alternatively, the site of sample collection is still within the main inflow channel with very low or absence of sustained sedimentation, similar to the WF location, which prevents the establishment of proper historical records over time. Even though, surface sediments at Clay Lake still show THg concentrations 26x higher than background concentrations.

2.3.4. MMHg profiles in sediment cores

Methylmercury concentration trends seem to follow THg, with the peak concentration of MMHg also being at the same depth as the peak of THg, except for the wetland near the Wabigoon Rapids, where the MMHg peak was at the 7.5 cm depth, 7 cm above the THg peak. This inconsistency of the MMHg profile compared to the THg could be

explained by active Hg methylation happening at this depth, or that MMHg from locations upstream was transported along the river and accumulated at Wabigoon Rapids wetland.

On average, 0.9 % of the total Hg is present in form of MMHg, which is slightly above the typical % MMHg reported for other sediments in freshwater environments of approximately 0.5 % (Bartlett & Craig, 1981; Mikac et al., 1999; Domagalski, 2001; Hammerschmidt & Fitzgerald, 2006; Cossa et al., 2014; Fleck et al., 2016), which suggests higher net Hg methylation occurring in this system. In particular, the Hydroelectric dam shows a consistent 0.2 % of MMHg along the whole core, except for the top sediments where the proportion increased to 2 % (Table A.5), suggesting higher MMHg production at the surface. Furthermore, Clay Lake showed consistently higher % MMHg compared to all the other locations (2.2% vs 0.8 %), suggesting that methylation may be enhanced at this location.

2.3.5. Repeatability of core sampling

Wabigoon Lake showed very uniform THg profiles between the two cores collected, having on average 0.038 ± 0.007 mg/kg and 0.042 ± 0.004 mg/kg, respectively (Table A.3). However, the 3 replicates collected at the wetland next to Wabigoon Lake show high variability in both THg and MMHg concentrations (Table A.4). While THg concentrations in the first two cores display similar depth profiles (t-test, $p = 0.8$), with an average of 0.099 and 0.097 mg/kg, respectively, methylmercury concentrations varied significantly (t-test, $p = 0.002$), and average MMHg concentrations differed by more than 10x (0.4 and 5.6 ng/g, respectively). The third core had significantly different THg (t-test, $p = 0.002$) and MMHg (t-test, $p = 0.03$) concentrations compared with the main core, with average THg and MMHg concentrations of 0.07 mg/kg and 1.9 ng/g, respectively. Wetlands are known to have high

spatial variations (Kim et al., 2015), which is also demonstrated by the varying levels of organic matter found in the 3 replicates (63 %, 92 % and 79 % of OM (Table A.11 to A.13), respectively), which may also be the reason for the observed difference in Hg concentrations. These variations observed are comparable to other studies where THg and MMHg concentrations varied spatially in wetlands (Miles & Ricca, 2010; Kim et al., 2015). Additionally, these differences are also reflected in varying % of MMHg, with averages of 0.4, 6.5 and 2.7 %, respectively, suggesting that even nearby wetland cores, collected within 15 m of each other, have different methylation environments and methylation within a wetland is not spatially uniform.

Replicate cores collected at the Hydroelectric dam show high variability among them (Table A.5), with the third replicate showing a considerably lower average THg concentration (3.0 mg/kg) compared to the other two (14.2 and 10.8 mg/kg, respectively). Downcore, some variation was observed with the first core having the peak THg of 60.9 mg/kg at 30.5 cm compared to two THg peaks of 39.0 mg/kg (13 cm) and 24.4 mg/kg (23.5 cm) in the second and no pronounce peak in the third core. The Hydroelectric dam does not experience a typical deposition of sediments due to receiving lumber, sawmill waste and other debris coming from the pulp and mill factory (German, 1969), resulting in large variations in organic matter content at each specific location and with depth. For example, core 3 showed varying percentages of organic matter (OM) between 14 to 75 % (Table A.14). As Hg is known to have a strong affinity to OM (Sanei et al., 2014), the large variation in THg concentrations at HD may be be explained by the differences in organic matter content caused by the irregular deposition of woody debris.

Cores from the wetland near Wabigoon Rapids also showed variable concentrations (t-test, p = 0.0004) with the second core showing lower mercury values (0.39 mg/kg) compared to the main core (1.7 mg/kg), with peak concentrations of 2.6 and 5.1 mg/kg, respectively (Table A.8). These differences could again be explained by the fact that wetlands are known to have a lot of variation within (Kim et al., 2015).

Clay Lake replicates were shorter (9 cm) than the main core collected at this location (21 cm). While the second core was similar to the top 9 cm of the main core (t-test, p = 0.36), showing an average of 0.37 mg/kg THg compared with 0.52 mg/kg, the third core was distinct (t-test, p = 0.0003), showing a considerably higher average concentration of 2.18 mg/kg and a peak concentration of 3.51 mg/kg compared with 0.77 and 0.93 mg/kg in the other replicates (Table A.10). Despite these differences, the top layers (3 cm) of the 3 cores show similar THg concentrations, averaging 0.9 ± 0.2 mg/kg in all cores, suggesting fairly uniform surface concentrations. The variability of the THg profiles in Clay Lake cores might be attributed to samples collected in the eastern basin, which is known to be shallow and unstratified (Jackson, 1980), resulting in atypical sedimentation in the area. This was also noted in past studies, where several cores collected within the east basin of Clay Lake had similar differences among each other (Rudd et al. (2021), supplemental data).

The high variability of THg between different replicates along the Wabigoon River system adds to the challenge of accurately establishing mercury levels in the system. However, despite the uncertainties around precise concentrations at each location, the Wabigoon River is clearly highly contaminated, with locations downstream from the pollution source still showing elevated THg concentrations compared to background levels.

2.4. Conclusion

Among locations investigated in this study, the wetland at the Wabigoon Rapids is identified as an area of concern. As a wetland, this location presents generally favourable conditions for Hg methylation (Hall et al., 2008). It further exhibited high THg concentrations in both sediments (between 0.79 and 2.72 mg/kg) and water (60.3 ng/L) that can potentially be available for Hg methylation. Additionally, MMHg concentrations in sediment are also elevated (9.01 to 13.4 ng/g) compared to other downstream locations, suggesting that this location could be a site of net MMHg production. Further analysis of this area and other similar wetlands in this river system should be performed to assess the full potential of Wabigoon River wetlands regarding MMHg production.

Methylmercury spatial variations point to Wabigoon Rapids being either a producer and/or sink for MMHg. Further analysis should be conducted at this location to fully understand its role regarding MMHg production, accumulation, and transport.

As well, the Hydroelectric dam and Clay Lake seem to be sites of net Hg methylation sites, based on the observed elevated % MMHg in core samples. Further analysis on these locations should also be performed to assess its role regarding MMHg production.

Mercury depth profiles show a decrease of THg concentrations above a downcore peak in most locations, indicating that Hg the Wabigoon River sediments may slowly decrease over time due to sediment accumulations, burying the THg peak caused by the historical pollution source. However, surface sediments still show THg concentrations on average 44x higher than background concentrations.

2.5. Tables

Table 2.1 : Distance to Pollution source (km,), THg in water (ng/L) and sediments (mg/kg), MMHg in water (ng/L) and sediments (ng/g) in several locations along the Wabigoon River System: Wabigoon Lake (WL), Wabigoon Lake wetland (WL WT), Hydroelectric Dam (HD), Minnitaki Bridge (MB), Wabigoon Rapids (WR), Wabigoon Rapids Wetland (WR WT), Wabigoon Falls (WF), Clay Lake Inflow (CL I) and Clay Lake West (CL W).

Location	Distance to pollution source (km)	THg in water (ng/L)	THg in sediments (mg/kg)	MMHg in water (ng/L)	MMHg in sediments (ng/g)	THg in core top 5 cm (mg/kg)	MMHg in core top 5 cm (ng/g)
WL TOP	-10	3.60		0.21			
WL BOT		3.67	0.07	0.31	0.16	0.04	0.59
WL WT	-6	6.41	0.07	0.11	1.46	0.12	0.65
HD	5	46.4	0.65	0.96	4.61	5.50	53.0
MB	30					0.03	0.56
WR WT	60	60.3	0.79	3.62	9.01	2.72	13.4
WR	62	28.1	0.55	2.51	34.1	0.13	10.2
WF	71					0.53	6.42
CL I	85	122	0.37	4.70	0.66	0.52	0.93
CL W	93		0.34		0.93		

2.6. Figures

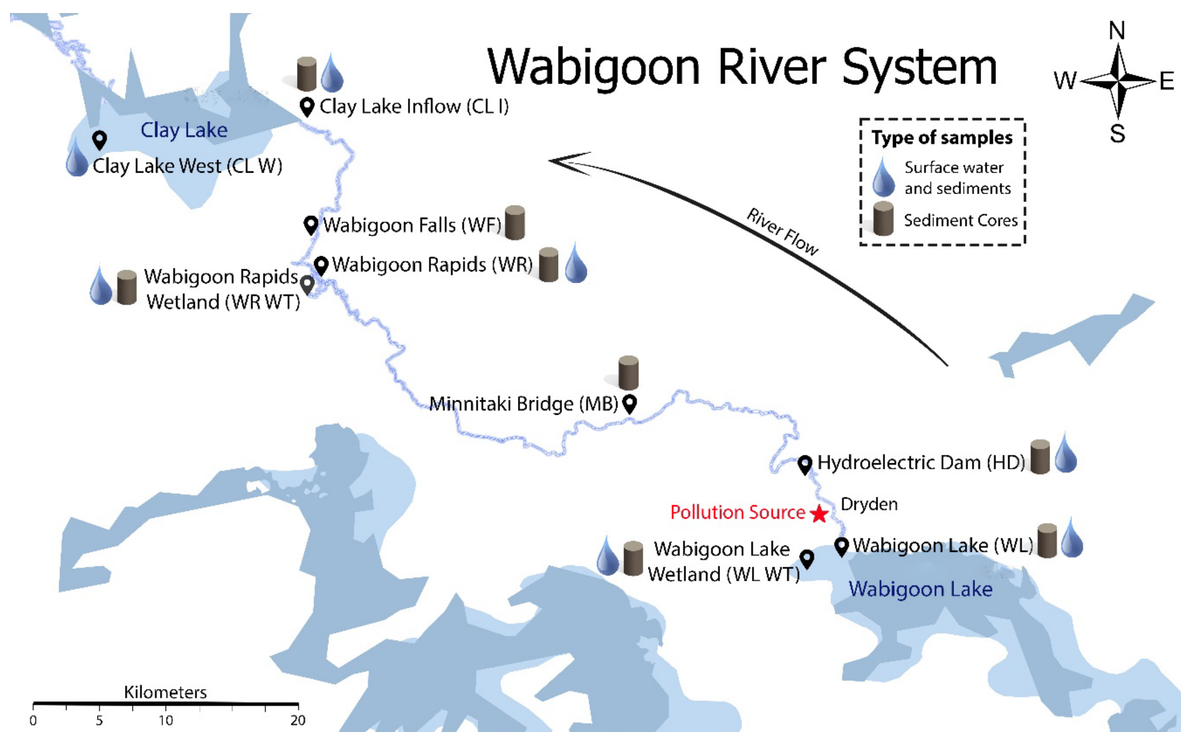

Figure 2.1 : Map of sampling locations along the Wabigoon River System with pollution source shown in red, core samples identified with a brown cylinder and surface samples identified with a blue drop. Core samples collected in Fall 2018. Surface water samples collected in August 2021 and sediment samples in July 2022.

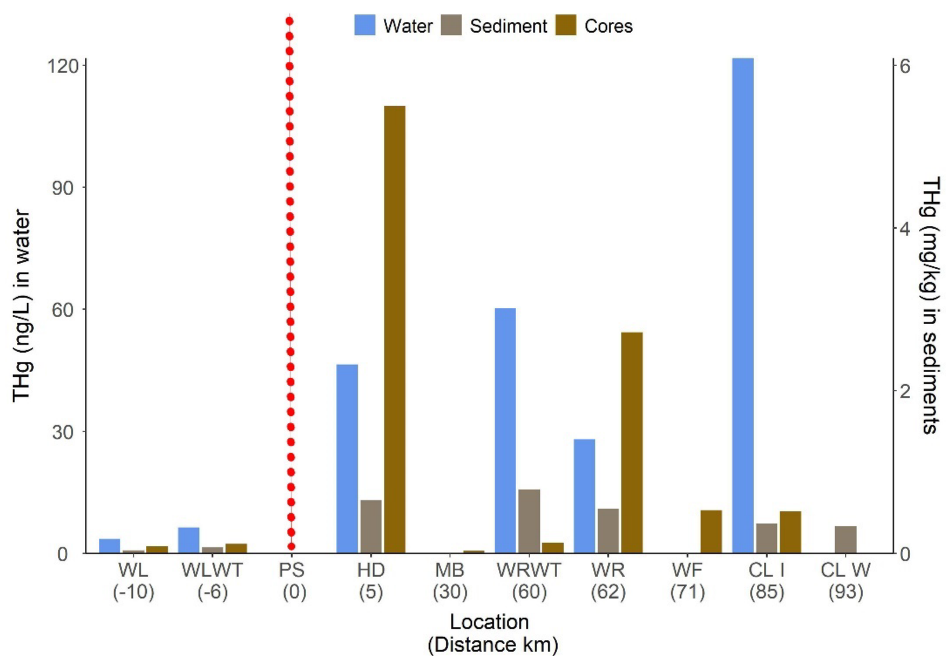

Figure 2.2 : THg in surface water (ng/L) and sediments and top 5 cm of cores (mg/kg) at difference locations along the Wabigoon River and distances from pollution source, with Pollution Source (PS) identified.

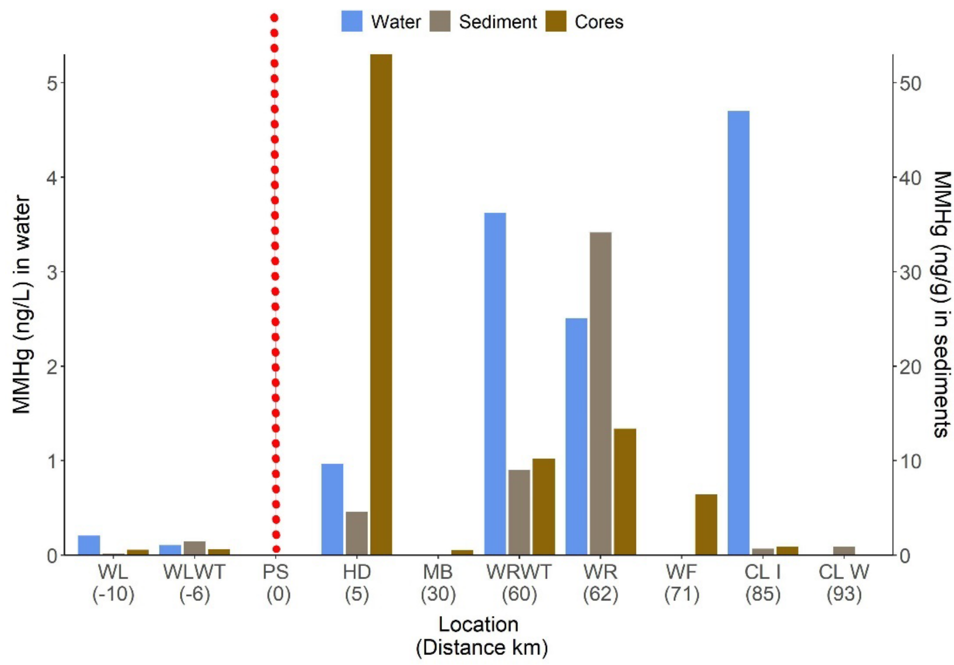

Figure 2.3 : MMHg in surface water (ng/L), sediments and cores (ng/g) at difference locations along the Wabigoon River and distance from pollution source, with Pollution Source (PS) identified.

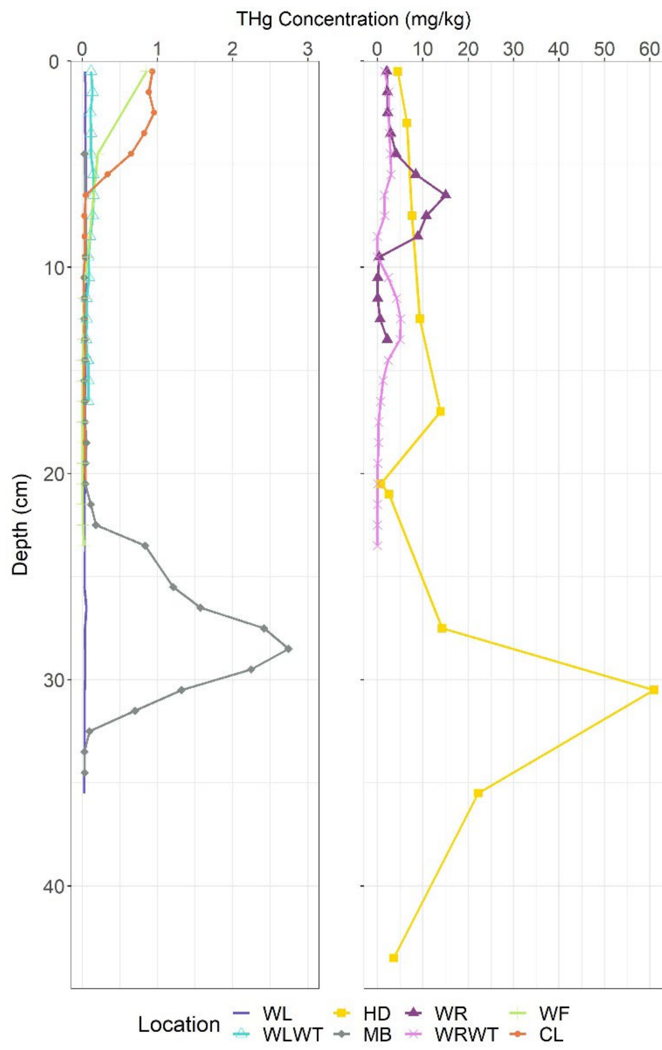

Figure 2.4 : THg concentration (mg/kg) depth profiles in sediment cores collected in 2018 from different locations along the Wabigoon River System.

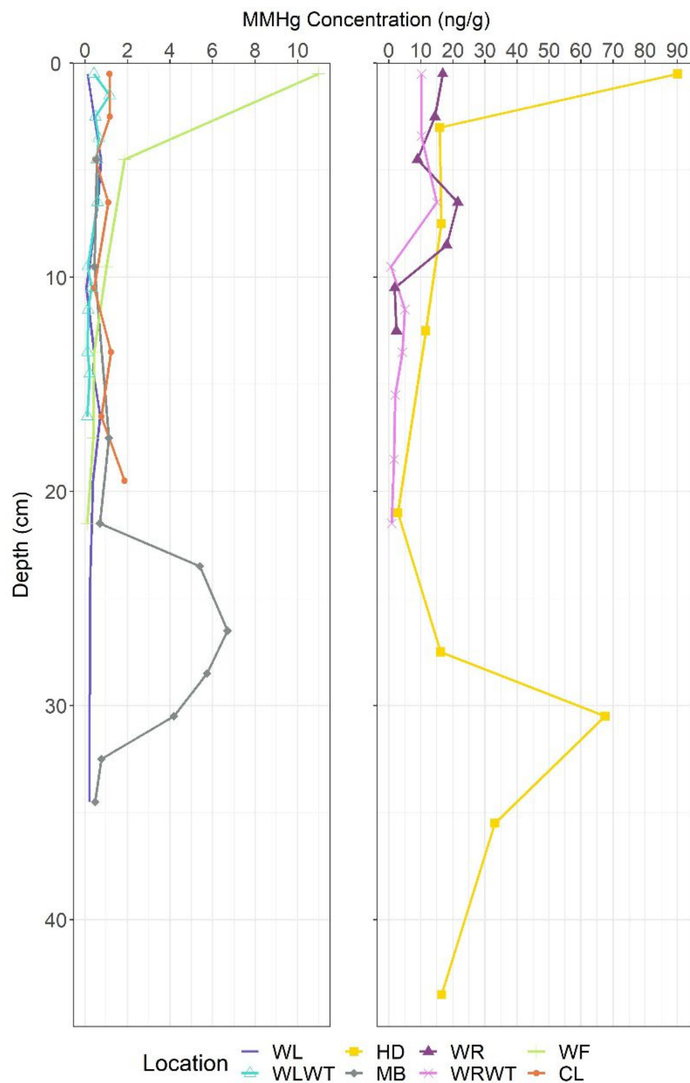

Figure 2.5 : MMHg concentrations (ng/g) depth profiles in sediment cores collected in 2018 from different locations along the Wabigoon River System.

Acknowledgments

This research was supported by a PhD Research Studentship (SFRH/BD/144532/2019), funded by the Portuguese Foundation for Science and Technology (FCT) to BB, as well as an NSERC Discovery grant to HH. Thanks to the Water Quality Center for the use of the ICP-MS; Doug Evans and Huy Dang for the use of the DMA; and Stephen McGovarin for the collection of the sediment cores analysed in this paper.

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Chapter 3: Assessment of mercury methylation and methylmercury demethylation potentials in water and sediments along the Wabigoon River system

Abstract

Monomethylmercury (MMHg) plays a crucial role in the accumulation of mercury (Hg) within the aquatic food chain. Since ambient levels of methylmercury are governed by the balance of simultaneous methylation and demethylation processes, determining *in situ* methylation and demethylation rates is critically important to understand the dynamics of formation and degradation of methylmercury in the system. This is especially important in the Wabigoon River system in Ontario, Canada, which was severely contaminated with Hg by a chlor-alkali facility operating in the 1960s and has present-day fish mercury concentrations still amongst the highest recorded in Canada. This work used a simultaneous addition of isotopically enriched Hg and MMHg tracers to ascertain Hg methylation and MMHg demethylation potentials in several locations across the Wabigoon River system. Among locations investigated in this study, the Hydroelectric dam located 5 km downstream from the pollution source was found to have the most favourable conditions for Hg methylation, being able to transform 4.2 % and 4.4 % of added Hg in water and sediments, respectively, to MMHg. This could correspond to 1.9 ng/L and 29 ng/g of new MMHg being produced from current ambient Hg in water and sediments at this location per day. Clay Lake, which is considered a sink for mercury (THg) and exhibits a seasonal anoxic environment at its bottom waters, was also found to generate significant amounts of MMHg, being able to produce 2.7 ng/L and 13 ng/g of MMHg per day in surface waters and bottom sediments, respectively. In contrast, Wabigoon Lake located upstream from the pollution source, may only form 0.13 ng/L and 2.7 ng/g per day in its bottom waters and sediments. Results from

the demethylation essays show that the half-life for methylmercury in sediments of riverbed and wetland locations is on average 2.1 days, indicating a rapid turnover and low persistence of methylmercury in the Wabigoon River sediments. Nonetheless, demethylation rates found at the sampled locations were consistently lower than rates reported in other systems. Notably, low demethylation rates were measured near the inflow of Clay Lake, where it took up to 144 days for MMHg to decrease by 50 %. Generally, most of the investigated locations downstream of the pollution source have the potential to generate methylmercury, which could be distributed throughout the Wabigoon River system and therefore require attention with respect to future remediation activities.

Keywords: *Mercury; Methylmercury; Methylation; Demethylation ; Methylation Potentials; Demethylation Potentials Wabigoon River; Sediments; Water*

3.1. Introduction

Mercury (Hg) toxicity is highly dependent on its chemical form, with monomethylmercury (MMHg) being one of the most toxic Hg species. Once it is formed, it enters the lower food chain by rapid diffusion and tight binding to proteins in aquatic biota (Lee & Fisher, 2016). It bioaccumulates in the food chain, attaining its highest concentrations in the tissues of top predatory fish due to biomagnifications through the trophic levels (Mason et al., 1996), resulting in fish and marine mammals at the highest trophic level being often unsafe for consumption. Sources of MMHg to aquatic systems include atmospheric deposition (such as precipitation), runoff from watersheds, in particular ones containing wetlands, and internal production (Rudd, 1995), with the latter being thought to be the dominant pathway for MMHg in freshwater systems (Regnell & Watras, 2019). In aquatic environments, inorganic Hg can be converted to methylmercury by both abiotic and biotic

mechanisms, though abiotic methylation is thought to have little significance compared with the biotic pathway (Ullrich et al., 2001). The discovery of the hgcAB gene cluster essential for mercury (Hg) methylation (Parks et al., 2013) has unveiled numerous microorganisms spanning diverse taxonomic groups with the capability of methylating mercury. However, Hg methylation is primarily considered an anaerobic process, predominantly carried out by microorganisms such as sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB), and methanogens (Barkay & Wagner-Döbler, 2005; Grégoire & Poulain, 2018; Regnell & Watras, 2019). Therefore, the dominant source of MMHg to freshwater systems is the methylation of inorganic Hg by anaerobic microorganisms. It is widely agreed that *in situ* rates of Hg methylation are controlled by microbial activity and Hg (II) bioavailability, which in turn depend on several environmental factors such as temperature, pH, redox conditions and organic carbon and sulfate concentrations (Lehnherr, 2014).

Undeniably, the methylation of Hg in the aquatic environment is a critical step toward accumulation of this toxic metal in the aquatic food chain. Determining *in situ* methylation rates is extremely important to understand MMHg concentrations in the environment and in fish. However, for many years, methylation rates in the environment remained unclear (Janssen et al., 2016). Advances of mass spectrometric methods permit the determination of methylation rates in sediment and water samples using isotopically enriched Hg as a tracer (Hintelmann et al., 2000), allowing a better understanding of Hg speciation in aquatic ecosystems. As environmental levels of methylmercury are regulated by simultaneous methylation and demethylation processes, estimating ambient methylmercury concentrations using only specific methylation rates tends to result in overpredictions (Eckley & Hintelmann, 2006). Hence, specific MMHg demethylation rates should also be estimated to better understand methylmercury dynamics.

The process of demethylating methylmercury involves both biotic and abiotic mechanisms in the degradation of methylmercury (Li & Cai, 2013). In abiotic demethylation processes, photodegradation, plays a central role (Barkay & Wagner-Döbler, 2005; Celo et al., 2006). Microbial demethylation of monomethylmercury (MMHg) occurs through two distinct mechanisms characterized by their volatile carbon products: reductive demethylation, yielding CH_4 and Hg^0, and oxidative demethylation, resulting in CO_2 and Hg(II) (Barkay & Wagner-Döbler, 2005). Demethylation appears to be widespread, with both pathways occurring equally in aerobic and anaerobic environments (Merritt & Amirbahman, 2009; Barkay & Gu, 2022).

Various bacterial strains have been identified in microbial demethylation processes (Grégoire & Poulain, 2018), with aerobic microorganisms primarily involved in reductive demethylation, while anaerobic microorganisms, such as sulfate-reducing bacteria (SRB), methanogens, and iron-reducing bacteria (IRB), participate in oxidative demethylation (Du et al., 2019). Specifically in sediments, MMHg demethylation is predominantly considered a biotic process, with microorganisms like SRB and methanogens playing primary roles (Du et al., 2019).

The efficiency of demethylation is influenced by many factors such as pH, temperature, redox potential and organic matter concentrations and characterization (Compeau & Bartha, 1984; Li & Cai, 2013; Du et al., 2019), with demethylation being favoured by aerobic and high salinity conditions (Compeau & Bartha, 1984) and hindered at lower pH (Ramlal et al., 1985).

The Wabigoon River is known for severe Hg contamination caused by a chlor-alkali facility in Dryden operating in the 1960s, with a total of more than 10 tonnes of Hg being

discharged into the local terrestrial and aquatic environments (Parks, 1976). Although the commercial fishery has been stopped, to this day traditional foods, including local fish, continue to be consumed by local communities, such as the First Nations of Asubpeeschoseewagong Netum Anishinabek (Grassy Narrows) and Wabaseemoong (White Dog) (Sellers, 2014). These Hg contaminated fish pose a severe health hazard to people that consume them as a large part of their diet. Methylmercury poisoning has many detrimental human health effects, primarily neurological which include sensory disturbances, constriction of visual fields, loss of muscle control, auditory disturbances, and tremors, among others. Besides that, in children, MMHg exposure in utero is associated with lower attention, and reduced memory and motor functions (Mergler et al., 2007). Even though the use and disposal of Hg in Dryden ceased in 1975 (Parks, 1976), to this day, the legacy Hg contamination continues to cause serious adverse health effects for the local communities (Philibert et al., 2022).

Although fish Hg concentrations from the Wabigoon River system have declined since the 1970s (Kinghorn et al., 2007; Neff et al., 2012), as of 2018 (McGovarin, 2020) Hg concentrations in walleye sampled at Wabigoon Rapids and Clay Lake still exceeded the Canadian guideline for commercially-sold fish (0.5 µg/g) (Health Canada, 2007). As of a consequence, further research is crucial to understand the deposition and transport of the historical Hg in this system, and more importantly, its methylation into MMHg that makes it available for bioaccumulation to this date. The objective of this work was to use isotopically enriched Hg and MMHg tracers to ascertain Hg methylation and MMHg demethylation potentials in several ecosystems across the Wabigoon River system. To our knowledge, methylation and demethylation potentials have never been measured in this river system,

making this a novel research project of significant importance for the research and local communities.

3.2. Methods

3.2.1. Study area

Water and sediment samples were collected at different locations along the Wabigoon River system including different types of ecosystems such as lakes, wetlands, and rivers. Coordinates for the locations can be found in Table 3.1 and are mapped in Figure B.1. Wabigoon Lake (WL) and a wetland next to it (WL WT) were deemed to be reference locations, as they were located upstream from the pollution source. Several locations were sampled downstream from the pollution source.

The Hydroelectric dam (HD) located just 5 km after the pollution source was selected as this location was suggested to provide favourable conditions for Hg conversion into MMHg (Pestana et al., 2019).

The Wabigoon Rapids (WR) located 62 km downstream from the pollution source, has previously been shown to have high Hg concentrations in sediments (McGovarin, 2020), being a potential source for distributing mercury throughout the Wabigoon River system.

A wetland near Wabigoon Rapids (WR WT), located 60 km downstream from the pollution source, is characterized by seasonal drying and flooding. Particularly the period immediately after inundation is often very productive in forming MMHg from stored Hg(II) (Eckley et al., 2017).

Clay Lake (CL) located 85 km downstream is a known sink of Hg. As recently as 2010, fish had the highest levels of mercury compared to other lakes in the Wabigoon system (Sellers,

2014), suggesting that Hg might be highly bioavailable at this lake and easily biomagnifies in fish. Water and sediment were collected at the Inflow (CL I) at 85 km and a sediment sample was collected in the deepest section of the west basin (CL W) at 92 km.

All water samples were collected in August 2021, except for the bottom waters of Wabigoon Lake (WL BOT) that was collected in July 2022. All sediment samples were collected in July 2022.

3.2.2. Sampling and field incubations

The top layer of bottom sediment samples from the lake locations was sampled using an Ekman style dredge and approximately 6 cm of surface sediment were collected into ziplock bags. Sediments from the riverine shoreline in riverine and wetland locations were collected by pushing 5 cm diameter PVC tubes by hand. The cores were transported to the field laboratory at ambient temperature and with overlying water to maintain *in situ* redox conditions as much as possible. Once in the field laboratory, the top 6 cm were homogenized, and river water was added to create a slurry. Around 20 g (wet weight) of slurry were subsampled into individual 250 mL glass beakers and spiked with either 1 or 3 µg of Hg enriched with ^{200}Hg and 10 ng or 30 ng of MM^{198}Hg (Trace Sciences International, see Table B.1 for isotope abundances) that was equilibrated with river water for several hours prior to the addition. Sediment samples from locations upstream of the pollution source were spiked with the lower amount (1 µg) of ^{200}Hg and (10 ng) MM^{198}Hg, increasing the total Hg concentration in sediment on average by 51 % and ambient MMHg concentrations between 4 to 228 % (Table B.2 and B.4). The enriched Hg spike (^{200}Hg) was added to the samples to track MM^{200}Hg formation while, MM^{198}Hg was added to track MM^{198}Hg degradation.

Sediment slurries were incubated at room temperature for 1 day. Slurries were subsampled after 4, 8, and 24-hours and frozen to stop the incubation. To account for abiotic methylation, one subsample was collected immediately after spike addition (t = 0 hrs). Additionally, a control sample was taken, where no spike was added.

Unfiltered surface water samples were collected using the clean hands/dirty hands protocol according to EPA method 1669 (EPA, 1996) into certified 250 mL fluorinated polyethylene wide mouth bottles (Brooks Rand Instruments; certified for < 0.4 ng/L Hg), except for the bottom water sample at the Wabigoon Lake where a peristaltic pump and a Teflon line was used for collection. Sample bottles from this location were over-filled and tightly caped to maintain anoxic conditions. All water samples were spiked in the field immediately after collection using an analytical syringe (Hamilton, 50 µL) with a ^{200}Hg isotope enriched spike that was equilibrated with river water for several hours prior to the injection. All water samples were spiked with the same amount of ^{200}Hg (4.5 ng), except for the bottom waters of Wabigoon Lake, where a lower amount of spike was added (2.5 ng), resulting on average in 100 % increase of the total Hg ambient concentration in water samples (Table B.3). Water samples were incubated at room temperature and individual incubations were stopped after 4, 8 and 24 hours using 2.5 mL of concentrated HCl and kept cold until transportation to the laboratory. Additionally, to account for abiotic methylation, a time-zero sample was obtained by adding HCl prior to spike addition as well as a control sample, where no spike was added.

Water samples were also spiked with MM^{198}Hg to determine MMHg demethylation potentials. However, obtained data were inconclusive, not showing any consistent trends over time.

3.2.3. Mercury species analysis

Total mercury (THg) in water and sediments was measured using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 8800). Prior to THg measurements approximately 200 mg of dried sediment were digested using a mixture of sulfuric/nitric acid (7:3 v/v) overnight at around 90 °C. For the water samples, around 20 mL of water was treated with a strong oxidant solution (150 µL of 0.2 N brominemonochloride – BrCl) overnight at room temperature to oxidize all forms of Hg into Hg^{2+}. An internal ^{199}Hg enriched standard solution (Trace Sciences International, see Table B.1 for isotopic abundances) was added to both water and sediment samples to correct for procedural recoveries.

To measure MMHg in both water and sediment samples, the sample preparation and measurement followed the EPA method 1630 (EPA, 1998). A water vapour distillation was used to separate MMHg from the sample matrices. Around 200 mg of homogenized dry sediment sample or 50 mL of unfiltered water sample were used for the distillation. $MM^{199}Hg$ synthesised from ^{199}HgO (Trace Sciences International, see Table B.1 for isotopic abundances) was added as an internal standard to correct for procedural recoveries. Prior to the distillation 500 µL of H_2SO_4 (9 M) and 200 µL of KCl (20 %) were added to all samples. The samples were distilled at 115 °C with a mercury free nitrogen gas flow of 60 mL/min until approximately 90 % of the sample was transferred to the receiving vessel (approximately 4 hours for water samples and 2 hours for sediment samples). Then 225 µL of sodium acetate buffer (2 M, pH = 4) and 30 µL of sodium tetraethylborate reagent (1 %) were added to each sample to ethylate all Hg species present, and the distillate was

measured using an automated methylmercury analyzer (Tekran® 2700) coupled to ICP-MS (Agilent 8800).

3.2.4. Calculation of methylation and demethylation potentials

Chromatographic data was collected from the ICP-MS and peak areas were used to calculate concentrations using matrix algebra (as described in Hintelmann and Ogrinc (2003)), where $MM^{202}Hg$ was used to represent the ambient MMHg concentrations.

The methylation potential is defined as the variation (in %) of increase of $MM^{200}Hg$ from the added ^{200}Hg spike over time. Since this depends highly on the concentration of THg added, the potentials were determined using the ratios of $MM^{200}Hg/^{200}Hg$ at each incubation time, as described in equation 3.1, where *ti* is time of incubation.

$$Methylation\ Potential\ (\%) = \frac{[MM^{200}Hg_{ti}]}{[^{200}Hg_{ti}]} \times 100 \tag{3.1}$$

To facilitate a more straightforward comparison of methylation activity with other systems, along with demethylation activity, a Hg methylation rate (*km* d^{-1}) was also calculated as described in equation 3.2.

$$km\ (d^{-1}) = \frac{[MM^{200}Hg_{24hr}] - [MM^{200}Hg_0]}{[^{200}Hg_{24hr}]\ t} \tag{3.2}$$

$MM^{198}Hg$ concentrations were plotted over time, with the assumption that demethylation follows an exponential decay (equation 3.3), where *kd* is the demethylation rate constant in d^{-1}.

$$[MM^{198}Hg] = [MM^{198}Hg]_0 \times e^{-kdt} \tag{3.3}$$

However, it is conceptually more intuitive to express the demethylation rate as the half-life of methylmercury in the sediment (equation 3.4)

$$Half\ life\ (days) = \frac{ln(2)}{kd} \tag{3.4}$$

3.2.5. Estimation of steady state MMHg levels

Specific Hg methylation (km d^{-1}) and MMHg demethylation rates (kd d^{-1}) were used to estimate steady state MMHg concentrations using equation 3.5.

$$[MMHg]_{Steady\ State} = \frac{km}{kd} \times [THg]_{ambient} \tag{3.5}$$

3.2.6. Complementary data

A multiprobe (YSI ProQuatro) with DO, temperature, conductivity, TDS, pH and salinity was used when sampling water samples (Table 3.4).

At each location a large volume (> 1 L) of water was collected and filtered using quartz filters (Whatman QMA - 2.2 µm) to determine the concentration of particulates. After filtration, filters were freeze dried and weighed (Table B.8).

To determine Dissolved Organic Carbon (DOC) concentrations in water, samples were filtered with a nylon syringe filter (0.45 µm) and analysed using a Shimadzu TOC-V_{CPH} instrument using NPOC analysis (Table 3.4).

Organic matter content (%) in sediments was measured as lost in ignition (LOI), by measuring mass loss after burning approximately 2 g of dried sediment at 500 °C for 4 hours (Table B.9).

3.2.7. QA/QC

Quality assurance was performed by analysis of IAEA-475 ($0.199 \pm 0.034 \times 10^{-3}$ mg/kg) marine sediment standard reference material for MMHg and SRM 1944 (3.4 ± 0.5 mg/kg) marine sediment standard reference material for THg. Concentrations of 0.154 ±

0.076×10^{-3} mg/kg (n=20) was obtained for IAEA-475 and 3.5 ± 0.4 mg/kg (n=4) for SRM 1944. The method detection limit for MMHg was 0.09 ng/g in sediments and 0.11 ng/L in water, based on 3 x standard deviation of the mean of the distillation blank. For THg concentrations the method detection limit was 0.04 ng/g in sediments and 0.10 ng/L in water, based on 3 x standard deviation of the mean of the digestion blank. For $MM^{200}Hg$, the instrument detection limit was 0.017 ng/L in water and 0.06 ng/g in sediments, resulting in a detection limit for MMHg conversion from the added spike of 0.19 % for water and 0.06 % for sediments. The instrument detection limit for $MM^{198}Hg$ was 0.04 ng/g.

3.3. Results & Discussion

3.3.1. Abiotic methylation

All locations, apart from the surface waters of Wabigoon Lake, exhibited instantaneous methylation immediately after the addition of the Hg spike (between 0.3 % and 1.9 % for water and 0.2 % and 0.4 % for sediments, Table 3.2), which reveals the ability to methylate mercury abiotically, without mediation by bacteria. Higher instantaneous MMHg conversion rates in water might suggest that abiotic Hg methylation could be relatively more important in the water column compared to sediments. However, due to the smaller pool of THg in water, this results in only 0.05 to 0.52 ng/L of MMHg being produced abiotically in the water column compared with 0.30 to 2.79 ng/g MMHg formed in sediments.

Larger organic components of dissolved organic matter such as fulvic and humic acids are reported to be the main cause of abiotic methylmercury formation (Celo et al., 2006). However, DOC did not correlate with abiotic methylation potentials found in the water of the Wabigoon River system ($R^2 = 0.0464$, $p = 0.643$, $n = 7$), neither did the concentration of

particulates in water (R^2 = 0.2602, p = 0.242, n = 7). However, we cannot rule out that the quality and type of aqueous organic matter varies along the river system and ultimately determines abiotic methylation.

A possible pathway of abiotic methylation in the Wabigoon River system is the reaction of Hg(II) with methylcobalamin coming from bacterial dead cells. This is supported by the fact that locations with higher abiotic methylation also show higher biotic methylation (R^2 = 0.6087, p = 0.038, n = 7). Proportionally higher instantaneous MMHg conversion in water than in sediments also points to a methycobalamin pathway since methycobalamin is known to be highly reactive in aqueous environments but not in sediments (Falter, 1999). In contrast, abiotic methylation potentials found in the sediments of the Wabigoon River system corelate with organic matter % (R^2 = 0.5865, p = 0.045, n = 7).

Even though abiotic methylation is thought to be largely aerobic (Ullrich et al., 2001), the anoxic waters at the bottom of Wabigoon Lake show one of the highest abiotic methylations (1.4%), indicating the existence of an anoxic abiotic pathway in this system.

3.3.2. Methylation potentials in water samples

The MMHg production over time (Figure 3.1) varied initially but stabilized after the 8 hr period, suggesting that the system approached a steady state, where MMHg is produced and eliminated at the same rate, resulting in a constant concentration. For this reason, we chose to compare the methylation potential after 24 hrs to obtain a better estimation of the net methylation capacity at each location.

It is of note that both biotic and abiotic methylations rates were below the detection limit in the surface water at Wabigoon Lake (WL TOP). This could be explained by the fact

that most surface waters are supersaturated in Hg^0 relative to the atmosphere, especially in summer, resulting in elemental Hg being readily lost from the aquatic environment at ambient temperatures, reducing the Hg(II) available for the synthesis of MMHg (Fitzgerald & Mason, 1996). In addition, methylating bacteria such as SRB are considered mesophilic anaerobes and may not be present in oxic environments (Widdel & Bak, 1992). As abiotic methylation in the Wabigoon River system may follow a methylcobalamin pathway from dead bacterial cells, the absence of methylating bacteria in oxic environments could also explain why abiotic methylation was not detected in surface waters of Wabigoon Lake. Another potential explanation for the absence of abiotic methylation could be that the quality of organic matter is very different in oxic and anoxic waters, impeding abiotic methylation in surface waters.

Comparing the % $MM^{200}Hg$ produced at the steady state (Table 3.3), the location that shows the highest methylation potential was the Hydroelectric dam, with 4.2 % of added ^{200}Hg being converted. The dam, functions as a man-made wetland, exhibiting stagnant waters. Additionally, the HD location is described to be replete with woody debris from the pulp and paper mill (German, 1969), which could lead to enhanced Hg methylation (Regnell et al., 2014). The stagnant water conditions from the dam combined with the existence of wood debris at this location may explain the high methylation potential found at this location.

Even though the Wabigoon Rapids is a fast-running water location it displays the second highest methylation potential of 3.7 % conversion. Since methylation potentials were found to be highly dependent on pH (R^2 = 0.8336, p = 0.0042, n = 7), with lower pH resulting in higher methylation potentials, this high value at the WR location may be a result of the

low pH (7.32, Table 3.4) at this location. Previous studies often found net methylation rates to be strongly dependent on pH, with lower pH values leading to an increase in the production of MMHg in freshwater environments (Miskimmin et al., 1992).

Water collected at the bottom of the Wabigoon Lake (WL BOT) showed an equally high methylation rate of 3.7 %. Since Hg methylation in freshwater sediments and water is known to be significantly higher under anaerobic conditions (Olson & Cooper, 1976; Regnell & Tunlid, 1991), this high value is likely a result of the low oxygen levels (0.16 mg/L) found at the bottom of the lake (Table 3.4), compared with 8.17 mg/L at the surface.

In general, steady state methylation potentials in water correlate well with the concentration of particulates (R^2 = 0.9338, p = 0.0016, n = 6), which could indicate that methylation in the water column may have a big abiotic component and be linked to particles (Ullrich et al., 2001). On the other hand, considering that some microbes are known to grow on suspended solids (that could be sediment particles, plants or minerals) in the water column (Konhauser, 2007), it could indicate that Hg methylation in the water column is a result of microbial activity in suspended particles.

Specific methylation rates measured in waters of the Wabigoon River system (between 0.001 and 0.04, average of 0.02 ± 0.01 d^{-1}; Table 3.3) are usually higher than rates observed in anoxic waters of other Canadian lakes such as Plastic Lake (*km* = 0.009 ± 0.001 d^{-1}) and Experimental Lakes Area (ELA) Lake L443 (*km* = 0.016 ± 0.006 d^{-1}), previously determined at the same time of the year (July – August), but lower compared to rates documented for ELA Lake L658 (*km* = 0.09 ± 0.04 d^{-1}) in July (Eckley & Hintelmann, 2006). There (limited) data may indicate that methylation potentials are generally higher in Wabigoon River waters compared to waters of other freshwater environments.

3.3.3. Methylation potentials in sediment samples

In contrast to water samples, the MMHg production in sediments did not seem to stabilize after the 8 hour period (Figure 3.2), indicating that in sediments it may take longer to reach a steady state. Although stabilization was not always attained by the end of the 24 hours incubation period, a trend toward steady state was discernible. Besides, natural *in situ* conditions may only be maintained in incubations for short periods (Eckley & Hintelmann, 2006), prompting us to select the 24 hour mark to compare locations.

The locations with the highest ability to produce MMHg were the Hydroelectric dam, with 4.4 % of Hg being converted, the west basin of Clay Lake (3.8 %) and Wabigoon Lake (3. 6%) (Table 3.5). Once again, the stagnant water conditions from the dam combined with the existence of wood debris at this location may explain the high methylation potential found at this location, while the high methylation rates found at the bottom of both lakes can be explained by the hypoxic environment (low levels of oxygen), which is known to enhance the net production of MMHg (Olson & Cooper, 1976). Water at the bottom of Clay Lake was found to have 2.2 mg/L of dissolved oxygen (DO) in late July, which is consistent with the oxygen trends reported previously (Rudd et al., 2021), where oxygen was progressively depleted in Clay Lake's bottom waters during the summer and fall, reaching lowest values in later September (0.0 mg/L DO) below 15 m. This seasonal change in DO concentrations agrees with other boreal lakes where anoxia was found to emerge in early summer, increasing until fall turnover (Zdorovennova et al., 2016). As Hg methylation is also known to experience seasonal changes consistent with the ascent of the hypoxic zone up the water column (Eckley & Hintelmann, 2006), this suggests that Hg methylation in Clay Lake will also

change seasonally, increasing over the summer months, due to the increase of the anoxic layer, reaching a peak in September.

While the organic matter content did not correlate well with Hg methylation potentials found in sediments ($R^2 = 0.1273$, $p = 0.4322$, $n = 7$), we cannot dismiss the possibility that the quality and type of organic matter is driving the different MMHg conversions observed, given that the characterization of organic matter plays a critical role in determining Hg(II) availability for methylation (Abdelhafiz et al., 2023).

Specific methylation rates at the Wabigoon River (WR, $km = 0.018\ d^{-1}$; Table 3.8) are substantially higher than observed rates in other studies, reporting for example $km = 0.000105$ and $0.000461\ d^{-1}$ for Hudson and Patuxent Rivers, respectively (Heyes et al., 2006). This may point to elevated methylation activity in Wabigoon riverbed sediments compared to other riverbed locations. As well, methylation rates in lake sediments ($km = 0.035 \pm 0.008\ d^{-1}$) are more than double than methylation rates reported in sediments of other lakes experiencing anoxic conditions (e.g., Ranger Lake, $km = 0.012\ d^{-1}$; Lake Vernon, $km = 0.016\ d^{-1}$; Hintelmann et al., 2000), indicating a higher level of Hg methylation in the sediments of the Wabigoon River System's lakes compared to other stratified lakes. However, methylation rates at wetland locations sampled ($km = 0.0235 \pm 0.0001\ d^{-1}$) were similar to values reported for littoral sediments of a Hg impacted reservoir (THg in littoral sediments = 181.4 ng/g) in the summer ($km = 0.0240 \pm 0.005\ d^{-1}$; Millard et al., 2023), suggesting that Hg methylation activity in wetlands along the Wabigoon River system is comparable to that observed in other impacted wetlands. Nonetheless, owing to higher THg concentrations at the Wabigoon River (786.8 vs 181.4 ng/g), equivalent methylation rates can lead to a greater amount of MMHg being produced at the Wabigoon River.

In general, elevated methylation rates observed in riverbed and lake sediments, in comparison to similar ecosystems elsewhere, imply a greater capacity for MMHg production in the Wabigoon River system.

3.3.4. Demethylation potentials in sediment samples

$MM^{198}Hg$ concentrations were plotted over time (Figure 3.3) and an exponential fit was applied resulting in demethylation rate constants (*kd*) of 0.005 to 0.5 d^{-1}, that correspond to half-lives of 144 and 1.5 days, respectively (Table 3.7). It is important to note that demethylation did not show a perfect exponential decay over time at all locations, with both wetland locations showing anormal $MM^{198}Hg$ values at the 4 hours mark. Knowing that wetlands are very heterogeneous (Kim et al., 2015) and that bacteria organize themselves in clusters (Konhauser, 2007), possibly the subsampling resulted in sediment sections with different consortia of demethylation bacteria, resulting in odd results at the 4 hour period for these locations.

Clay Lake showed almost no demethylation activity, with both Inflow and West locations only demethylating 1 and 2 % of $MM^{198}Hg$ per day, respectively. Methylmercury in Clay Lake may stay in the system for up to 144 days (half-life), possibly accelerating accumulation of MMHg in biota. Apart from Clay Lake, all the other sampled locations seem to be more active demethylation sites with half-lives between 1.4 to 4.8 days. The wetland at Wabigoon Rapids showed the highest demethylation activity, with a half-life of only 33 hours, meaning that half of the MMHg present that this location would degrade shortly after 1 day. Apart from both lakes and the wetland at Wabigoon Rapids, all the other locations had similar demethylation rates with 24 to 28 % of $MM^{118}Hg$ being degraded after 1 day. Both Wabigoon and Clay lakes showed higher half-lives of 4.8 and 9.6 days, respectively. The

bottom of Wabigoon Lake and Clay Lake's west basin are both stratified zones exhibiting anoxic waters in the summer with 0.16 and 2.2 mg/L of dissolved oxygen, respectively, at the time of collection (July 2022). The presence of an anoxic environment could explain the low demethylation found in these locations, since the degradation of MMHg is favoured by aerobic conditions (Korthals & Winfrey, 1987). Nonetheless, anaerobic organisms such as SRB, methanogens and IRB are also important demethylators (Du et al., 2019), explaining the occurrence, however low, of demethylation at the bottom of both lakes.

Higher demethylation rates did not correlate with lower ambient methylmercury concentrations (R^2 = 0.066, p = 0.577, n = 7), suggesting that methylation rates may still be the more important controlling factor of ambient mercury concentrations. However, the sampling took place during the summer, where Hg methylation is known to be enhanced due to higher temperatures (Bubb et al., 1993), while demethylation is favoured by lower temperatures (Bodaly et al., 1993), leaving open the possibility that demethylation could become a more important factor for net MMHg concentrations in the Wabigoon River during winter months.

Ideally, demethylation assays would only add low concentrations of MMHg to avoid a disturbance of environmental conditions. However, spike additions must also be high enough to allow safe detection of the MMHg spike against the ambient background. Considering the ambient methylmercury concentrations expected at the sampling locations, sediment samples received on average 1.1 ng/g of $MM^{198}Hg$ spike, which increased the ambient concentrations on an average by 103 % (Table B.4). Although a doubling of ambient concentrations might have affected the estimation of demethylation rates, older studies (Ramlal et al., 1986) found that spike concentrations of up to 44 µg/g did not affect the rate

of methylmercury degradation, leading us to assume that the present demethylation rates are a good indicator of natural demethylation potentials in the Wabigoon River.

The specific demethylation rates observed at the Wabigoon River (WR, kd = 0.312 d^{-1}) were approximately half of those reported in sediments of the Hudson River (kd = 0.66 d^{-1}; Heyes et al., 2006), indicating a higher persistence of MMHg in riverbed sediments in the Wabigoon River compared with other riverbed locations. Moreover, demethylation rates in anoxic lake sediments (kd = 0.11 ± 0.04 d^{-1}) were lower than those reported in sediments of other lakes experiencing anoxic conditions (Ranger Lake, kd = 0.417 d^{-1}; Lake Vernon, kd = 0.528 d^{-1}; Hintelmann et al., 2000), suggesting reduced degradation of MMHg in the sediments of the Wabigoon River System's lakes compared to other stratified lakes. Demethylation rates at sampled wetland locations (kd = 0.43 ± 0.07 d^{-1}) were comparable but still lower than values reported for littoral sediments of a Hg impacted reservoir in the summer (kd = 0.526 ± 0.158 d^{-1}; Millard et al., 2023). Notably, the demethylation rate observed in Clay Lake Inflow (kd = 0.005 d^{-1}) is remarkably low, being still twice as low as the lowest demethylation rate recorded in sediments of an Italian Lagoon (kd ~ 0.01 d^{-1}; Hines et al., 2012). The generally lower demethylation rates observed in Wabigoon River sediments, in contrast to similar ecosystems elsewhere, imply higher preservation of MMHg in the Wabigoon River system.

3.3.5. Estimation of MMHg production amounts

The methylation potentials reveal the ability for certain locations to produce MMHg. However, to establish which locations present the highest capacity for distributing methylmercury throughout the Wabigoon River system we need to account not only for the ability to produce MMHg but also for the amount of available Hg in that location. For an

initial approximation, we considered both the methylation potential and the concentrations of total ambient Hg present at the different locations (Table 3.3 and 3.5).

Even though water at the bottom of the Wabigoon Lake showed a high methylation potential (3.7 %), this location had low concentrations of THg (3.67 ng/L), resulting in a lower potential for MMHg production at this location. The Hydroelectric dam displayed not only the highest methylation rate (4.2 %), but also relatively high concentrations of THg (46.4 ng/L) in water, being able to potentially produce 1.93 ng of MMHg per liter/day. At the same time, despite having a low methylation rate (2.2 %), Clay Lake had a high concentration of THg (122 ng/L), resulting in a potential production of 2.70 ng of MMHg per liter/day in superficial waters of this lake.

Similar, to the water column, the Hydroelectric dam location showed high methylation potential in sediments (4.4 %), as well as high concentrations of THg (651.5 ng/g), resulting in a potential production of 28.6 ng of MMHg per gram/day. Despite having a low methylation rate (2.4 %), sediments at the wetland near Wabigoon Rapids had a high concentration of THg (786.8 ng/g), resulting in possibly 19.3 ng of MMHg being produced per gram/day. Also noteworthy are sediments from the west basin of Clay Lake, having a methylation potential of 3.8 % and concentration of THg of 337.1 ng/g, which could produce 12.7 ng of MMHg per gram/day.

3.3.6. Comparison with MMHg ambient levels

It is noteworthy that the estimation of MMHg production per day was consistently lower than the ambient MMHg concentrations in water (Table 3.3), except for the Hydroelectric dam where the estimation of MMHg produced in both water and sediments was higher than ambient MMHg. This points to the Hydroelectric dam being a MMHg

producer, but not a sink, suggesting that most of MMHg produced at the Hydroelectric dam may be transported to locations downstream, explaining the increase of ambient MMHg in water over distance along the Wabigoon River system. It also suggests that the *in situ* methylation alone does not explain all of the ambient concentrations in water found downstream of the Hydroelectric dam.

In sediment samples, the estimation of MMHg produced per day is consistently higher than measured ambient MMHg levels (Table 3.5). This difference may be explained by the fact that Hg^{2+} added as a spike in incubation experiments is generally better available than ambient inorganic Hg species. For example, Hg^{2+} adsorbed to solids is less bioavailable for methylation reactions compared with newly added spike Hg^{2+} (Li & Cai, 2013). However, the ambient MMHg concentration at Wabigoon Rapids is higher than estimated by MMHg production rates. This may suggest that Wabigoon Rapids is a sink for MMHg, meaning that MMHg produced upstream (such as at the Hydroelectric dam) accumulates at WR. Interestingly, MMHg concentrations decreased downstream of WR, which may indicate that MMHg concentrations at Clay Lake are mainly controlled by *in situ* production at the bottom of the lake, rather than imported from upstream locations.

3.3.7. Estimation of steady state MMHg concentrations in sediments using specific methylation and demethylation rates

Applying the obtained specific Hg methylating and MMHg demethylation rates, we would predict steady-state concentrations of ambient methylmercury in the Wabigoon River sediments to range from 4.83 to 201 ng/g (Table 3.8). However, the measured methylmercury ambient concentrations plateaued between 0.16 and 34.1 ng/g, with only

the Wabigoon Rapids location showing comparable ambient MMHg concentrations to steady state predictions (34.1 ng/g vs 32.1 ng/g).

As demethylation rates using enriched MMHg tracers have demonstrated reliability as indicators of natural demethylation (Ramlal et al., 1986; Hintelmann et al., 2000), the observed discrepancy is likely attributed to over estimations in methylation rates, due to higher availability of the Hg^{2+} tracer compared to natural Hg^{2+} (Li & Cai, 2013). Furthermore, it is possible that the system never reaches a steady state. It is conceivable that processes other than demethylation, such as erosion, advection, and diffusion, are diminishing MMHg levels, preventing accumulation to the theoretically possible concentrations. On the other hand, the observation that the Wabigoon Rapids location showed comparable ambient MMHg concentrations to steady state predictions suggests that such processes may have a lower contribution at this location. Instead, MMHg levels at this location are likely predominantly controlled by methylation and demethylation processes.

3.4. Conclusions

Overall, all downstream locations from the historic pollution source at Dryden investigated in this study are capable of methylmercury production and export (Figure 3.4), with the Hydroelectric dam, the wetland near Wabigoon Rapids and Clay Lake having the highest production potentials. The Hydroelectric dam has the most favourable conditions for Hg methylation in both water and sediments, being able to produce 1.93 ng/L and 28.6 ng/g of MMHg, respectively, per day. Being a sink of Hg, Clay Lake is able to produce 2.70 ng of MMHg in its surface waters and 12.7 ng/g in its bottom sediments. Additionally, the wetland at Wabigoon Rapids is able to produce 0.66 ng/g of MMHg in water and 19.3 ng/g in sediments. These three locations and similar locations along the Wabigoon River have the

potential for formation and downstream distribution of methylmercury and should be paid special attention in future remediation actions.

It's important to note that the present assessment likely overestimate MMHg production in the Wabigoon River, especially in sediments. Firstly, these rates reflect MMHg production during the summer, when Hg methylation is often at a peak due to higher rates of growth and metabolic activity (Korthals & Winfrey, 1987). Hence, seasonal rates may vary, and average annual rates are likely lower than those measured here. Secondly, even after equilibration, the Hg spike is often more bioavailable for methylation reaction compared to natural, ambient Hg (Hintelmann et al., 2000). For example, not all of the ambient THg present is available for methylation, because up to 80 % of the THg between Dryden and Clay Lake is bound to inorganic particles (Rudd et al., 2021), which would reduce its bioavailability (Farrell et al., 1998). Nevertheless, on a relative scale the assessed methylation potentials are a good indicator of potential MMHg production along the Wabigoon River system.

The calculated half-life for methylmercury in sediments of riverbed and wetland locations was 2.1 days, suggesting a rapid turnover and low persistence of methylmercury in sediments of the Wabigoon River. However, both lakes showed high half-lives for methylmercury in sediments, between 4.8 to 144 days, suggesting that lake sediments may be more prone to methylmercury accumulation. Nevertheless, the consistently lower demethylation rates observed in Wabigoon River sediments, compared to rates reported in sediments of similar ecosystems, suggest a heightened preservation of MMHg in the Wabigoon River system. In particular, the inflow of Clay Lake showed a very low

demethylation rate, with methylmercury being able to stay in the system for up to 144 days, posing a enhanced potential for MMHg accumulation in biota.

It is crucial to highlight that the present assessment sampled a limited number of locations along the Wabigoon River system. We cannot rule out the possibility that other locations not sampled may have equal or even higher methylation capacities, thereby also contributing to the production and transport of MMHg in the Wabigoon River system.

3.5. Figures

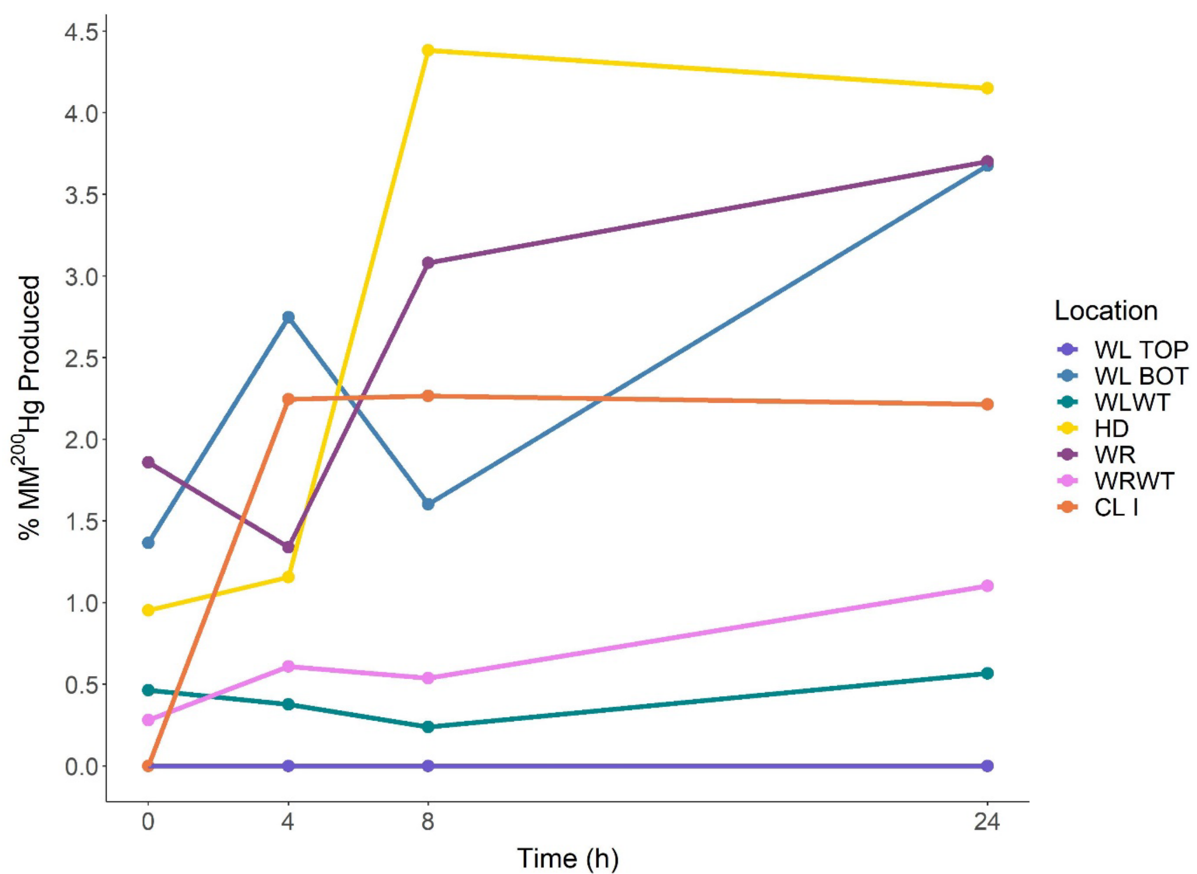

Figure 3.1 : % MM^{200}Hg produced from the added spike over time in water samples collected at different locations across the Wabigoon River System. Full data can be found in the Appendix (Table B.5).

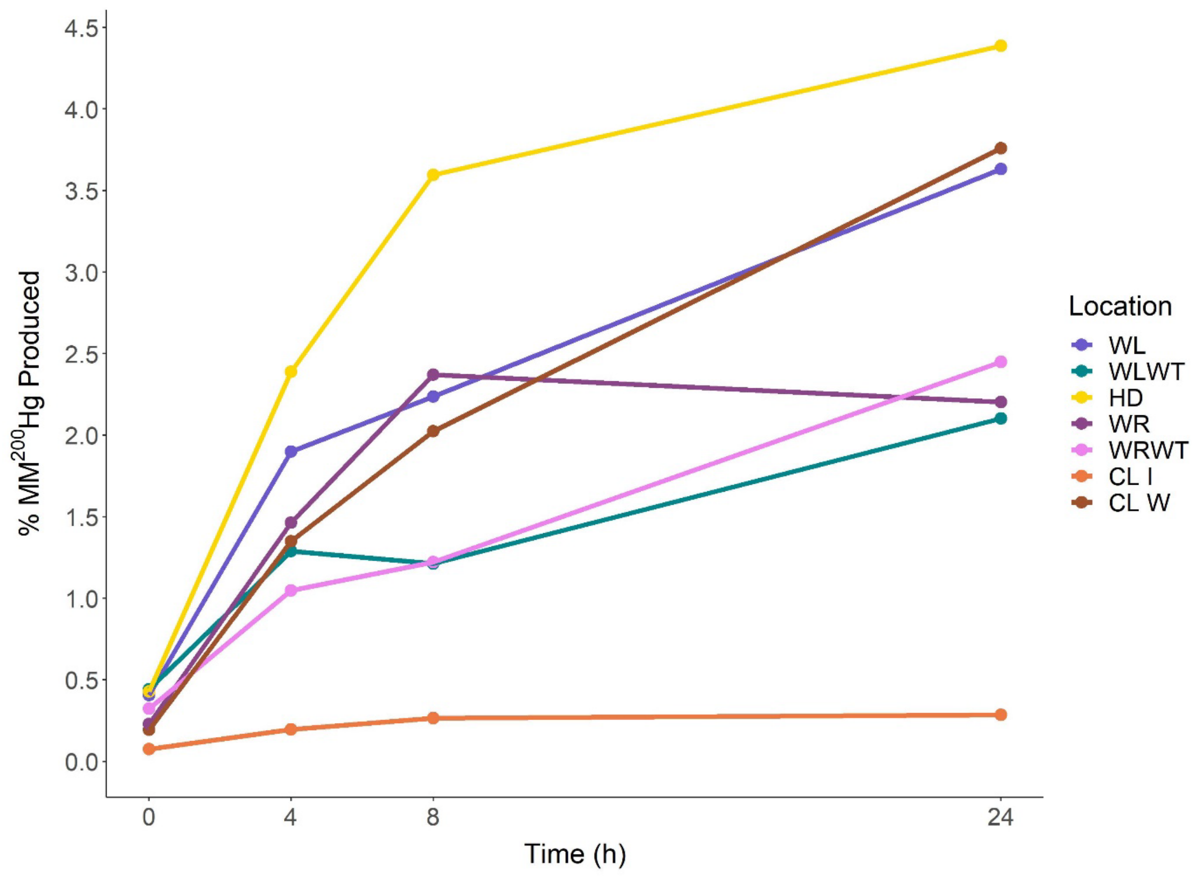

Figure 3.2 : % MM^{200}Hg produced from the added spike over time in sediment samples collected at different locations across the Wabigoon River System. Full data can be found in the Appendix (Table B.6).

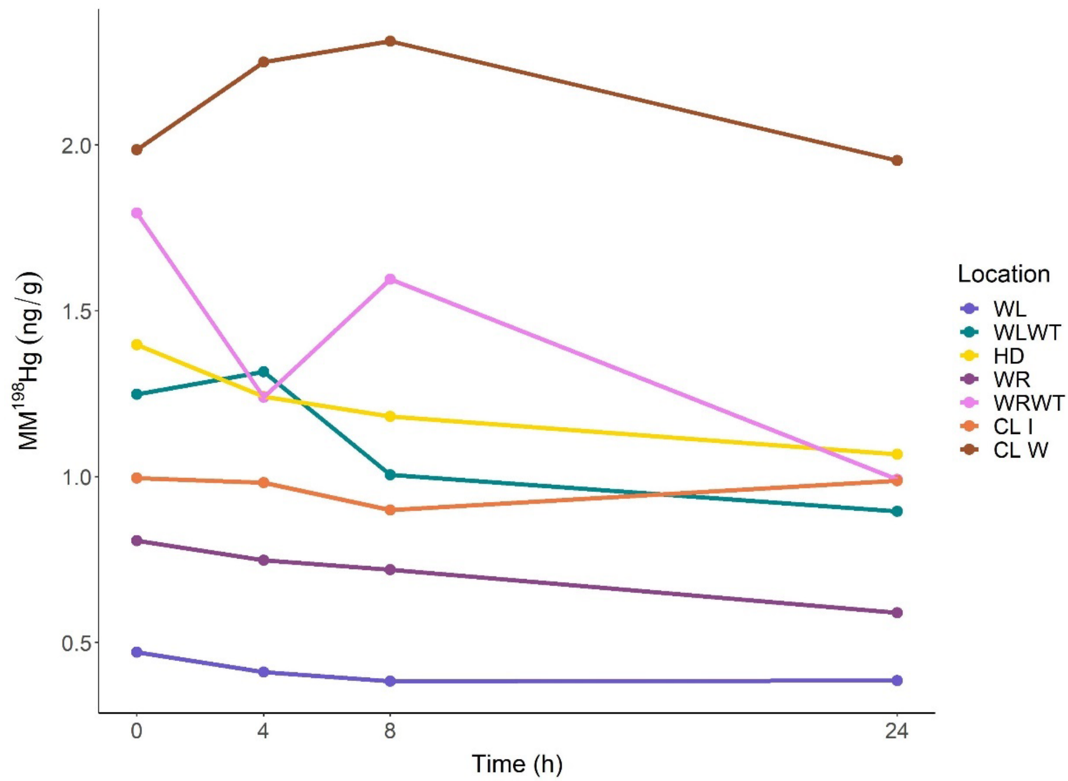

Figure 3.3 : $MM^{198}Hg$ (ng/g) over time in sediment samples collected at different locations across the Wabigoon River System. Full data can be found in Supplements (Table B.7).

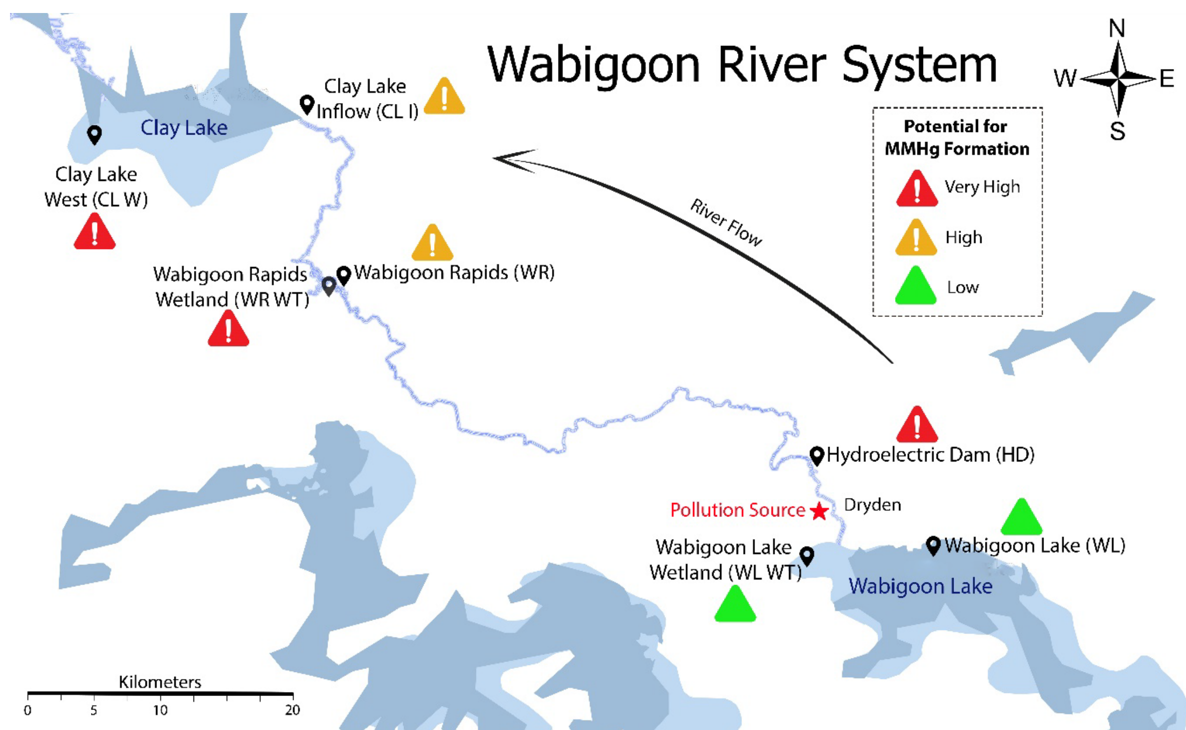

Figure 3.4 : Comparison of MMHg formation potential for various locations along the Wabigoon River system that were investigated in this study.

3.6. Tables

Table 3.1 : Coordinates for water and sediment sampling locations.

Location	Sample	Latitude	Longitude
Wabigoon Lake TOP	water, sediment	49°41'57.44" N	92°47'26.30" W
Wabigoon Lake BOT	water	49°45'14.18" N	92°44'24.50" W
Wabigoon Lake Wetland	water	49°45'57.31" N	92°53'21.37" W
	sediment	49°45'54.32" N	92°53'15.25" W
Hydroelectric Dam	water	49°48'56.23" N	92°52'35.97" W
	sediment	49°48'54.50" N	92°52'45.30" W
Wabigoon Rapids	water	49°55'33.96" N	93°21'08.64" W
	sediment	49°55'34.10" N	93°21'09.54" W
Wabigoon Rapids Wetland	water	49°55'34.14" N	93°21'01.47" W
	sediment	49°55'34.06" N	93°26'06.13" W
Clay Lake Inflow	water	50°03'07.09" N	93°25'03.68" W
	sediment	50°03'07.09" N	93°25'04.29" W
Clay Lake West	sediment	50°03'10.65" N	93°32'54.06" W

Table 3.2 : Abiotic methylation rates as % ^{200}Hg converted and amounts of MMHg produced instantaneously from ambient Hg in water and sediments at different locations along the Wabigoon River System

Location	**Water**		**Sediments**	
	%MM^{200}Hg	**MMHg produced (ng/L)**	**%MM^{200}Hg**	**MMHg produced (ng/g)**
WL TOP	<DL	n.d.		
WL BOT	1.4	0.05	0.4	0.30
WLWT	0.5	0.03	0.4	0.33
HD	1.0	0.44	0.4	2.79
WR	1.9	0.52	0.2	1.26
WRWT	0.3	0.17	0.3	2.54
CL I	<DL	n.d.	<DL	n.d.
CL W			0.2	0.65

Table 3.3 : MMHg production rates (% $MM^{200}Hg$), specific Hg methylation rates (km d^{-1}), ambient THg concentrations (ng/L), amounts of MMHg produced from ambient Hg (ng/L per day) and ambient MMHg concentrations (ng/L) in water samples at different locations along the Wabigoon River System.

Location	% $MM^{200}Hg$ produced	*km* (d^{-1})	Ambient THg (ng/L)	MMHg produced per day (ng/L)	Ambient MMHg (ng/L)
WL TOP	<DL	0.001	3.60	n.d.	0.21
WL BOT	3.7	0.04	3.67	0.13	0.31
WLWT	0.6	0.001	6.41	0.04	0.11
HD	4.2	0.03	46.4	1.93	0.96
WR	3.7	0.02	28.1	1.04	2.51
WRWT	1.1	0.009	60.3	0.66	3.62
CL I	2.2	0.02	122	2.70	4.70

Table 3.4 : Complementary data for water samples including Temperature, Dissolved Oxygen, pH, Salinity, Dissolved Organic Carbon and particulates.

Location	T (ºC)	DO (mg/L)	pH	Sal (ppt)	DOC (mg/L)	Particulates (g/L)
WL TOP	n.d.	8.17	7.95	0.05	10.58	0.07
WL BOT	17.7	0.16	7.48	0.05	10.84	0.04
WLWT	22.9	9.38	8.22	0.05	12.30	1.59
HD	22.9	5.74	7.34	0.11	12.75	0.15
WR	22.1	6.27	7.32	0.10	11.34	0.11
WRWT	21.8	12.5	7.74	0.09	14.45	1.01
CL I	22.1	7.72	7.62	0.09	11.43	0.88

Table 3.5 : MMHg production rates (% $MM^{200}Hg$), ambient THg concentrations (ng/g), amounts of MMHg produced from ambient Hg (ng/g per day) and MMHg ambient concentrations (ng/g) in sediment samples at different locations along the Wabigoon River System.

Location	% $MM^{200}Hg$ produced	Ambient THg (ng/g)	MMHg produced per day (ng/g)	Ambient MMHg (ng/g)
WL BOT	3.6	74.0	2.69	0.16
WLWT	2.1	74.3	1.56	1.46
HD	4.4	651	28.6	4.61
WR	2.2	547	12.1	34.1
WRWT	2.4	787	19.3	9.01
CL I	0.3	365	1.04	0.66
CL W	3.8	337	12.7	0.93

Table 3.6 : Complementary data for sediment samples: Organic Matter calculated by LOI and water content (%).

Location	OM %
WL	8
WLWT	28
HD	25
WR	11
WRWT	17
CL I	3
CL W	10

Table 3.7 : % demethylation per day, constant of demethylation (kd) and half-time in hours and days for the different sediment samples from several locations along the Wabigoon River.

Location	% demethylation per day	*kd* (d^{-1})	half-life (hours)	half-life (days)
WL	18%	0.144	116	4.8
WLWT	28%	0.360	46	1.9
HD	24%	0.240	69	2.9
WR	27%	0.312	53	2.2
WRWT	45%	0.504	33	1.4
CL I	1%	0.005	3466	144
CL W	2%	0.072	231	9.6

Table 3.8 : Specific methylation (km) and demethylation (kd) rates, ratio of rates (km/kd), estimation of steady state MMHg concentrations (ng/g) and ambient MMHg concentrations (ng/g) at different sediment samples from several locations along the Wabigoon River.

Location	*km* (d^{-1})	*kd* (d^{-1})	*km/kd*	Steady state MMHg ambient estimation (ng/g)	MMHg ambient (ng/g)
WL	0.028	0.144	0.19	14.3	0.16
WLWT	0.023	0.360	0.06	4.83	1.46
HD	0.033	0.240	0.14	89.0	4.61
WR	0.018	0.312	0.06	32.1	34.1
WRWL	0.024	0.504	0.05	36.9	9.01
CL I	0.002	0.005	0.40	145	0.66
CL W	0.043	0.072	0.60	201	0.93

Acknowledgements

This research was supported by a PhD Research Studentship (SFRH/BD/144532/2019) to BB, funded by the Portuguese Foundation for Science and Technology (FCT) as well as an NSERC Discovery grant to HH. The Trent University Water Quality Centre is acknowledged for the use of the ICP-MS equipment to perform the mercury analysis; the IISD Experimental Lakes Area for accommodation and use of a field lab during the 2022 field season; Tyler Roy for the DOC analysis; and Andrew Williams and Adelheid Willsie for assistance in field sampling.

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Chapter 4: Wet and dry cycle simulation and influence on Hg methylation

Abstract

Understanding the environmental variables influencing methylmercury (MMHg) production, and hence the bioavailability of mercury (Hg) to fish, is key to identifying strategies that can be used to reduce MMHg levels in fish. Seasonal flooding is one of the variables that is deemed to stimulate MMHg production, most likely due to remobilization of inorganic mercury and sulfate during flooding. The Wabigoon River, located in the northwest Ontario, is known for historical mercury (Hg) contamination, showing elevated concentrations of both inorganic and organic mercury in its system. Knowing that the Wabigoon River system experiences seasonal water flow and level variations and that the intensification of the hydrological cycle is predicted to increase under climatic change, this study aimed to examine the influence of wetting and drying cycles on Hg methylation on riverbed and wetland locations in the Wabigoon River through a laboratory simulation. The wetting and drying cycle resulted in a decreasing trend of % MMHg over time, with an average decrease of 70 ± 24 % during the 6 day experiment. Results from the preliminary laboratory simulation suggested that drying may also be an important factor in controlling MMHg levels and that riverbank locations in the Wabigoon River are more susceptible to increased Hg methylation after flooding.

Keywords: *Mercury; Methylmercury; wetting cycle, Methylation variation*

4.1. Introduction

Mercury (Hg) is a toxic heavy metal, where its toxicity is highly dependent on its speciation, with methylmercury (MMHg) being the most toxic form due to its accumulation in the food chain. The determining factor of mercury concentration in aquatic biota is the MMHg concentration in water and sediments, which is controlled by methylation and demethylation processes (Morel et al., 1998). Understanding the variables influencing MMHg production, and hence the bioavailability of Hg to fish, is key to identifying strategies that can be used to reduce MMHg levels in fish.

Seasonal variations in MMHg production generally have been attributed to temperature effects, where higher temperatures enhance methylation, resulting in a peak in Hg methylation rates in aquatic systems during the summer months (Korthals & Winfrey, 1987; Bubb et al., 1993). Additionally, seasonal variations in MMHg concentrations are also strongly linked to changes in redox state, with MMHg levels in hypolimnetic waters of seasonally stratified lakes increasing during summer stratification and decreasing following fall turnover (Eckley & Hintelmann, 2006). While seasonal variations in MMHg production appear to be mainly related to temperature and redox effects, as well as seasonal changes in productivity and hence nutrient availability (Ullrich et al., 2001), seasonal MMHg variation has also been attributed to seasonal flooding.

The influence of wetting and drying cycles on Hg Methylation was identified in early studies (Bodaly et al., 1984) where high mercury levels were found after inundation of lakes concurrent with an increase in fish mercury concentrations. Later studies (Hecky et al., 1991) also concluded, using indirect data, that there was a large increase in net methylation following flooding by reservoir development in northern Manitoba. More recently, Eckley et

al. (2017) found that areas experiencing seasonal variations in water levels have substantially higher MMHg concentrations and Hg methylation rates compared to permanently inundated areas and that the elevated values were mostly driven by increased partitioning of THg into the porewater-phase during flooding events, raising its bioavailability for methylating organisms. Furthermore, Coleman Wasik et al. (2015) showed that flooding increases the concentration of MMHg by both stimulating its release from sediments and *in situ* production, where drought-induced sulfate was found to be a determining factor in increased MMHg production in a sulfate-impacted experimental peatland.

It is thought that the flooding of vegetation and soils induces oxidative releases of absorbed inorganic Hg (Ullrich et al., 2001), making newly inundated soils short-term sources of mercury to downstream systems, where mercury is now available to be methylated. In addition, inundating a terrain causes the flooded organic carbon in soils and plants to decompose, releasing large amounts of organic matter and nutrients that stimulate microbial methylation activity (St.Louis et al., 2004). Furthermore, wetting cycles can also increase sulfate release, making it available to stimulate in-situ SRB activity and Hg methylation. Subsequent sulfate export to downstream aquatic systems (such as lakes and wetlands) (Coleman Wasik et al., 2015), could potentially increase MMHg not only in flooded areas, but also in undisturbed downstream locations.

The Wabigoon River, located in northwestern Ontario, is known for its historical mercury contamination (Parks, 1976). Elevated present-day concentrations of both inorganic and organic mercury in the system are thought to be a result of remobilization of legacy inorganic mercury from riverbank erosion that stimulates methylmercury production (Rudd et al., 2021). Consequently, further research is crucial to understand the variables

influencing MMHg production in order to identify mitigation strategies to reduce MMHg levels in fish in the area.

Even though the flow of the Wabigoon River between Dryden and Clay Lake is controlled by dams at the outflows of Wabigoon and Eagle Lakes (German, 1969), historical hydrometric data from the Water Survey of Canada (site 05QD006) (Water Survey of Canada, 2023), show seasonal flow and level changes at the Wabigoon River near Quibell, 5 km downstream from the Wabigoon Rapids (Figure C.1). These temporal data show that the Wabigoon River experiences seasonal flooding in the summer months following snow melt, with peak river flow and level occurring in June and July every year. Knowing that wetting and drying cycles stimulate Hg methylation, it was suggested that controlling the river flow would reduce downstream flooding that simulates MMHg production (Rudd et al., 2021), however no studies were performed to test the response of the Wabigoon River to flooding.

Given that the intensification of the hydrological cycle is predicted to increase under climatic change (Bapiri et al., 2010), understanding how Hg methylation in the Wabigoon River system will respond to wetting and drying cycles is extremely important. This study aimed to examine the influence of wetting and drying cycles on the Hg methylation on riverbed and wetland locations in the Wabigoon River through a laboratory simulation of wetting and drying cycles.

4.2. Methods

4.2.1. Sampling

Cores of 6 cm depth were collected at locations affected by seasonal flooding. One core was collected at the Wabigoon Rapids riverbed (WR) and another one at Wabigoon

Rapids wetland (WRWT) in July 2022 (Figure 4.1), using a hand corer (5 cm diameter PVC tubes). Sediment cores were collected in areas that were not inundated by water at the time of collection, about 0.5 m above the current water level. Sediment cores were kept covered to maintain ambient conditions until transportation to the laboratory. At each location, approximately 250 mL of river or wetland water was also collected to perform the wetting experiment.

4.2.2. Wetting and drying cycle simulation

The 6 cm dry cores were divided into two sections (top 3 cm and bottom 3 cm) to evaluate differences in sediment layers. Each 3 cm slice was then vertically separated into 3 equal segments of around 67 g (dw) each and different treatments were applied (as described in Figure 4.2). One segment to serve as a comparator, where no river water was added, hereafter referred as "dry", while the other two sediment segments went through the wetting cycle, hereafter referred to as "wet" samples. One of the wet segments received 3 µg of an enriched ^{200}Hg spike (referred as spiked, Trace Sciences International, see Table C.1 for isotopic abundances) to track the formation of $MM^{200}Hg$ from the added spike over time. No spike was added to the other wet segment (referred as non-spiked) and MMHg fluctuations were monitored by measuring ambient MMHg concentrations over time. The 3 subsamples were separated into 3 different glass beakers, and river water was added to "wet" sediments to create a slurry consisting on average of 45 ± 8 % of water. The dry samples were stirred with a spoon for a similar physical disturbance as the wet samples.

Dry and wet samples were allowed to air dry at room temperature over a period of 6 days, selected to approach dryness of the wet samples. This strategy was adopted to assess

the impact of the drying process to two levels of wetting (natural wet vs supersaturated conditions).

Each treatment was subsampled for Hg and MMHg analysis immediately after wetting with river water (T0), and after 1, 24, 72 hours and finally after 6 days. Although most studies on drying–rewetting effects are limited to only one cycle (Bapiri et al., 2010) this study applied a second wetting cycle after the initial 6-day drying period to monitor the effects of repeated drying–rewetting cycles. On day 6, river water was added again to the wet samples and subsamples were collected immediately and after 1 hour. The comparator samples were subsampled in parallel, but with no river water addition on T0 or day 6. The sampling times were chosen to assess the short-term temporal impacts of wetting on MMHg production.

4.2.3. Laboratory analysis

Total mercury (THg) was measured using an Inductively Coupled Plasma Mass Spectrometer -ICP-MS (Agilient 8800). Approximately 200 mg of dried sediment were digested using a 7:3 (v/v) mix of concentrated sulfuric and nitric acid overnight at 90 °C. 50 ng of ^{199}Hg (Trace Sciences International, see Table C.1 for isotopic abundances) was added as an internal standard to correct for procedural recoveries.

To measure methylmercury in the sediment samples, the sample preparation followed the EPA method 1630 (EPA, 1998). Briefly, a water vapour distillation was used to separate MMHg from the sample matrix. For the distillation, 500 µL of H_2SO_4 (9 M) and 200 µL of KCl (20 %) were added to 200 mg of homogenized dry sediment, where 100 pg of MM^{199}Hg, synthesised from ^{199}HgO (Trace Sciences International, see Table C.1 for isotopic abundances), was added as an internal standard to correct for procedural recoveries. The

samples were distilled at 115 °C with a mercury-free nitrogen gas flow of 60 mL/min until approximately 90% of the sample was transferred to the receiving vessel (approximately 2 hours). Following addition of 225 µL of sodium acetate buffer (2 M, pH = 4) and 30µL of sodium tetraethylborate reagent (1 %) to ethylate all Hg species present, the distillate was measured using an Automated Methylmercury Analyzer (Tekran® 2700) coupled to ICP-MS (Agilent 8800).

Chromatographic data were collected from the ICP-MS and peak areas were used to calculate concentrations using matrix algebra (as described in Hintelmann and Ogrinc (2003)), where $MM^{202}Hg$ was used to represent the ambient MMHg concentrations.

Water content (%) was measured as the mass loss after heating the samples at 60 °C for 48 hours (Table C.4).

Organic matter content (%) in sediments was measured as lost-on-ignition (LOI), by measuring mass loss after burning approximately 1 g of dried sediment at 500 °C for 4 hours (Table C.5).

4.2.4. QA/QC

Quality assurance was performed by analysing CRM IAEA-475 ($0.199 \pm 0.034 \times 10^{-3}$ mg/kg) marine sediment for MMHg and SRM 1944 (3.4 ± 0.5 mg/kg) marine sediment for THg. A concentration of $0.154 \pm 0.076 \times 10^{-3}$ mg/kg (n = 20) was obtained for IAEA-475 and 3.5 ± 0.4 mg/kg (n = 4) for SRM 1944. The method detection limit for MMHg and THg were 0.09 ng/g and 0.04 ng/g, respectively, based on 3 x standard deviations of the mean of the distillation or digestion blanks. The instrument detection limit for $MM^{200}Hg$ was 0.05 ng/g,

resulting in a detection limit for the conversion of the added ^{200}Hg spike to MM^{200}Hg of 0.07 %.

4.3. Results & Discussion

4.3.1. Water content variation

The dry samples were not entirely "dry" at the beginning of the experiment, reflecting the natural wetness of the dry cores at the time of collection (water content of 28 ± 7 %). By the end of the drying experiment the water content of dry samples decreased to 3 ± 1 %.

Wet samples were wetted to a water content on average of 45 ± 8 % at the beginning of the experiment. After a 6-day drying period, the "wet" samples dried down to around 5 ± 1 %. At day 6 the "wet" samples went through a second wetting cycle with water content increasing again to 45 ± 5 % and decreasing to 15 ± 8 % after 1hr. It's interesting to note that the second wetting cycle showed a faster drying process, with samples showing an average of just 15 ± 8 % after 1hr, compared to 49 ± 10 % during the 1st wetting after the same period. This difference in drying speeds may be explained by the lower amount of mass (20 g dw) on day 6, after subsequent subsampling throughout the experiment, that resulted in a higher surface area of the sediment being in contact with the air enhancing the drying speed.

Water content (%) over time can be found in the Appendix (Table C.4, Figure C.2 to C.5).

4.3.2. *Changes in MMHg concentrations*

Methylmercury concentrations did not show a discernible trend over time. In fact, the concentrations seem to stay flat during the 6-day period of the first wetting cycle (Figure 4.3). At the beginning of the wetting cycles the wet samples from the riverbed (WR) location showed around 10 ± 5 ng/g of MMHg, staying consistent around that value until the end of the wetting cycle (day 6), while the wet samples from the wetland location (WRWT) started at 3 ± 2 ng/g on T0 followed by a small decrease to 0.8 ± 0.7 ng/g on day 6. These results are comparable to results found elsewhere (Strickman & Mitchell, 2017), who observed that drying-rewetting cycles in surface-flow artificial wetlands did not enhance MMHg accumulation at the wetland margins. They theorised that their unexpected results were attributed to their experimental design, which did not result in a complete dryout, while other studies that link wetting and drying cycles with increased MMHg production have been based on extreme droughts or complete dryout (Feng et al., 2014; Coleman Wasik et al., 2015). Similarly, our samples did not start from complete dryness, having around 28 ± 7 % of water content before wetting, suggesting that indeed Hg methylation enhancement only occurs after a complete dryout.

After day 6, the wet samples were rewetted to a water content of 45 ± 5%, resulting in MMHg concentrations suddenly dropping to 0.3 ± 0.1 ng/g for both locations one hour after rewetting. The second wetting cycle seemed to influence Hg methylation negatively (as seen by ambient MMHg concentrations). This could be a consequence of the bioavailable Hg pool being completely depleted in the first wetting cycle, especially considering that a small amount of sediment was used to perform the experiment (around 67g dw per treatment), or that the higher surface area at day 6 prohibited the creation of an anoxic environments to

support Hg methylation by anaerobic bacteria, while anoxic conditions largely favour MMHg demethylation (Olson & Cooper, 1976).

The dry samples showed a similar stagnant trend, maintaining around 7 ± 5 ng/g of MMHg in the riverbed location during the first wetting cycle, while the wetland location showed 1.5 ± 0.1 ng/g on T0, with a slow decrease to 0.64 ± 0.02 ng/g on day 6 (Figure 4.3). These results suggest that the magnitude of the wetting (28 % vs 45 % of water content) does not influence MMHg concentrations and that the drying process is the determining factor regulating MMHg concentrations. However, when comparing average Hg concentrations of the wet samples with the concentration of the dry core at the time of collection, wet MMHg concentrations at riverbed and wetland locations, were 14 % and 60 % higher, respectively. Although not tested, if we assume that the steady state concentrations measured at time of sampling would remain unchanged if the dry sediment would be maintained at *in situ* moisture levels, then an increase in MMHg concentrations in both locations would be observed due to wetting.

4.3.3. Changes in the proportion of MMHg

The percentage of THg that is present in form of MMHg (% MMHg) is a good relative indicator of MMHg production rates in ecosystems (Gilmour et al., 1998) and has been used in several studies as a measure of a system's net methylation efficiency (Eckley et al., 2017). Since both locations showed different levels of MMHg concentrations, normalization to the amount of inorganic Hg available was applied to examine the % MMHg variation over time to infer MMHg production.

The normalization with THg ambient concentrations lessened the difference between the riverbed and the wetland locations (R^2 = 0.5770, p = 0.0495, n = 7), and between the dry

and wet treated samples (R^2 = 0.9528, p = 0.00017, n = 7), showing similar % MMHg at all times sampled across all treatments, depths, and locations. A decreasing trend of % MMHg over time was observed, with an average decrease of 70 ± 24 % for all samples and treatments, with the exception of the dry treatment of the top layer at Wabigoon Rapids riverbed location, where an increase of 55 % is observed during the 6 day period. In general, at the beginning of the wetting cycle the proportion of MMHg in both wet and dry samples was approximately 10 ± 5 %, which slowly decreased to 3 ± 2 % at the end of the first wetting cycle (day 6). After day 6, the wet samples were rewetted to a water content of 45 ± 5%. However, % MMHg continued to decrease in both wet and dry samples to 0.5 ± 0.6 % one hour after rewetting. Once again, the second wetting cycle seemed to have a negative influence on Hg methylation (as seen by the % MMHg).

That both wet and dry samples showed a similar trend in % MMHg over time could be explained by the fact that the dry samples were not completely dry at the beginning of the experiment and still experienced a drying process similar to the wet samples. This may suggest that MMHg levels might be less influenced by the magnitude of the wetting, but more so by the drying process. Considering that the drying process in the second wetting cycle was faster and resulted in a faster decrease in MMHg concentrations and proportions, it also suggests that the rate of the drying process may have an impact on net MMHg concentrations. Additionally, the decreasing trend in % MMHg could be explain by the fact that wetting and drying cycles are known to induce MMHg degradation as well (Xie et al., 2020). Since the degradation of MMHg is generally favoured by aerobic conditions (Ullrich et al., 2001), it is plausible that the drying process stimulated MMHg demethylation over Hg methylation, resulting in the decline observed.

However, when comparing % MMHg of the wet samples with the % MMHg of the dry core at the time of collection, MMHg proportions of wet samples were on average 45 % higher at the riverbed location and 48 % lower at the wetland location than % MMHg in the dry core. Once again, if we assume that the steady state MMHg proportion measured at time of sampling would remain unchanged if the dry sediment would be maintained at *in situ* moisture levels, then an increase in % MMHg in the riverbed location would be observed due to wetting.

4.3.4. Changes in Hg methylation rates

To better understand the influence of the wetting cycle on Hg methylation, one of the subsamples was spiked with isotope enriched ^{200}Hg to track MMHg formation.

In terms of $MM^{200}Hg$ being produced from the added ^{200}Hg spike (Figure 4.5), the wetting cycle did not have noticeable influence on Hg methylation rates, resulting in usually less than 0.6 % of $MM^{200}Hg$ being produced at all locations and depths across all times. The only exception was the top 3 cm of the riverbed location, where up to 4.3 % of the added Hg spike was converted to MMHg. These results show that Hg methylation is predominant in the top layer of the Wabigoon Rapids riverbed, showing on average 1.53 % of $MM^{200}Hg$ being produced during the 6-day period of the 1st wetting cycle, which is significantly higher than the $MM^{200}Hg$ conversion during the same period in the other samples. Curiously, the second wetting cycle seems to have a negative effect on Hg methylation across all samples with methylation rates below the detection limit one hour after the second wetting.

The absence of a relationship between observed methylation rates and MMHg concentrations ($R^2 = 0.1782$, $p = 0.0252$, $n = 28$) and % MMHg ($R^2 = 0.0005$, $p = 0.9046$, $n = 27$) suggests that net MMHg concentrations are being controlled by additional factors other

than methylation rates alone. As ambient MMHg concentrations are known to reflect the balance between methylation and demethylation processes (Schäfer et al., 2010), these results indicate that the MMHg variations observed are mostly likely strongly influenced by the demethylation process that could also been provoked by the wetting cycle (Xie et al., 2020).

4.3.5. Spatial differences

While the primary objective of this experiment was to determine the effects of the wetting cycle on Hg methylation, we also assessed the degree of differentiation between ecosystems (river vs wetland) and how these differences may influence MMHg production.

In general, the wetland (WRWT) location showed not only lower THg ambient concentrations (57 ± 32 ng/g) than the riverbed (WR) location (246 ± 96 ng/g), but also lower ambient MMHg concentrations (1 ± 3 vs 10 ± 6 ng/g). However, normalizing to % MMHg as a proxy for Hg methylation activity shows that the riverbed might be slightly more productive in MMHg formation compared to the wetland location (average of 4.9 % vs 3.5 %), even though they follow the same pattern of decreasing % MMHg over time. When comparing concentrations of total mercury and % MMHg in both locations no correlations were found ($R^2 = 0.0028$, $p = 0.7514$, $n = 40$ for WR and $R^2 = 0.0292$, $p = 0.2797$, $n = 42$ for WRWT), indicating that % MMHg in both locations is being controlled by factors other than Hg bioavailability alone.

Additionally, methylation essays also show that Hg methylation is stronger at the riverbed location, showing on average 1.03 % of $MM^{200}Hg$ being produced during the 6-day period of the 1st wetting cycle, which was more than five times higher than the % $MM^{200}Hg$ produced during the same period in the wetland location (0.19 %).

While both locations showed similar concentrations of organic matter (10.9 ± 0.1% and 11 ± 1% for WR and WRWT, respectively – Table C.5) we cannot rule out that the quality and type of organic matter differs in both locations and is driving the distinct MMHg productions observed. In turn, the different responses of the two locations may be a result of prior adaptations of the bacterial communities to the moisture conditions found in wetlands (Fierer et al., 2003).

4.3.6. Variations with depth

When comparing ambient MMHg concentrations at both depths the wetland location showed no statistical differences between the 0-3 cm and 3-6 cm layers (t-test, $p = 0.69$), while concentrations in the two layers at the riverbed location were distinct (11.8 vs 9.4 ng/g; t-test, $p = 0.03$), suggesting elevated MMHg production in the top layer of the riverbed location.

However, when looking at % MMHg, the wetland location showed differences between the two depths (t-test, $p = 0.01$), with the 3 – 6 cm layer showing higher % MMHg overall (4.6 ± 0.4 vs 2.3 ± 0.1 %), while the riverbed location showed no statistical difference (t-test, $p = 0.44$), suggesting that methylation is enhanced in the bottom layer of the wetland location. The opposing results when looking at concentrations and % MMHg to infer on MMHg production could suggest that Hg methylation proceeds similarly in top 6 cm of wetted sediments during a wet cycle, which is consistent with previous studies, where MMHg production was found to be consistently higher in the upmost sediments (0-5 cm) just below the sediment-water interface (Schäfer et al., 2010). However, data from the methylation essays with isotope enriched Hg seem to indicate that Hg methylation predominantly occurs in the top 3 cm of the riverbed location (average of 1.53 % vs 0.4 % of

$MM^{200}Hg$) and in the lower 3 cm of the wetland location (average of 0.31 % vs 0.1 % of $MM^{200}Hg$). Nevertheless, due to the creation of the slurry paste, the distribution of microorganism could have been altered resulting in inconclusive results.

4.4. Conclusions

In general, results from the wetting and drying cycle experiment suggest that the extent and magnitude of the wetting does not have a substantial effect on the rate of Hg methylation and that possibly MMHg levels are mainly affected by the drying process. Additionally, results indicate that MMHg concentrations strongly depend on the demethylation process that seems to be enhanced during the drying. While it is widely recognized that wetlands possess many environmental factors that promote Hg methylation and are recognized as important sites of methylmercury production (Hall et al., 2008), results show that, due to differences in organic matter quality or prior adaptations of the bacterial communities to the moisture conditions found in wetlands (Fierer et al., 2003), flooded river banks could become an area of concern for Hg methylation as they are more susceptible to wetting and drying cycles.

However, it is important to note, that owning to experimental design choices the results obtained in this experiment may not accurately reflect the conditions found in nature. Specifically, sediment cores were not entirely dry at the start of the experiment, which could have prevented a proper oxidation of Hg, organic matter, sulfur, and nutrients that is required to induce Hg methylation during wetting (Gilmour et al., 2004).

Additionally, the current study was conducted by creating a slurry, which may not be the ideal method to simulate the real conditions of MMHg formation in a natural sediment environment. It is known that microorganisms are organized in biogeochemically stratified

environments, known as microbial mats, where the survival of a given individual microorganism is dependent on the metabolic activity of others coexisting in the environment (Konhauser, 2007). Methylating bacteria, such as sulfate reducers and methanogens, are often found in the deepest layers of these mats, being highly supported by the microbial remnants of surface communities (Konhauser, 2007). Since the activity and structure of the microbial community is highly related to MMHg production (Eckley et al., 2017), physical disturbances applied to our samples, such as creation of a slurry and stirring with a spoon, alter the structure of the microbial community and could have influenced the ability of microorganisms to methylate Hg.

Moreover, our weeklong experiment might not have been sufficiently long to accurately assess the influence of flooding in MMHg production. For instance, a lag period is to be expected before microbial growth is stimulated (leading to Hg methylation) after wetting. While it is thought that the respiration rate and bacterial growth reaches a stable level within 3 days after wetting (Bapiri et al., 2010), previous history (number of wetting cycles), the composition of the microbial community of the soil and the type of sediment will result in different timed responses (Bapiri et al., 2010). Additionally, it has been found that THg release from soils after flooding is not always immediate (Coleman Wasik et al., 2015). The combination of these factors can result in a delay before MMHg concentrations increase in wetted sediments. For example, a previous study (Kelly et al., 1997) observed a lag period of 2 weeks before MMHg concentrations increased in the flooded pond.

That said, future research should be performed to correctly understand the response of the Wabigoon River sediments to wetting and drying cycles.

4.5. Future Work

Future experiments should airdry the sediment before starting the wetting process and have an additional wet control sample that stays permanently wet throughout the experiment.

To simulate the real conditions of MMHg formation in an actual sediment environment, future studies should recreate the wetting and drying cycle in a soil column experiment, maintaining the integrity of cores (Hindle, 2005). Water can be added from the top maintaining the core wet with a plug at the bottom, allowing for the cores to air dry from the top. At the end of the wetting and drying cycle the cores are sliced and THg and MMHg concentrations are measured at different depths. While this approach maintains the microbial structure in the soil, providing a more reliable estimation of Hg methylation, it only permits one time measurement at the end of the experiment, not allowing for a temporal analysis of the influence of the wet and dry cycles on MMHg production. Additionally, as the cores are destroyed for analysis after the experiment, repeated cycles are not possible.

Another approach that could have been employed is to sample sediment cores before and after a natural wetting cycle at locations that are seasonally inundated, permanently inundated and permanently dry. This approach would ensure that the microbial community is not affected due to physical alterations and will also account for other environmental changes in the ecosystem that influence MMHg production. For example, it incorporates the death of the vegetation that causes a large amount of organic carbon to become available for decomposition, which is known to enhance methylation rates (Balogh et al., 2002). This decomposition activity can also lead to an increase of anaerobic habitat, which is also known to enhance mercury methylation (Regnell & Tunlid, 1991). For example, a recent study

(Eckley et al., 2017) collected sediment, porewater and surface water samples reflecting the different seasonal water tables during winter (low), spring (high), summer (high) and fall (low) following a drawdown and measured THg, MMHg, Hg methylation rates and several ancillary parameters. The results showed that sediment and porewater MMHg concentrations were more than 3-times higher in areas experiencing water-level fluctuations compared to permanently inundated sediments, which was also confirmed by the methylation essays performed. However, some drawbacks of this approach are 1) it requires year-round seasonal collection, which is specially complicated at remote locations, 2) cannot control the flooding conditions and 3) requires additional studies on the hydrology of the system to select appropriate sampling locations and periods.

Ultimately the best approach would have been to do a whole ecosystem flooding experiment comparable to those executed at the Experimental Lakes Area in northwestern Ontario (Kelly et al., 1997). With this type of approach, the flooding conditions can be controlled, such as intensity of the flooding, flooded area, duration and number of wetting cycles. The above-mentioned experiment flooded the wetland to a depth of 1.3 m above the previous pond level by damming the outflow, where the surrounding peatland was inundated, increasing the surface area of the pond by a factor of 3 and the water volume by a factor of 6. The experiment lasted for 2 years and each year the reservoir was drained to preflood levels in late fall to simulate the winter drawdown of many northern hydroelectric reservoirs. The average MMHg concentration in the reservoir after flooding was about 10-fold higher than pre flooding. Another study examined the longer term (9 year) effects of flooding in said experimentally flooded wetland (St.Louis et al., 2004). This longer period of examination was able to identify that the persistence of the MMHg production is relatively short-lived (2-3 years), presumably due to microbial demethylation. They also hypothesized

that the very high rates of Hg methylation that occurred in the few years following inundation may have depleted the bioavailable Hg pool there, resulting in lower net Hg methylation after 2-3 years. This leads us to believe that a longer experimental period, of several years, is necessary to correctly determine the influence of flooding in MMHg production.

Nonetheless, to perform a whole ecosystem flooding experiment in the Wabigoon River system is not possible. As a result, the best approach to correctly identifying the influence of wetting cycles in this system might be to collect sediment cores before and after seasonal flooding in a span of at least 2 years, including permanently dried and permanently wet control samples.

Furthermore, as MMHg concentrations were found to be highly dependent on both the methylation and demethylation processes, which in turn are simultaneously affected by wetting-drying cycles, demethylation rates should also be accessed to correctly identify the influence of wetting cycles on net MMHg production.

4.6. Figures

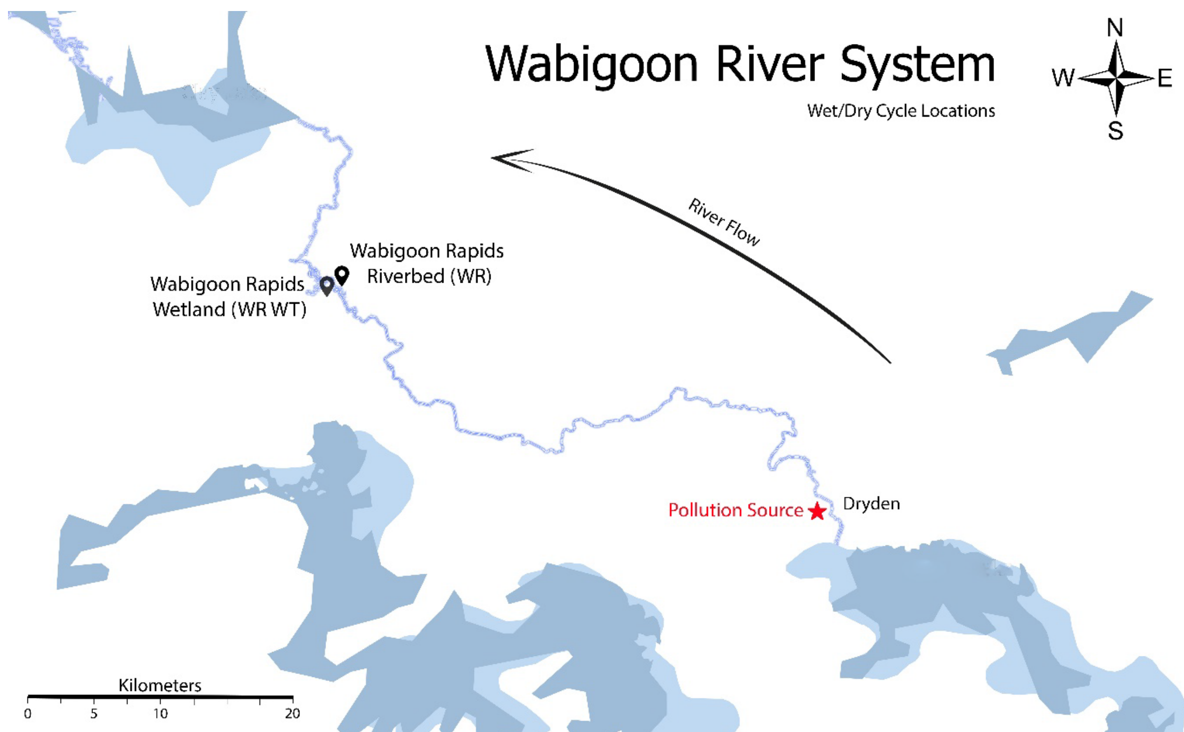

Figure 4.1 : Map of the sampling locations for Sediment Cores collected for Wetting Cycle simulation, with Pollution Source identified in red.

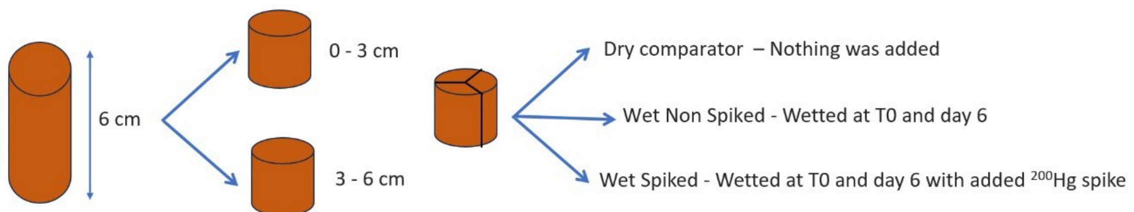

Figure 4.2 : Visual description of the separation and treatments applied to the cores.

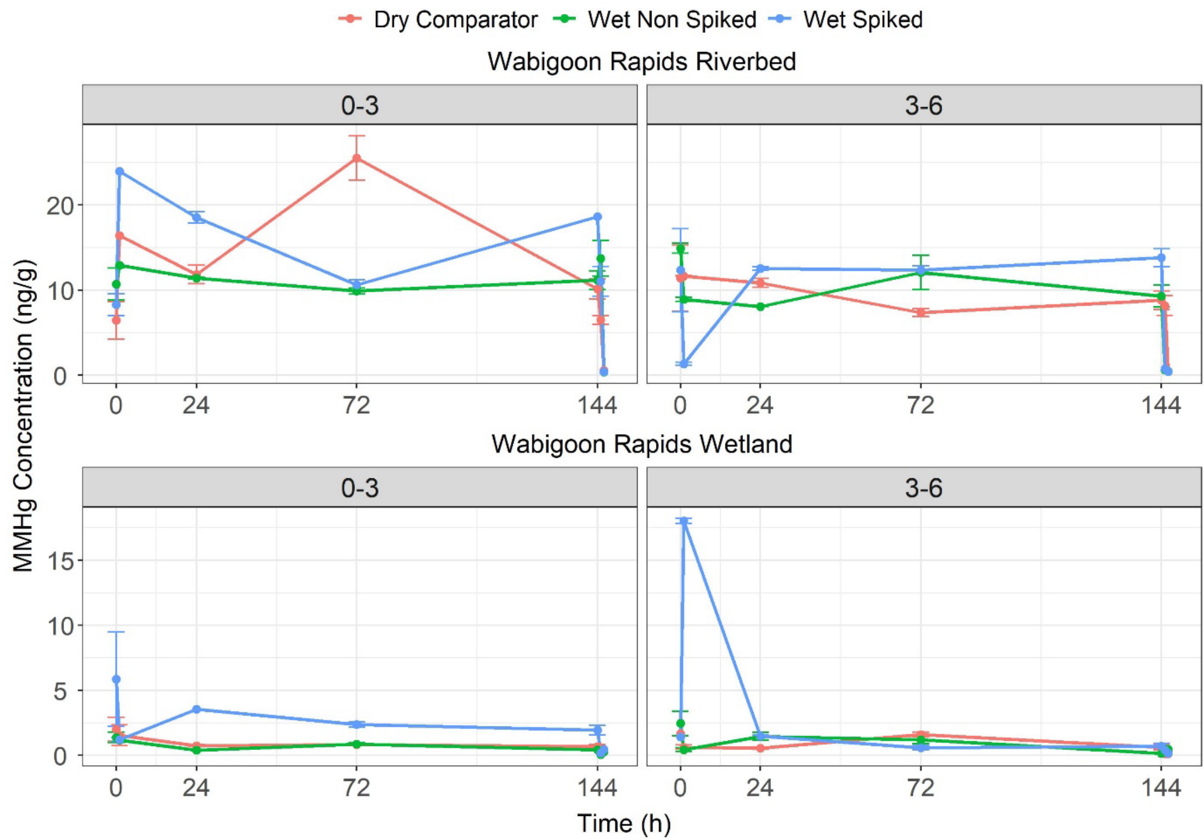

Figure 4.3 : MMHg ambient concentrations (ng/g) over time for the Wabigoon Rapids riverbed (WR) and Wabigoon Rapids Wetland (WRWT) Locations at both depths (0-3 cm and 3-6 cm), across the different treatments (Dry Comparator, Wet Non Spiked and Wet Spiked).

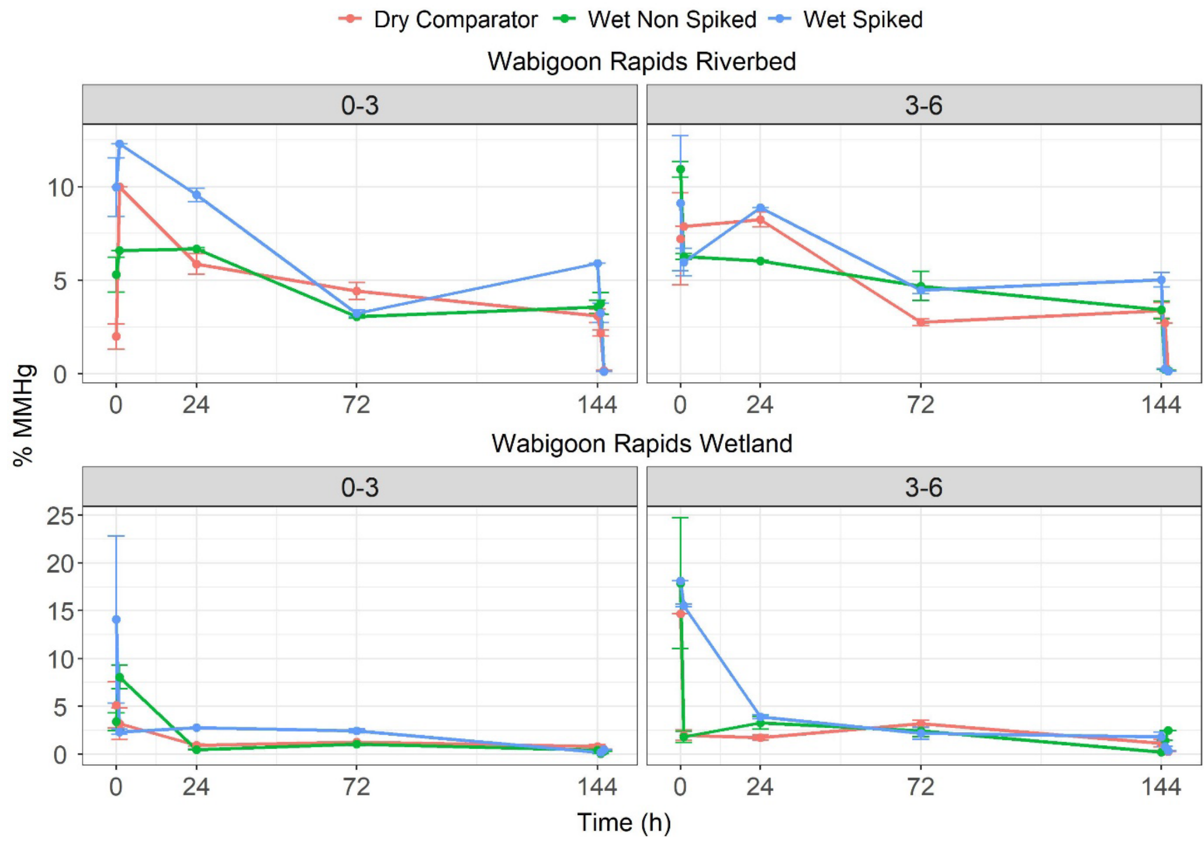

Figure 4.4 : % MMHg over time for the Wabigoon Rapids riverbed (WR) and Wabigoon Rapids Wetland (WRWT) Locations at both depths (0-3 cm and 3-6 cm), across the different treatments (Dry Comparator, Wet Non Spiked and Wet Spiked).

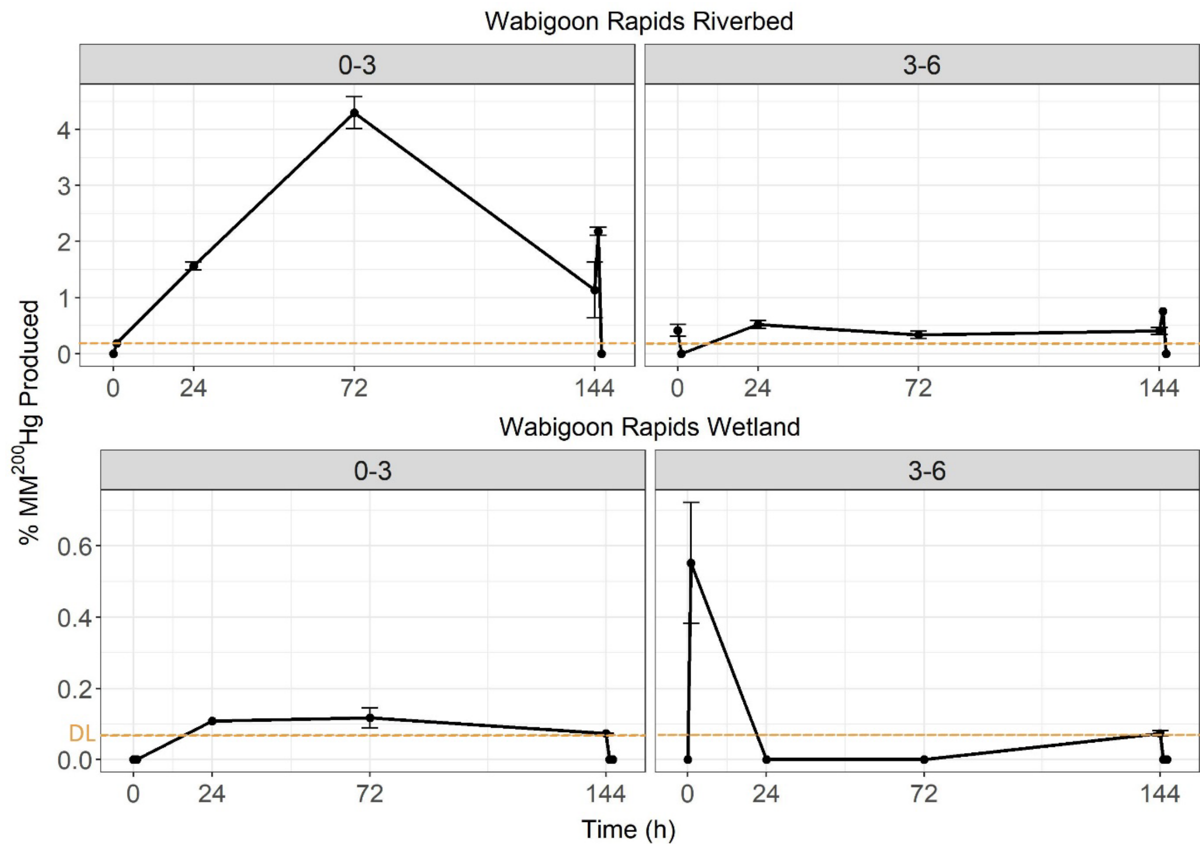

Figure 4.5 : % MM^{200}Hg produced from the added ^{200}Hg spike over time for the Wabigoon Rapids riverbed (WR) and Wabigoon Rapids Wetland (WRWT) locations at both depths (0-3 cm and 3-6 cm), with detection limit (DL) identified in orange.

Acknowledgements

This research was supported by a PhD Research Studentship (SFRH/BD/144532/2019) to BB, funded by the Portuguese Foundation for Science and Technology (FCT) as well as an NSERC Discovery grant to HH. Thanks to the Water Quality Center for the use of the ICP-MS equipment to perform the analysis; the IISD Experimental Lakes Area for accommodation and use of field lab during field season; and Adelheid Wilsie for assistance in field sampling.

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Chapter 5: General Discussion & Conclusion

More than six decades after the initial pollution the Wabigoon River is still contaminated with Hg. Downstream locations from the pollution source showed THg concentrations that are at least 8x higher in water (28.1 to 122 ng/L), and more than 5x higher in surface sediments (0.37 to 0.79 mg/kg), while the top 5 cm of core samples showed concentrations up to 134 times higher than the reference location (0.13 to 5.5 mg/kg), with the exception of Minnitaki Bridge that showed concentrations similar to background levels (0.032 mg/kg), either due to high sedimentation at this location or high presence of sand and gravel in surface sediments. Methylmercury concentrations downstream from the pollution source also showed MMHg concentrations that are at least 5x higher in water (0.96 to 4.70 ng/L) and more than 4x in surface sediments (0.66 to 34.1 ng/g), while the top 5 cm of core samples showed MMHg concentrations up to 89 times higher than the refence location (0.93 to 53.0 ng/g), again with the exception of Minnitaki Bridge that showed MMHg levels similar to the reference (0.56 ng/g), consistent with the low total mercury values found at this location.

Depth profiles of sediment cores showed a buried peak of THg concentrations, with lower concentrations at the top at most locations, suggesting a slow decline of Hg levels in the Wabigoon River system due to sediment accumulation that has buried the historical pollution-induced THg peak. Nevertheless, surface sediments continue to exhibit high THg concentrations, on average 44 times higher than background levels. However, since none of the cores in the current study were dated, exact temporal assignment was not possible,

adding uncertainty to the interpretation. Future temporal analysis should be aided with core dating to correctly infer on sedimentation of the historical pollution.

Assessment of current levels of both total mercury and methylmercury in water and sediments at selected locations along the Wabigoon River (Chapter 2) identified the Wabigoon Rapids wetland, the Hydroelectric dam and Clay Lake as potential sources of methylmercury in the system, while the Wabigoon Rapids was identified as either a sink or source or both of methylmercury. Further analysis of specific methylation rates (Chapter 3) confirmed that the Wabigoon Rapids wetland, the Hydroelectric dam and Clay Lake are indeed locations with high capacity for methylmercury production. With the Hydroelectric dam showing the most favourable conditions for Hg methylation in both water and sediments and potentially being able to produce 1.93 ng/L and 28.6 ng/g of MMHg, respectively, per day. Clay Lake, due to being a sink for THg and accumulating up to 122 ng/L of mercury in its surface waters, could produce 2.70 ng/L of MMHg in its surface waters and 12.7 ng/g in its bottom sediments. Additionally, the wetland at Wabigoon Rapids is potentially able to produce 0.66 ng/g of MMHg in water and 19.3 ng/g in sediments. These three sites exemplify the potential for the generation and downstream distribution of methylmercury. These and other similar locations deserve special consideration in future remediation efforts. Furthermore, the Wabigoon Rapids wetland also possesses methylation capacities, being able to produce 1.04 ng/L and 12.1 ng/g per day. However, the methylation potentials do not fully explain the high ambient methylmercury concentrations found at Wabigoon Rapids, suggesting again that this location is likely also a sink for methylmercury within the system.

In general, elevated methylation rates observed in sediments and waters of the Wabigoon River system, in comparison to similar ecosystems elsewhere, suggest that MMHg production is an important factor in the Wabigoon River and must be considered when assessing to MMHg accumulation to biota.

Nevertheless, the current assessment likely overstates MMHg production in the Wabigoon River, especially within sediments. Firstly, these rates reflect MMHg production during the summer, when Hg methylation often peaks due to elevated rates of growth and metabolic activity (Korthals & Winfrey, 1987). Consequently, seasonal variations may occur, and average annual methylation rates are likely lower than those observed here. Secondly, the assessment method of using isotope enriched Hg(II) additions to calculate methylation rates, may return upper maxima for methylation rates. Even after equilibration, the mercury spike is often more bioavailable for methylation reactions compared to natural, ambient mercury (Hintelmann et al., 2000). For instance, not all ambient mercury present is accessible for methylation, as up to 80% of the THg between Dryden and Clay Lake is bound to inorganic particles (Rudd et al., 2021), reducing its bioavailability (Farrell et al., 1998). Nevertheless, on a relative scale, the current methylation potentials serve as a valuable indicator for identifying potential MMHg sources along the Wabigoon River system.

The establishment of specific demethylation rates (Chapter 3) further the understanding of methylmercury dynamics in the system. The calculated half-life for methylmercury in sediments of riverbed and wetland locations was 2.1 days on average, suggesting a rapid turnover and low persistence of methylmercury in sediments of the Wabigoon River. However, both lakes show high half-lives for methylmercury in sediments, between 4.8 to 144 days, suggesting that MMHg is more persistent in lakes. While anaerobic

organisms such as SRB, methanogens and IRB partipate in both methylation and demethylation processes (Du et al., 2019), they seem to favour Hg methylation, resulting in accumulation of methylmercury in anoxic environments, e.g., the bottom of lakes.

In particular, the inflow of Clay Lake shows very low demethylation rates, with methylmercury being able to stay in the system for up to 144 days. Combined with the high methylation rates found here, MMHg bioaccumulation may be enhanced at this location.

The rapid turnover of methylmercury of just 33 and 69 hours for the Wabigoon Rapids wetland and Hydroelectric dam, respectively, implies that specific methylation rates at these locations might overestimate the net MMHg production. However, these locations still show elevated concentrations of methylmercury, of between 9.01 to 13.4 ng/g at Wabigoon Rapids wetland and between 4.61 to 53.0 ng/g at the Hydroelectric dam, suggesting an overall MMHg accumulation at these locations.

In general, the consistently lower demethylation rates observed in Wabigoon River sediments, compared to rates reported in sediments of similar ecosystems, suggest a heightened preservation of MMHg in the Wabigoon River system.

Because both mercury methylation and MMHg demethylation are significantly influenced by temperature, with Hg methylation known to be stimulated at high temperatures (Bubb et al., 1993), while demethylation is favoured at low temperatures (Bodaly et al., 1993), methylation and demethylation essays should be performed in the winter to assess the seasonal variability of factors driving ambient methylmercury concentrations.

Furthermore, the combination of specific mercury (Hg) methylation (*km*) and methylmercury (MMHg) demethylation rates (*kd*) with modelling and geographic information system (GIS) tools can offer a potent method for estimating MMHg production along the Wabigoon River System. For instance, utilizing GIS to assess wetland areas in the Wabigoon River and assuming that wetlands in the system exhibit similar Hg methylation capacities as observed in the Wabigoon Rapids wetland, the *km* at WR can be employed to estimate the overall MMHg production in the wetland area.

Finally, findings from the wetting cycle experiment (Chapter 4) may suggest that the extent or magnitude of wetting does not significantly impact the rate of mercury methylation, offering the added possiblility that MMHg levels are primarily influenced by the drying process. Furthermore, lab the results indicate that MMHg concentrations are strongly influenced by the demethylation process, which appears to be enhanced during the drying process. However, the results from this lab experiment should not be directly extrapolated to real ecosystems. Due to necessary experimental design choices, the study may not have truthfully mimicked natural conditions and processes. Nevertheless, future work should include the assessment of specific demethylation rates to accurately determine the impact of wetting cycles on the overall production of methylmercury.

Additionally, the results may still suggest that flooded riverbanks are more subjected to wetting and drying cycles due to differences in organic matter quality or prior adaptations of bacterial communities to the moisture conditions found in wetlands (Fierer et al., 2003), posing a concern for Hg methylation.

As the current investigation into wetting and drying cycles was conducted through a laboratory simulation, it is not without its limitations. To accurately discern the impact of

wetting cycles in this system, future work should involve collecting sediment cores before and after seasonal flooding over a period of at least two years, including control samples from permanently dried and permanently wet conditions.

In conclusion, this study achieved to 1) establish current levels of both total mercury and methylmercury in water and sediments, showing that locations downstream from the pollution source still have elevated mercury concentrations and identifying the Wabigoon Rapids as a sink for methylmercury (Chapter 2); 2) show, through analysis of sediment cores, a slow recovery of the Wabigoon River System due to sediment accumulation that has buried the historical pollution-induced THg peak. However, surface sediments still have high THg concentrations, on average 44 times higher than background levels (Chapter 2); 3) identify types of locations that may produce MMHg within the Wabigoon River, with the Hydroelectric dam, the wetland at Wabigoon rapids and Clay Lake being examples of potential MMHg sources (Chapter 3), with Clay Lake showing simultaneously high methylation an low demethylation rates; and finally, 4) assess the influence of wetting and drying cycles on the Hg methylation on riverbed and wetland locations in the Wabigoon River System suggesting that riverbed locations are more susceptible to wetting and drying cycles (Chapter 4).

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Appendix A: Supplementary data for Chapter 2

A.1. Tables

Table A.1 : Coordinates for sampling locations.

Location	Sample	Latitude	Longitude
Wabigoon Lake	Core 1	49°45’52.51” N	92°50’40.63” W
	Core 2	49°45’52.39” N	92°50’41.37” W
	Sediment, surface water	49°41’57.44” N	92°47’26.30” W
	Bottom water	49°45’14.18” N	92°44’24.50” W
Wabigoon Lake Wetland	Core 1, Core 2, Core 3	49°45’55.85” N	92°53’22.63” W
	Surface water	49°45’57.31” N	92°53’21.37” W
	Sediment	49°45’54.32” N	92°53’15.25” W
Hydroelectric Dam	Core 1	49°48’55.70” N	92°52’35.10” W
	Core 2	49°48’56.80” N	92°52’36.60” W
	Core 3	49°48’58.80” N	92°52’37.10” W
	Surface water	49°48’56.23” N	92°52’35.97” W
	Sediment	49°48’54.50” N	92°52’45.30” W
Minnitaki Bridge	Core	49°51’20.40” N	93°03’59.00” W
Wabigoon Rapids	Core	49°55’42.00” N	93°21’07.90” W
	Surface water	49°55’33.96” N	93°21’08.64” W
	Sediment	49°55’34.10” N	93°21’09.54” W
Wabigoon Rapids Wetland	Core 1	49°55’32.90” N	93°21’33.00” W
	Core 2	49°55’35.20” N	93°21’31.40” W
	Surface water	49°55’34.14” N	93°21’01.47” W
	Sediment	49°55’34.06” N	93°26’06.13” W
Wabigoon Falls	Core	49°58’32.56” N	93°23’54.33” W
Clay Lake Inflow	Core 1, Core 2, Core 3	50°03’17.01” N	93°24’51.20” W
	Surface water	50°03’07.09” N	93°25’03.68” W
	Sediment	50°03’07.09” N	93°25’04.29” W
Clay Lake West	Sediment	50°03’10.65” N	93°32’54.06” W

Table A.2 : Abundances (%) of Hg isotopes used as internal standard for the ICP-MS measurement.

Abundance %	196	198	199	200	201	202	204
^{199}Hg	<0.02	1.63 ± 0.02	91.95 ± 0.05	4.92 ± 0.03	0.66 ± 0.01	0.73 ± 0.01	0.11 ± 0.01

Table A.3 : THg and MMHg concentrations (mg/kg), as well as % MMHg with depth (cm) for the Wabigoon Lake (WL) core 1 and THg (mg/kg) with depth (cm) for WL 2. Cores collected in Fall 2018.

Wabigoon Lake Cores				
depth (cm)	1			2
	THg (mg/kg)	MMHg (mg/kg)	% MMHg	THg (mg/kg)
0.5	0.0378	0.0001	0.3	
1.5	0.0433			
2.5	0.0405	0.0004	1.1	
3.5	0.0401			
4.5	0.0443	0.0008	1.7	
5.5	0.0503			
6.5	0.0430	0.0006	1.4	
7.5	0.0447			
8.5	0.0385			0.0379
9.5	0.0465			0.0415
10.5	0.0413	0.0001	0.1	0.0483
11.5	0.0564			0.0382
12.5	0.0428			0.0393
13.5	0.0416	0.0004	1.0	0.0460
14.5	0.0431	0.0004	0.9	0.0403
15.5	0.0410			0.0393
16.5	0.0390	0.0007	1.8	0.0439
17.5	0.0351			0.0398
18.5	0.0375			0.0420
19.5	0.0338	0.0004	1.1	0.0476
20.5	0.0349			0.0459
21.5	0.0289			0.0464
22.5	0.0324			0.0385
23.5	0.0301			0.0425
24.5	0.0321	0.0002	0.7	0.0380
25.5	0.0277			
26.5	0.0566			0.0376
27.5	0.0363			
28.5	0.0372			
29.5	0.0404	0.0002	0.5	
30.5	0.0341			
31.5	0.0318			
33.5	0.0328			
34.5	0.0276	0.0002	0.8	
35.5	0.0253			
Ave.	**0.0385**	**0.0004**	**1.0**	**0.0418**

Table A.4 : THg and MMHg concentrations (mg/kg), as well as % MMHg with depth (cm) for the Wabigoon Lake Wetland (WLWT) cores collected in Fall 2018.

Wabigoon Lake Wetland Cores									
depth (cm)	**1**			**2**			**3**		
	THg (mg/kg)	**MMHg (mg/kg)**	**% MMHg**	**THg (mg/kg)**	**MMHg (mg/kg)**	**% MMHg**	**THg (mg/kg)**	**MMHg (mg/kg)**	**% MMHg**
0.5	0.1159	0.0004	0.4	0.0624	0.0105	17	0.0613	0.0007	1.1
1.5	0.1353	0.0012	0.9	0.0858			0.0619		
2.5	0.1098	0.0005	0.4	0.1104	0.0029	2.6	0.0736		
3.5	0.1173	0.0006	0.5	0.0749			0.0875	0.0013	1.4
4.5	0.1144	0.0006	0.5	0.0818	0.0073	8.9	0.0600		
5.5	0.1480			0.0993	0.0042	4.2	0.0587	0.0019	3.3
6.5	0.1529	0.0006	0.4	0.0689			0.1032	0.0013	1.3
7.5	0.1392			0.0794	0.0031	4.0	0.0556		
8.5	0.1061			0.0882	0.0045	5.1	0.0559		
9.5	0.0837	0.0001	0.2	0.1351			0.0703		
10.5	0.0921	0.0003	0.3	0.1762	0.0070	4.0	0.0605	0.0043	7.0
11.5	0.0612	0.0001	0.2				0.0708		
12.5	0.0580								
13.5	0.0511	0.0001	0.2				0.0981	0.0020	2.0
14.5	0.0684	0.0002	0.3						
14.5	0.0800								
15.5	0.0828								
16.5	0.0780	0.0001	0.1						
Ave.	**0.0997**	**0.0004**	**0.4**	**0.0966**	**0.0056**	**6.5**	**0.0706**	**0.0019**	**2.7**

Table A.5 : THg and MMHg concentrations (mg/kg), as well as % MMHg with depth (cm) for the Hydroelectric Dam (HD) core 1 and THg (mg/kg) with depth (cm) for HD cores 2 and 3. Cores collected in Fall 2018.

Hydroelectric Dam Cores					
depth (cm)	**1**			**2**	**3**
	THg (mg/kg)	**MMHg (mg/kg)**	**% MMHg**	**THg (mg/kg)**	**THg (mg/kg)**
0.5	4.485	0.090	2.0	1.202	1.811
1.5				1.256	2.807
2.5				1.664	
3.0	6.513	0.016	0.2		
3.5				2.025	
4.0					6.687
4.5				1.329	
5.5				1.071	
6.5				2.159	
7.5	7.648	0.016	0.2	3.826	13.226
8.5				5.570	
9.5				18.309	
10.5				30.942	
11.0					10.860
12.5	9.376	0.012	0.1		
13.0				38.950	
15.0					1.372
17.0	13.912				
17.5				11.140	
19.0					0.198
20.5	0.785				
21.0	2.569	0.003	0.1		
21.5				0.594	0.175
22.5				11.116	0.157
23.5				24.378	0.100
24.5				16.506	0.049
25.5				14.216	0.040
26.5					0.053
27.5	14.3	0.016	0.1		
28.7				0.107	
30.5	60.9	0.067	0.1		
35.5	22.2	0.033	0.1		
40.5				1.046	
43.5	3.597	0.017	0.5		
Ave.	**14.18**	**0.022**	**0.2**	**10.78**	**2.992**

Table A.6 : THg, MMHg concentrations (mg/kg) and % MMHg with depth (cm) for the Minnitaki Bridge Core collected in Fall 2018.

Minnitaki Bridge Core			
depth (cm)	**1**		
	THg (mg/kg)	**MMHg (mg/kg)**	**% MMHg**
4.5	0.032	0.001	1.7
9.5	0.051	0.0004	0.9
10.5	0.030		
11.5	0.031		
12.5	0.028		
13.5	0.039		
14.5	0.038		
15.5	0.024		
16.5	0.036		
17.5	0.035	0.001	3.2
18.5	0.057		
19.5	0.045		
20.5	0.041		
21.5	0.115	0.001	0.6
22.5	0.184		
23.5	0.836	0.005	0.6
25.5	1.209		
26.5	1.574	0.007	0.4
27.5	2.417		
28.5	2.743	0.006	0.2
29.5	2.247		
30.5	1.322	0.004	0.3
31.5	0.703		
32.5	0.096	0.001	0.8
33.5	0.030		
34.5	0.034	0.0005	1.4
Ave	**0.538**	**0.003**	**1.0**

Table A.7 : THg, MMHg concentrations (mg/kg) and % MMHg with depth (cm) for the Wabigoon Rapids Core collected in Fall 2018.

Wabigoon Rapids Core			
depth (cm)	**1**		
	THg (mg/kg)	**MMHg (mg/kg)**	**% MMHg**
0.5	2.075	0.017	0.8
1.5	2.203		
2.5	2.261	0.014	0.6
3.5	2.945		
4.5	4.116	0.009	0.2
5.5	8.450		
6.5	15.046	0.022	0.1
7.5	10.734		
8.5	8.888	0.018	0.2
9.5	0.388		
10.5	0.098	0.002	1.9
11.5	0.073		
12.5	0.577	0.002	0.4
13.5	2.185		
Ave	**4.289**	**0.012**	**0.6**

Table A.8 : THg and MMHg concentrations (mg/kg), as well as % MMHg with depth (cm) for the Wabigoon Rapids Wetland (WR WT) core 1 and THg (mg/kg) with depth (cm) for WR WT 2. Cores collected in Fall 2018.

Wabigoon Rapids Wetland Cores				
depth (cm)	1			2
	THg (mg/kg)	MMHg (mg/kg)	% MMHg	THg (mg/kg)
0.5	1.632	0.010	0.6	0.152
1.5	2.523			0.153
2.5	2.493			0.144
3.5	2.620	0.010	0.4	0.100
4.5	2.909			0.087
5.5	2.987			0.067
6.5	1.525	0.015	1.0	0.087
7.5	1.599			0.063
8.5	0.032			1.561
9.5	0.026	0.001	2.7	2.636
10.5	2.395			0.020
11.5	4.245	0.005	0.1	0.032
12.5	5.122			0.018
13.5	5.019	0.004	0.1	
14.5	2.388			
15.5	1.250	0.002	0.2	
16.5	0.688			
17.5	0.297			
18.5	0.276	0.002	0.6	
19.5	0.079			
20.5	0.075			
21.5	0.042	0.001	2.1	
22.5	0.029			
23.5	0.021			
Ave.	**1.678**	**0.006**	**0.9**	**0.394**

Table A.9 : THg, MMHg concentrations (mg/kg) and % MMHg with depth (cm) for the Wabigoon Falls Core collected in Fall 2018

Wabigoon Falls Core			
depth (cm)	1		
	THg (mg/kg)	MMHg (mg/kg)	% MMHg
0.5	0.856	0.0110	1.3
4.5	0.206	0.0019	0.9
9.5	0.073	0.0010	1.4
10.5	0.037		
11.5	0.019		
12.5	0.017		
13.5	<DL	0.0004	n.d
14.5	<DL		
15.5	<DL		
16.5	<DL		
17.5	<DL	0.0004	n.d
18.5	<DL		
19.5	<DL		
20.5	<DL		
21.5	<DL	0.0001	n.d
22.5	<DL		
23.5	0.023		
Ave	**0.176**	**0.002**	**1.2**

Table A.10 : THg and MMHg concentrations (mg/kg), as well as % MMHg with depth (cm) for the Clay Lake (CL) core 1 and THg (mg/kg) with depth (cm) for CL cores 2 and 3. Cores collected in Fall 2018.

Clay Lake Cores					
depth (cm)	1			2	3
	THg (mg/kg)	MMHg (mg/kg)	% MMHg	THg (mg/kg)	THg (mg/kg)
0.5	0.9305	0.0011	0.1	0.7715	1.0434
1.5	0.8873			0.7496	1.1823
2.5	0.9545	0.0012	0.1	0.5193	1.3624
3.5	0.8252			0.2864	1.8484
4.5	0.6504	0.0005	0.1	0.2586	2.5312
5.5	0.3369			0.0858	3.5104
6.5	0.0486	0.0011	2.3	0.0912	3.3928
7.5	0.0276			0.4203	1.8930
8.5	0.0310			0.1319	2.8659
9.5	0.0329				
10.5	0.0210	0.0004	2.0		
11.5	0.0235				
12.5	0.0295				
13.5	0.0276	0.0012	4.5		
14.5	0.0436				
14.5	0.0287				
15.5	0.0253				
16.5	0.0246	0.0008	3.1		
18.5	0.0311				
19.5	0.0353	0.0019	5.3		
20.5	0.0459				
Ave.	**0.2410**	**0.0010**	**2.2**	**0.3683**	**2.1811**

A.2. Organic Matter

Organic matter content (%) in sediments was measured as Lost on Ignition (LOI), where between 2 to 0.5 g of dried sediment were burned at 500 °C for 4 hours, and mass loss was measured as described in equation A1.

$$OM\ (\%) = \frac{(initial\ mass - final\ mass)}{initial\ mass} \times 100\ \% \qquad \text{(A1)}$$

Table A.11 : Organic matter content (% LOI) in Wabigoon Lake Wetland Core 1.

WLWT 1 (cm)	OM %
0.5	57
1.5	57
2.5	60
3.5	61
4.5	61
6.5	55
9.5	56
10.5	61
11.5	80
13.5	84

Table A.12 : Organic matter content (% LOI) in Wabigoon Lake Wetland Core 2.

WLWT 2 (cm)	OM %
0.5	89
2.5	91
4.5	94
5.5	94
7.5	94
8.5	93
10.5	92

Table A.13 : Organic matter content (% LOI) in Wabigoon Lake Wetland Core 3.

WLWT 3 (cm)	OM %
0.5	83
3.5	84
5.5	71
6.5	75
10.5	77
13.5	85

Table A.14 : Organic matter content (% LOI) in Hydroelectric Dam Core 3.

HD 3 (cm)	OM %
0.5	30
4	43
7.5	52
11	72
15	75
19	74
23.5	15
26.5	14

Table A.15 : Organic matter content (% LOI) in Minnitaki Bridge Core.

MB (cm)	OM %
4.5	8
9.5	7
12.5	7
15.5	7
19.5	8
22.5	9
25.5	6
28.5	8
30.5	7
34.5	6

Appendix B: Supplementary data for Chapter 3

B.1. Figures

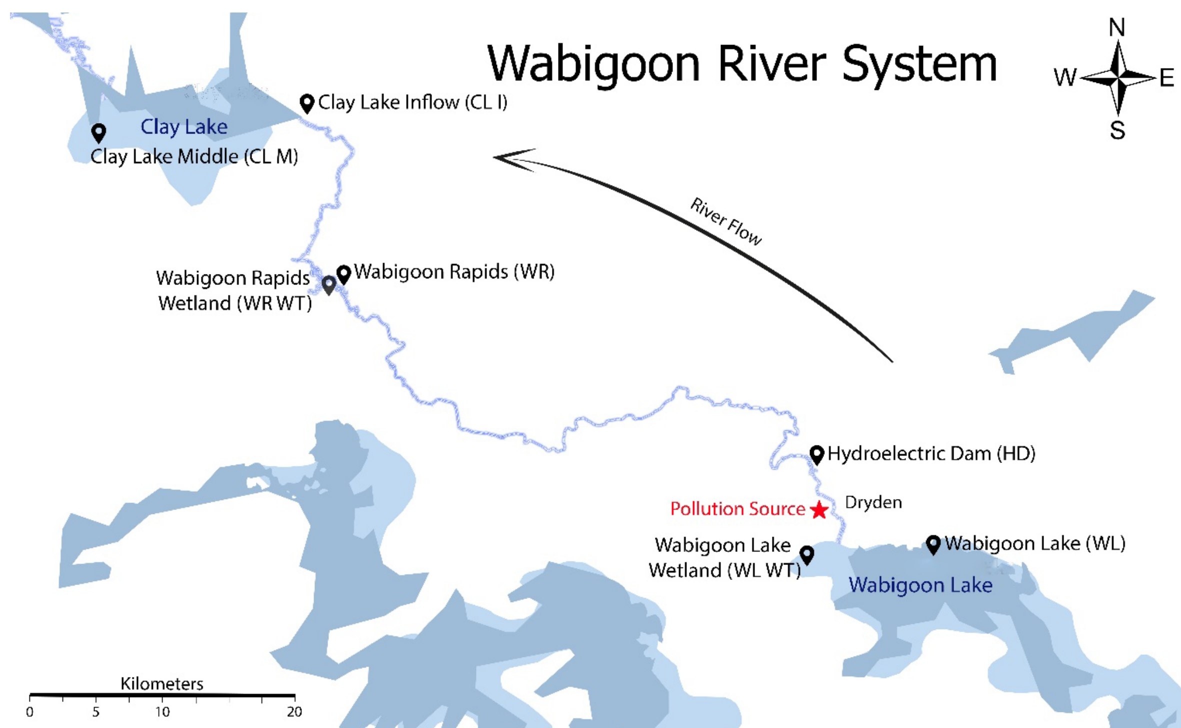

Figure B.1 : Map of the sampling locations along the Wabigoon River System with pollution source shown in red.

B.2. Tables

Table B.1 : Abundances (%) and uncertainties for the enriched isotopes used in methylation essays.

Abundance (%)	196	198	199	200	201	202	204
^{199}Hg and MM^{199}Hg standards	<0.02	1.63	91.95	4.92	0.66	0.73	0.11
^{200}Hg spike	n.d.	0.11	1.00	98.29	0.32	0.24	0.04
MM^{198}Hg spike	0.22	94.26	0.55	3.50	0.51	0.79	0.17

Table B.2 : Ambient THg, ^{200}Hg field spike and % of field spike compared to ambient THg concentrations in sediment samples at different locations along the Wabigoon River System.

Location	THg (ng/g dw)	^{200}Hg field spike (ng/g dw)	% of field spike
WL	74.0	37.5	51
WLWT	74.3	131	176
HD	651	107	16
WR	547	71.6	13
WRWT	787	130	17
CL I	365	113	31
CL W	337	184	55

Table B.3 : Ambient THg, ^{200}Hg field spike and % of field spike compared to ambient THg concentrations in water samples at different locations along the Wabigoon River System.

Location	THg (ng/L)	^{200}Hg field spike (ng/L)	% of field spike
WL TOP	3.60	12.94	359
WL BOT	3.67	3.40	93
WLWT	6.41	10.25	160
HD	46.4	10.48	23
WR	28.1	10.90	39
WRWT	60.3	11.69	19
CL I	122	12.60	10

Table B.4 : Ambient MMHg, MM^{198}Hg field spike and % of field spike compared to ambient MMHg concentrations in sediment samples at different locations along the Wabigoon River System.

Location	MMHg (ng/g dw)	MM^{198}Hg field spike (ng/g dw)	% of field spike
WL	0.16	0.4	228
WLWT	1.46	1.3	90
HD	4.61	1.1	23
WR	34.1	1.3	4
WRWT	9.01	0.7	8
CL I	0.66	1.1	171
CL W	0.93	1.8	198

Table B.5 : $T^{200}Hg$, $MM^{200}Hg$ and % MMHg produced in water at different incubation times at different locations along the Wabigoon River System.

Water Samples				
Location	**Time (h)**	**$T^{200}Hg$ (ng)**	**$MM^{200}Hg$ (ng)**	**% MMHg produced**
WL TOP T0	0	11.599	<DL	<DL
WL TOP T1	4	12.124	<DL	<DL
WLTOP T2	8	16.879	<DL	<DL
WLTOP T3	24	11.159	<DL	<DL
WL BOT T0	0	2.767	0.038	1.4
WL BOT T1	4	2.089	0.057	2.7
WL BOT T2	8	4.947	0.079	1.6
WL BOT T3	24	3.785	0.139	3.7
WLWT T0	0	10.216	0.047	0.5
WLWT T1	4	10.303	0.039	0.4
WLWT T2	8	10.199	0.024	0.2
WLWT T3	24	10.271	0.058	0.6
HD T0	0	10.605	0.101	1.0
HD T1	4	10.337	0.119	1.2
HD T2	8	10.391	0.455	4.4
HD T3	24	10.572	0.439	4.2
WR T0	0	10.673	0.199	1.9
WR T1	4	10.935	0.146	1.3
WR T2	8	10.829	0.334	3.1
WR T3	24	11.147	0.413	3.7
WRWT T0	0	11.007	0.031	0.3
WRWT T1	4	12.631	0.077	0.6
WRWT T2	8	11.705	0.063	0.5
WRWT T3	24	11.417	0.126	1.1
CL I T0	0	12.713	<DL	<DL
CL I T1	4	14.462	0.325	2.2
CL I T2	8	11.889	0.269	2.3
CL I T3	24	11.338	0.251	2.2

Table B.6 : $T^{200}Hg$, $MM^{200}Hg$ and % MMHg produced in sediments at different incubation times at different locations along the Wabigoon River System.

Sediment Samples				
Location	**Time (hr)**	**$T^{200}Hg$ (ng)**	**$MM^{200}Hg$ (ng)**	**% MMHg produced**
WL T0	0	40.937	0.166	0.4
WL T1	4	37.126	0.705	1.9
WL T2	8	36.111	0.808	2.2
WL T3	24	35.887	1.303	3.6
WL WT T0	0	101.140	0.448	0.4
WLWT T1	4	144.558	1.862	1.3
WLWT T2	8	142.816	1.732	1.2
WLWT T3	24	133.738	2.813	2.1
HD T0	0	117.598	0.504	0.4
HD T1	4	108.007	2.580	2.4
HD T2	8	101.931	3.665	3.6
HD T3	24	99.409	4.360	4.4
WR T0	0	76.158	0.175	0.2
WR T1	4	70.840	1.036	1.5
WR T2	8	68.249	1.618	2.4
WR T3	24	71.288	1.570	2.2
WRWT T0	0	124.023	0.401	0.3
WRWT T1	4	132.742	1.389	1.0
WRWT T2	8	128.766	1.574	1.2
WRWT T3	24	136.140	3.333	2.4
CL I T0	0	121.602	0.091	0.1
CL I T1	4	106.440	0.208	0.2
CL I T2	8	111.710	0.296	0.3
CL I T3	24	113.267	0.323	0.3
CL W T0	0	162.910	0.316	0.2
CL W T1	4	185.717	2.508	1.4
CL W T2	8	193.491	3.917	2.0
CL W T3	24	194.781	7.324	3.8

Table B.7 : MM^{198}Hg (ng/g) over time in sediment samples from different locations along the Wabigoon River.

Location	Time (h)	MM^{198}Hg (ng/g)
WL	0	0.470
WL	4	0.410
WL	8	0.384
WL	24	0.385
WLWT	0	1.249
WLWT	4	1.316
WLWT	8	1.005
WLWT	24	0.895
HD	0	1.398
HD	4	1.241
HD	8	1.181
HD	24	1.068
WR	0	0.807
WR	4	0.748
WR	8	0.720
WR	24	0.589
WRWT	0	1.794
WRWT	4	1.240
WRWT	8	1.595
WRWT	24	0.992
CL I	0	0.995
CL I	4	0.982
CL I	8	0.900
CL I	24	0.988
CL W	0	1.986
CL W	4	2.250
CL W	8	2.312
CL W	24	1.953

B.3. QA/QC

B.3.1. Detection Limits

The method detection limit for MMHg using ICP-MS detection was calculated based on 3 standard deviations of the distillation blank mean, resulting in 6 pg for sediments and 5 pg for water samples. Based on sample sizes of 0.2g of sediment or 50 mL of water this gives detection limits of 0.09 ng/g for sediments and 0.11 ng/L for water.

The method detection limit for THg using ICP-MS detection was calculated based on 3 standard deviations of the digestion blank mean, resulting in 2 pg for sediments and 1.9 pg for water samples. Based on sample sizes of 0.2 g of sediment or 20 mL of water this gives detection limits of 0.04 ng/g in sediments and 0.10 ng/L in water.

The detection limit for the conversion of $MM^{200}Hg$ from the added ^{200}Hg spike was calculated based on the precision of the isotope ratio measurement as described in equation B1.

$$Instrument\ DL\ (\%) = 3\ \times RSD \frac{^{200}Hg}{^{202}Hg}\ isotope\ ratio\ \times natural\ abundance \tag{B1}$$
$$= 3\ \times 1.2\ \%\ \times 0.231$$
$$= 0.8\ \%$$

The instrument DL represents the magnitude of required concentration change to be able to measure the increase of $MM^{200}Hg$ from the added spike. Knowing that the average ambient concentration of MMHg in the Wabigoon River system are 2.05 ng/L and 7.28 ng/g in water and sediments, respectively, this translates in 0.017 ng/L and 0.06 ng/g of $MM^{200}Hg$ that must be produced in order for additional MMHg to be detected by the instrument. Finally, knowing that the Hg(II) spike was 9 ng/L in water and 110 ng/g in sediment samples, this results in a conversion detection limit of 0.19 % in water samples and 0.06 % in sediment samples.

The detection limit for $MM^{198}Hg$ was calculated based on the precision of the isotope ratio measurement as described in equation B2.

$$Instrument\ DL\ (\%) = 3\ \times RSD \frac{^{198}Hg}{^{202}Hg}\ isotope\ ratio\ \times natural\ abundance \tag{B2}$$
$$= 3\ \times 1.9\ \%\ \times 0.0997$$
$$= 0.6\ \%$$

The instrument DL represents the required concentration to be able to measure the added $MM^{198}Hg$. Knowing that the average ambient concentration of MMHg in the Wabigoon River sediments is 7.28 ng/g, this translates in 0.04 ng/g of $MM^{198}Hg$ spike that needs to be present in the samples to be detected by the instrument.

B.4. Calculation of Complementary data

B.4.1. Particulates in water

Particulate concentration (g/L) in water was calculated based on the volume of water (L) filtered using quartz fibre filters (Whatman QMA– 2.2µm), the number of filters used for each location and the mass of the filters after filtration, knowing that the mass of a clean filter is 0.1483 g, as shown in equation B3.

$$Particulates\ (g/L) = \frac{mass\ after\ filtration - 0.1483 \times number\ of\ filters}{Liters\ of\ water} \quad (B3)$$

Table B.8 : Data for calculation of particulate (g/L) in water samples.

Location	number of filters	mass after filtration	Liters of water	particulates (g/L)
WL TOP	4	0.6668	10	0.07
WL BOT	2	0.4393	10	0.04
WLWT	1	0.1585	0.1	1.59
HD	10	1.5258	10	0.15
WR	2	0.3296	3	0.11
WRWT	1	0.1608	0.16	1.01
CL	4	1.7625	2	0.88

B.4.2. Organic matter

Organic matter content in sediments was measured as Lost on Ignition (LOI %), where around 2 g of dried sediment were burned at 500 °C for 4 hours, and mass loss was measured as described in equation B3.

$$OM\ (\%) = \frac{(initial\ mass - final\ mass)}{initial\ mass} \times 100\ \% \qquad (B3)$$

Table B.9 : Organic matter content (%) by LOI in sediment samples.

Location	OM %
WL	8
WLWT	28
HD	25
WR	11
WRWT	17
CL I	3
CL W	10

B.5. Statistics

For the statistical analysis, methylation potentials that were below the conversion detection limit were assumed to be somewhere between zero and the detection limit and half of the conversion limit value was attributed.

Appendix C: Supplementary data for Chapter 4

C.1. Tables

Table C.1 : Abundances (%) and uncertainties for the isotope enriched Hg used in methylation essays.

Abundance %	196	198	199	200	201	202	204
^{199}Hg (internal standard)	<0.02	1.63	91.95	4.92	0.66	0.73	0.11
^{200}Hg (Hg spike)	n.d.	0.11	1.00	98.29	0.32	0.24	0.04

Table C.2 : Ambient THg, ambient MMHg data for the different treated samples: dry = Dry Comparator; ns = Wet Non Spiked; s = Wet Spiked.

Location	Depth	Type	Name	Time (hrs)	THg ambient (ng/g)	MMHg ambient (ng/g) Rep 1	MMHg ambient (ng/g) Rep 2	MMHg ambient (ng/g) Rep 3	MMHg ambient (ng/g) Average	MMHg ambient (ng/g) SD	% MMHg Average	% MMHg SD
WR	0-3	dry	T0	0	322.90	4.23	8.62		6	2	2.0	0.7
WR	0-3	dry	T1	1	163.79	16.38			16.4		10.0	
WR	0-3	dry	T24	24	201.68	11.27	10.83	13.35	12	1	5.9	0.5
WR	0-3	dry	T3d	72	577.23	28.10	22.89		25	3	4.4	0.5
WR	0-3	dry	T6d	144	327.59	11.32	8.95		10	1	3.1	0.4
WR	0-3	dry	Rewet	145	297.50	6.97	5.99		6.5	0.5	2.2	0.2
WR	0-3	dry	Rewet 1hr	146	330.29	0.56			0.6		0.2	
WR	0-3	ns	T0	0	202.01	8.82	12.58		11	2	5.3	0.9
WR	0-3	ns	T1	1	195.78	12.89			12.9		6.6	
WR	0-3	ns	T24	24	170.71	11.27	11.49		11.4	0.1	6.7	0.1
WR	0-3	ns	T3d	72	322.93	10.21	9.53		9.9	0.3	3.1	0.1
WR	0-3	ns	T6d	144	312.36	12.26	10.04		11	1	3.6	0.4
WR	0-3	ns	Rewet	145	365.73	15.81	11.62		14	2	3.7	0.6
WR	0-3	ns	Rewet 1hr	146	303.87	0.36			0.36		0.1	
WR	0-3	s	T0	0	83.04	6.74	8.18	9.92	8	1	10	2
WR	0-3	s	T1	1	194.92	23.94			23.9		12.3	
WR	0-3	s	T24	24	193.81	19.21	17.84		18.5	0.7	9.6	0.4
WR	0-3	s	T3d	72	328.63	11.22	9.98		10.6	0.6	3.2	0.2
WR	0-3	s	T6d	144	315.90	18.63			18.6		5.9	
WR	0-3	s	Rewet	145	339.80	12.75	9.26		11	2	3.2	0.5
WR	0-3	s	Rewet 1hr	146	345.35	0.39			0.4		0.1	
WRWT	0-3	dry	T0	0	38.92	1.06	2.93		2.0	0.9	5	2
WRWT	0-3	dry	T1	1	48.35	0.74	2.34		1.5	0.8	3	2
WRWT	0-3	dry	T24	24	78.86	0.83	0.66		0.7	0.1	0.9	0.1

WRWT	0-3	dry	T3d	72	65.83	0.86	0.82		0.84	0.02	1.28	0.03
WRWT	0-3	dry	T6d	144	81.16	0.82	0.51		0.7	0.2	0.8	0.2
WRWT	0-3	dry	Rewet	145	102.66	0.28			0.3		0.3	
WRWT	0-3	dry	Rewet 1hr	146	74.68	0.39			0.4		0.5	
WRWT	0-3	ns	T0	0	40.76	1.77	1.00		1.4	0.4	3	1
WRWT	0-3	ns	T1	1	14.61	1.36	1.00		1.2	0.2	8	1
WRWT	0-3	ns	T24	24	80.70	0.41	0.38		0.40	0.02	0.5	0.02
WRWT	0-3	ns	T3d	72	81.27	0.99	0.74		0.9	0.1	1.1	0.15
WRWT	0-3	ns	T6d	144	102.75	0.52	0.36		0.4	0.1	0.4	0.07
WRWT	0-3	ns	Rewet	145	99.68	0.05			0.1		0.1	
WRWT	0-3	ns	Rewet 1hr	146	91.82	0.26			0.3		0.3	
WRWT	0-3	s	T0	0	41.54	2.22	9.47		6	4	14	9
WRWT	0-3	s	T1	1	51.99	1.35	1.07		1.2	0.1	2.3	0.3
WRWT	0-3	s	T24	24	126.98	3.53			3.5		2.8	
WRWT	0-3	s	T3d	72	96.98	2.57	2.15		2.4	0.2	2.4	0.2
WRWT	0-3	s	T6d	144	n.d.	2.32	1.56		1.9	0.4	n.d	n.d.
WRWT	0-3	s	Rewet	145	108.58	0.18			0.2		0.2	
WRWT	0-3	s	Rewet 1hr	146	96.79	0.47			0.5		0.5	
WR	3-6	dry	T0	0	158.00	7.5	15.3		11	4	7	2
WR	3-6	dry	T1	1	147.93	11.6	11.7		11.65	0.01	7.87	0.01
WR	3-6	dry	T24	24	131.55	11.4	10.3		10.8	0.5	8.2	0.4
WR	3-6	dry	T3d	72	267.46	7.8	6.9		7.3	0.5	2.7	0.2
WR	3-6	dry	T6d	144	260.97	9.9	7.7		9	1	3.4	0.4
WR	3-6	dry	Rewet	145	300.94	9.3	7.01		8	1	2.7	
WR	3-6	dry	Rewet 1hr	146	258.97	0.4			0.4		0.2	
WR	3-6	ns	T0	0	136.44	15.5	14.33		14.9	0.6	10.9	0.4
WR	3-6	ns	T1	1	142.05	9.1	8.7		8.9	0.2	6.3	0.2
WR	3-6	ns	T24	24	133.56	8.0	8.1		8.06	0.03	6.03	0.02
WR	3-6	ns	T3d	72	257.12	9.5	12.3	14.40	12	2	4.7	0.8
WR	3-6	ns	T6d	144	272.94	10.6	8.0		9	1	3.4	0.5
WR	3-6	ns	Rewet	145	273.11	0.6			0.6		0.2	
WR	3-6	ns	Rewet 1hr	146	263.25	0.4			0.4		0.2	
WR	3-6	s	T0	0	135.42	7.45	17.22		12	5	9	4
WR	3-6	s	T1	1	22.17	1.48	1.16		1.3	0.2	6.0	0.7
WR	3-6	s	T24	24	141.15	12.73	12.31		12.5	0.2	8.9	
WR	3-6	s	T3d	72	276.83	12.81	11.84		12.3	0.5	4.5	0.2
WR	3-6	s	T6d	144	274.96	14.86	12.74		14	1	5.0	0.4
WR	3-6	s	Rewet	145	279.62	0.72			0.7		0.3	
WR	3-6	s	Rewet 1hr	146	289.64	0.40			0.4		0.1	
WRWT	3-6	dry	T0	0	11.28	1.65			1.7		14.7	
WRWT	3-6	dry	T1	1	31.59	0.79	0.45		0.6	0.2	2.0	0.5
WRWT	3-6	dry	T24	24	31.36	0.63	0.46		0.5	0.1	1.7	0.3
WRWT	3-6	dry	T3d	72	50.38	1.78	1.43		1.6	0.2	3.2	0.3

WRWT	3-6	dry	T6d	144	53.46	0.73	0.32	0.79	0.6	0.2	1.2	0.4
WRWT	3-6	dry	Rewet	145	29.07	0.20			0.2		0.4	
WRWT	3-6	dry	Rewet 1hr	146	45.90	0.23			0.2		0.5	
WRWT	3-6	ns	T0	0	13.76	1.52	3.40		2.5	0.9	18	7
WRWT	3-6	ns	T1	1	24.18	0.58	0.30		0.4	0.1	1.8	0.6
WRWT	3-6	ns	T24	24	44.34	1.75	1.16		1.5	0.3	3.3	0.7
WRWT	3-6	ns	T3d	72	49.27	1.53	0.89		1.2	0.3	2.5	0.7
WRWT	3-6	ns	T6d	144	67.37	0.16	0.12		0.14	0.02	0.2	0.03
WRWT	3-6	ns	Rewet	145	33.50	0.48			0.5		1.4	
WRWT	3-6	ns	Rewet 1hr	146	18.41	0.46			0.5		2.5	
WRWT	3-6	s	T0	0	7.75	1.40			1.4		18.1	
WRWT	3-6	s	T1	1	116.04	17.85	18.21		18.0	0.2	15.5	0.2
WRWT	3-6	s	T24	24	37.90	1.56	1.40		1.5	0.1	3.9	0.2
WRWT	3-6	s	T3d	72	26.82	0.75	0.42		0.6	0.2	2.2	0.6
WRWT	3-6	s	T6d	144	39.36	0.91	0.52		0.7	0.2	1.8	0.5
WRWT	3-6	s	Rewet	145	47.30	<DL	0.38		0.4	n.d	0.8	n.d.
WRWT	3-6	s	Rewet 1hr	146	43.07	0.15			0.1		0.3	

Table C.3 : $T^{200}Hg$, $MM^{200}Hg$ data for the different spiked samples.

Location	Depth	Name	Time (hrs)	$T^{200}Hg$ (ng/g)	$MM^{200}Hg$ (ng/g) Rep 1	$MM^{200}Hg$ (ng/g) Rep 2	$MM^{200}Hg$ (ng/g) Rep 3	$MM^{200}Hg$ (ng/g) Average	% $MM^{200}Hg$ produced Average	% $MM^{200}Hg$ produced SD
WR	0-3	T0	0	22.60	<DL	0.10	<DL	0.10	0.44	n.d.
WR	0-3	T1	1	51.85	<DL	0.10		0.10	0.19	n.d.
WR	0-3	T24	24	50.24	0.75	0.82		0.79	1.57	0.07
WR	0-3	T3d	72	86.36	3.96	3.46		3.71	4.3	0.3
WR	0-3	T6d	144	82.45	1.35	0.53		0.94	1.1	0.5
WR	0-3	Rewet	145	80.59	1.82	1.70		1.76	2.18	0.07
WR	0-3	Rewet 1hr	146	87.68	<DL			<DL	<DL	n.d.
WR	3-6	T0	0	44.30	0.14		0.23	0.19	0.4	0.1
WR	3-6	T1	1	16.96	<DL	<DL	<DL	<DL	<DL	n.d.
WR	3-6	T24	24	48.33	0.22	0.29		0.25	0.52	0.07
WR	3-6	T3d	72	90.25	0.25	0.36		0.30	0.34	0.06
WR	3-6	T6d	144	90.40	0.31	0.43		0.37	0.41	0.06
WR	3-6	Rewet	145	84.64	<DL		0.64	0.64	0.8	n.d.
WR	3-6	Rewet 1hr	146	95.80	<DL			<DL	<DL	
WRWT	0-3	T0	0	27.38	<DL	<DL		<DL	<DL	n.d.
WRWT	0-3	T1	1	37.53	<DL	<DL		<DL	<DL	
WRWT	0-3	T24	24	87.62	<DL	0.09		0.09	0.1	
WRWT	0-3	T3d	72	103.51	0.09	0.15		0.12	0.12	0.03
WRWT	0-3	T6d	144	89.05	0.07	0.07		0.07	0.0741	0.0001
WRWT	0-3	Rewet	145	88.60	<DL			<DL	<DL	
WRWT	0-3	Rewet 1hr	146	101.03	<DL			<DL	<DL	
WRWT	3-6	T0	0	25.20	<DL	<DL		<DL	<DL	n.d.
WRWT	3-6	T1	1	34.01	0.25	0.13		0.19	0.6	0.2
WRWT	3-6	T24	24	48.54	<DL	<DL		<DL	<DL	n.d.
WRWT	3-6	T3d	72	61.05	<DL	<DL		<DL	<DL	
WRWT	3-6	T6d	144	78.21	0.05	0.06		0.06	0.07	0.01
WRWT	3-6	Rewet	145	74.18	<DL		0.08	0.08	<DL	
WRWT	3-6	Rewet 1hr	146	90.89	<DL			<DL	<DL	

C.2. Figures

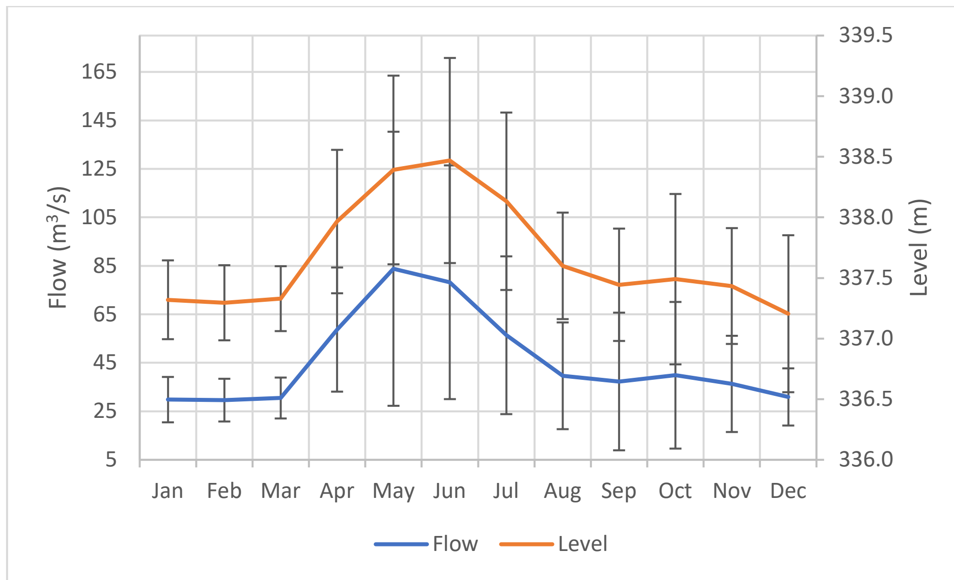

Figure C.1 : Representation of Historical Hydrometric Data of Mean Flow (m^3/s) and Mean Level (m) at Wabigoon River near Quibell (Water Survey of Canada site 05QD006). Flow data between 1953 and 2021, Level data between 2002 and 2021.

C.3. Conversion Detection Limit

The detection limit for the conversion of $MM^{200}Hg$ from the added ^{200}Hg spike was calculated based on the precision of the isotope ratio measurement as described in equation C1.

$$Instrument\ DL\ (\%) = 3\ \times RSD\ isotope\ ratio\ \ 200/202\ \times natural\ abundance \qquad (C1)$$

$$= 3\ \times 1.2\ \%\ \times 0.231$$

$$= 0.8\ \%$$

The instrument DL represents the magnitude of concentration change required to be able to detect the formation of $MM^{200}Hg$ from the added spike. Knowing that the average ambient concentration of MMHg in the samples analysed is on average 5.80 ng/g, this translates to approximately 0.05 ng/g of $MM^{200}Hg$ that must be produced in order to be detected by the instrument. Finally, knowing that the $^{200}Hg(II)$ spike led to a sediment

concentration of 67 ng/g of $^{200}Hg(II)$, this results in a conversion detection limit of 0.07% $MM^{200}Hg$ in the samples.

C.4. Water content

Water content was measured by heating the samples at 60 ºC for 48 hours to accelerate the evaporation process and water content was defined as the mass loss during evaporation as described in equation C2.

$$Water\ content\ (\%) = \frac{initial\ mass - final\ mass}{initial\ mass} \times 100\ \% \tag{C2}$$

Table C.4 : Initial and final masses to calculate water content (%) in wetting cycle samples.

Location	Depth	Type	Name	Time (hrs)	initial mass (g)	final mass (g)	% water
WR	0-3	dry	T0	0	5.0893	3.2349	36
WR	0-3	dry	T1	1	3.6172	2.2898	37
WR	0-3	dry	T24	24	4.7946	3.3417	30
WR	0-3	dry	T3d	72	3.0649	2.7132	11
WR	0-3	dry	T6d	144	1.9609	1.8863	4
WR	0-3	dry	Rewet	145	1.6454	1.5960	3
WR	0-3	dry	Rewet 1hr	146	1.6014	1.5557	3
WR	0-3	ns	T0	0	8.5216	3.4415	60
WR	0-3	ns	T1	1	6.2921	2.4072	62
WR	0-3	ns	T24	24	9.7712	4.3792	55
WR	0-3	ns	T3d	72	5.1703	2.8036	46
WR	0-3	ns	T6d	144	1.6352	1.5638	4
WR	0-3	ns	Rewet	145	5.8730	3.0935	47
WR	0-3	ns	Rewet 1hr	146	2.2582	2.0321	10
WR	0-3	s	T0	0	9.6976	5.6473	42
WR	0-3	s	T1	1	7.6572	3.2079	58
WR	0-3	s	T24	24	9.7238	4.6438	52
WR	0-3	s	T3d	72	3.8982	2.2490	42
WR	0-3	s	T6d	144	1.5034	1.4061	6
WR	0-3	s	Rewet	145	6.0077	3.2039	47
WR	0-3	s	Rewet 1hr	146	5.3890	3.9227	27
WRWT	0-3	dry	T0	0	3.6372	2.7924	23
WRWT	0-3	dry	T1	1	4.3481	3.3085	24
WRWT	0-3	dry	T24	24	4.5210	3.8065	16
WRWT	0-3	dry	T3d	72	3.8561	3.6658	5

WRWT	0-3	dry	T6d	144	3.0926	2.9498	5
WRWT	0-3	dry	Rewet	145	3.2067	3.0920	4
WRWT	0-3	dry	Rewet 1hr	146	2.1747	2.0869	4
WRWT	0-3	ns	T0	0	7.1178	4.3713	39
WRWT	0-3	ns	T1	1	7.6684	4.5446	41
WRWT	0-3	ns	T24	24	6.9733	4.6093	34
WRWT	0-3	ns	T3d	72	3.0163	2.4211	20
WRWT	0-3	ns	T6d	144	2.3296	2.2218	5
WRWT	0-3	ns	Rewet	145	4.1881	2.2366	47
WRWT	0-3	ns	Rewet 1hr	146	3.8083	2.8271	26
WRWT	0-3	s	T0	0	8.0429	4.8090	40
WRWT	0-3	s	T1	1	7.3686	4.2200	43
WRWT	0-3	s	T24	24	6.3073	3.7326	41
WRWT	0-3	s	T3d	72	6.2253	4.4232	29
WRWT	0-3	s	T6d	144	2.6602	2.5414	4
WRWT	0-3	s	Rewet	145	3.1463	1.7172	45
WRWT	0-3	s	Rewet 1hr	146	1.7501	1.6516	6
WR	3-6	dry	T0	0	4.8915	3.3082	32
WR	3-6	dry	T1	1	6.2924	4.4254	30
WR	3-6	dry	T24	24	5.9940	5.0274	16
WR	3-6	dry	T3d	72	2.3634	2.2988	3
WR	3-6	dry	T6d	144	2.8955	2.8079	3
WR	3-6	dry	Rewet	145	1.4816	1.4267	4
WR	3-6	dry	Rewet 1hr	146	0.8130	0.7927	2
WR	3-6	ns	T0	0	7.6808	3.4007	56
WR	3-6	ns	T1	1	6.7521	2.6307	61
WR	3-6	ns	T24	24	6.1635	2.9893	51
WR	3-6	ns	T3d	72	5.1087	2.9791	42
WR	3-6	ns	T6d	144	2.4884	2.4127	3
WR	3-6	ns	Rewet	145	2.0750	1.0191	51
WR	3-6	ns	Rewet 1hr	146	0.6390	0.6240	2
WR	3-6	s	T0	0	6.3300	3.1728	50
WR	3-6	s	T1	1	8.3948	5.3713	36
WR	3-6	s	T24	24	9.1309	4.7235	48
WR	3-6	s	T3d	72	2.3445	1.7003	27
WR	3-6	s	T6d	144	2.7014	2.5993	4
WR	3-6	s	Rewet	145	6.5696	3.4303	48
WR	3-6	s	Rewet 1hr	146	2.9755	2.5773	13
WRWT	3-6	dry	T0	0	4.3597	3.4198	22
WRWT	3-6	dry	T1	1	3.7785	2.9161	23
WRWT	3-6	dry	T24	24	5.4774	4.6361	15
WRWT	3-6	dry	T3d	72	2.5238	2.3848	6
WRWT	3-6	dry	T6d	144	3.6884	3.5290	4
WRWT	3-6	dry	Rewet	145	3.6332	3.5014	4
WRWT	3-6	dry	Rewet 1hr	146	5.8401	5.5993	4
WRWT	3-6	ns	T0	0	10.3302	6.6950	35

WRWT	3-6	ns	T1	1	9.1541	5.6009	39
WRWT	3-6	ns	T24	24	7.3179	4.5147	38
WRWT	3-6	ns	T3d	72	4.4917	3.3662	25
WRWT	3-6	ns	T6d	144	2.7597	2.6330	5
WRWT	3-6	ns	Rewet	145	5.2732	3.4629	34
WRWT	3-6	ns	Rewet 1hr	146	5.2828	4.3524	18
WRWT	3-6	s	T0	0	9.1975	5.4544	41
WRWT	3-6	s	T1	1	7.3547	3.7057	50
WRWT	3-6	s	T24	24	8.1690	5.0806	38
WRWT	3-6	s	T3d	72	11.2896	7.4275	34
WRWT	3-6	s	T6d	144	3.9691	3.7107	7
WRWT	3-6	s	Rewet	145	4.2091	2.4632	41
WRWT	3-6	s	Rewet 1hr	146	3.1486	2.6642	15

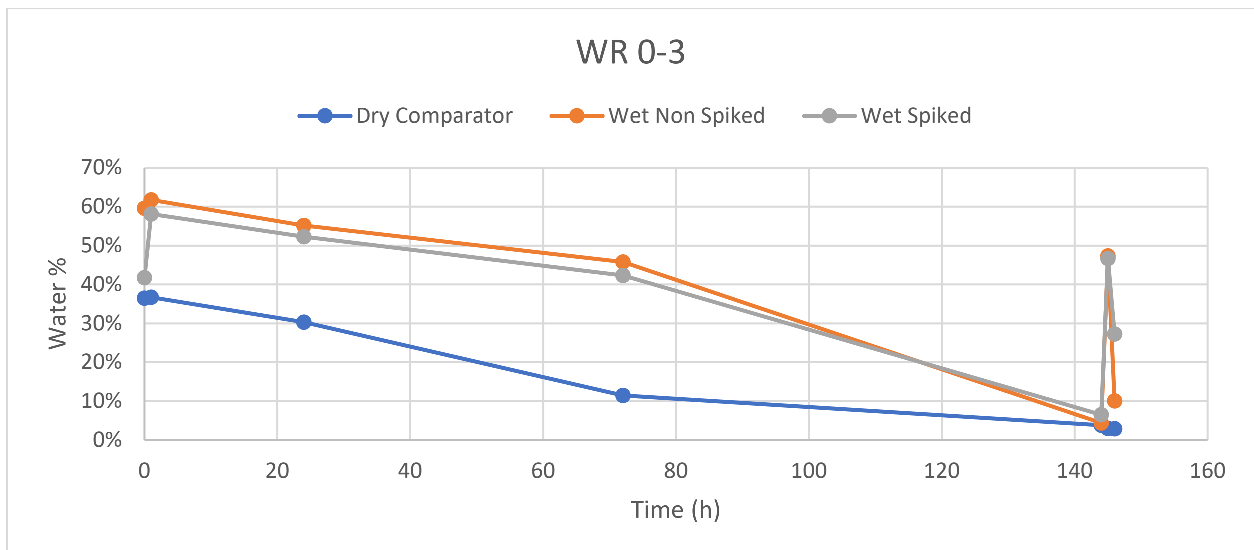

Figure C.2 : Water content (%) over time for the Wabigoon Rapids Riverbed (WR) location at the 0-3 cm depth, for the dry comparator, wet non-spiked and wet spiked samples.

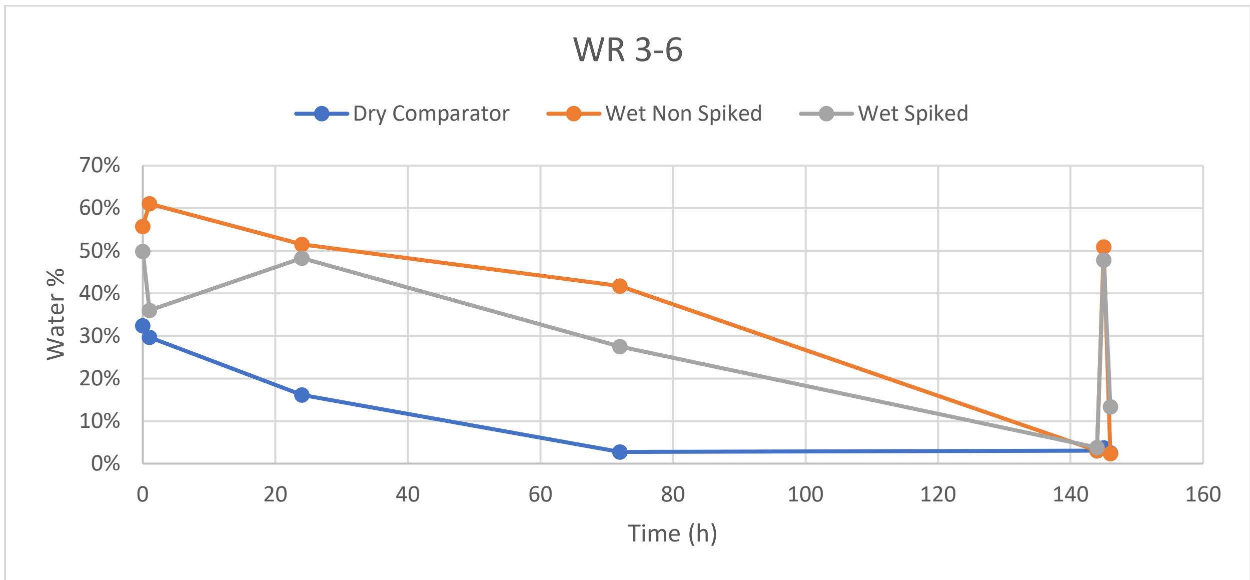

Figure C.3 : Water content (%) over time for the Wabigoon Rapids Riverbed (WR) location at the 3-6 cm depth, for the dry comparator, wet non-spiked and wet spiked samples.

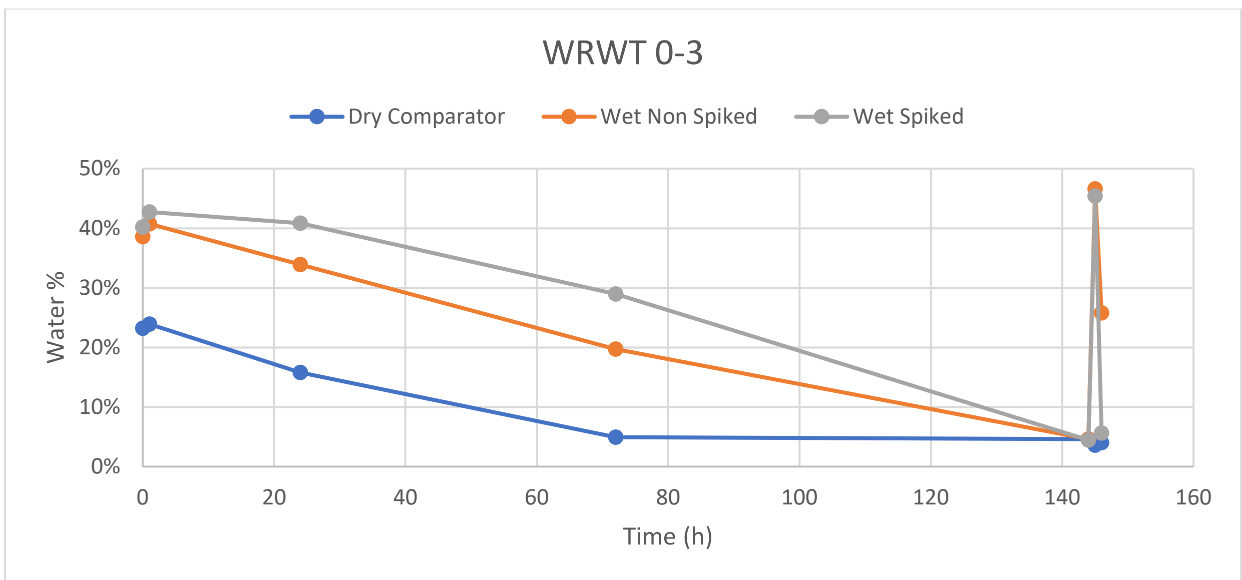

Figure C.4 : Water content (%) over time for the Wabigoon Rapids Wetland (WRWT) location at the 0-3 cm depth, for the dry comparator, wet non-spiked and wet spiked samples.

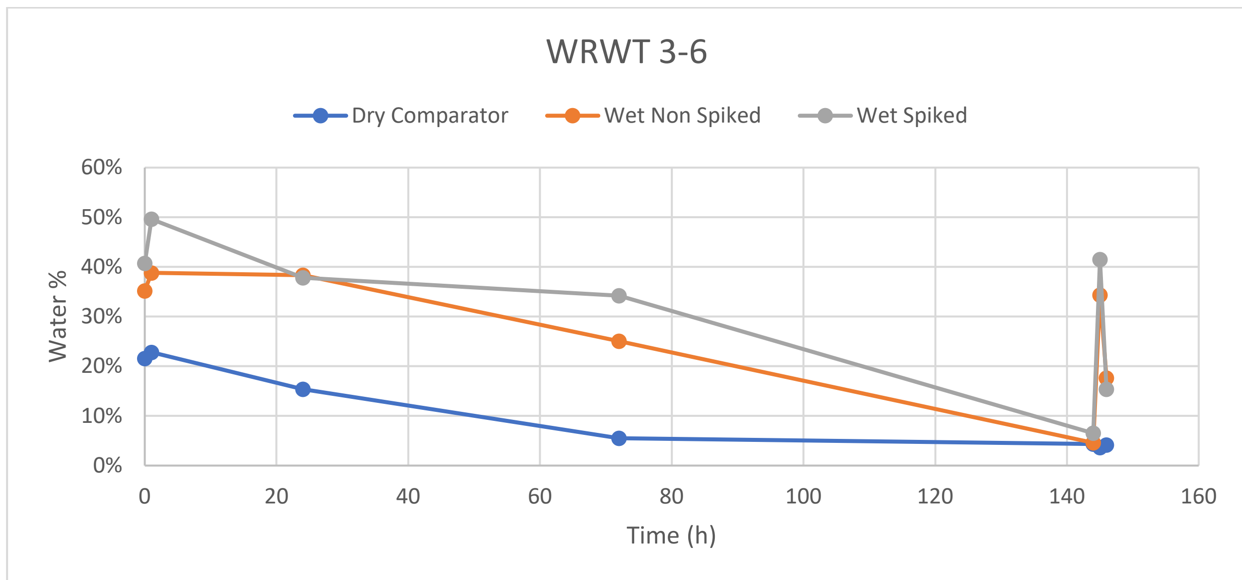

Figure C.5 : Water content (%) over time for the Wabigoon Rapids Wetland (WRWT) location at the 3-6 cm depth, for the dry comparator, wet non-spiked and wet spiked samples.

C.5. Organic Matter

Organic matter content (OM %) in sediments was measured as Lost on Ignition (LOI), where around 1 g of dried sediment were burned at 500 °C for 4 hours, and mass loss was measured as described in equation C3.

$$OM\ (\%) = \frac{(initial\ mass - final\ mass)}{initial\ mass} \times 100\ \% \qquad \text{(C3)}$$

Table C.5 : Organic matter content (%) by LOI in sediment samples

Sample	OM (%)
WR 0-3 cm	11
WR 3-6 cm	11
WRWT 0-3 cm	12
WRWT 3-6 cm	10